NRG ONCOLOGY

NRG-GI007

(ClinicalTrials.gov Identifier NCT #04391049)

Phase I Trial with Expansion Cohort of OBP-301 (Telomelysin) and Definitive Chemoradiation for Patients with Locally Advanced Esophageal And Gastroesophageal Cancer Who are Not Candidates for Surgery

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PHASE I TRIAL WITH EXPANSION COHORT OF OBP-301 (TELOMELYSIN™) AND DEFINITIVE CHEMORADIATION FOR PATIENTS WITH LOCALLY ADVANCED ESOPHAGEAL AND GASTROESOPHAGEAL CANCER WHO ARE NOT CANDIDATES FOR SURGERY

This trial is sponsored by the National Cancer Institute (NCI) and will be led by NRG Oncology.

Coordinating Center:
NRG Oncology
Four Penn Center; 1600 JFK Blvd; Suite 1020; Philadelphia, PA 19103

Study Team (19-MAY-2023)

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Protocol Agent

Agent	Supply	NSC #	IND #	IND Sponsor
OBP-301	Oncolys BioPharma	809930	19404	NRG
Carboplatin	Commercial	241240		
Paclitaxel	Commercial	673089		

Participating Sites (19-MAY-2023)

☒ U.S.

☐ Canada

☐ Approved International Member Sites

Document History

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Amendment 6	**July 10, 2023**
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<table>
<tr><td colspan="3">CONTACT INFORMATION (19-MAY-2023)</td></tr>
<tr><td>For regulatory requirements:</td><td>For patient enrollments:</td><td>For study data submission:</td></tr>
<tr><td>Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal. Regulatory Submission Portal: (Sign in at www.ctsu.org, and select Regulatory > Regulatory Submission.)

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support.

Contact the CTSU Regulatory Help Desk at 1-866-651-2878, or CTSURegHelp@coccg.orgfor regulatory assistance.</td><td>Refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN). OPEN is accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org.

Contact the CTSU Help Desk with any OPEN-related questions by phone or email : 1-888-823-5923, or ctsucontact@westat.com.</td><td>Data collection for this study will be done exclusively through Medidata Rave. Refer to the data submission section of the protocol for further instructions.</td></tr>
<tr><td colspan="3">The most current version of the study protocol and all supporting documents must be downloaded from the protocol-specific page located on the CTSU members’ website (https://www.ctsu.org).

Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the Roster Maintenance application and in most cases viewable and manageable via the Roster Update Management System (RUMS) on the CTSU members’ website.</td></tr>
<tr><td colspan="3"><u>For clinical questions (i.e., patient eligibility or treatment-related)</u>
Contact the Study Data Manager of the Lead Protocol Organization</td></tr>
<tr><td colspan="3"><u>For non-clinical questions (i.e., unrelated to patient eligibility, treatment, or clinical data submission)</u> contact the CTSU Help Desk by phone or e-mail:
CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.</td></tr>
</table>

TABLE OF CONTENTS

NRG-GI007
SCHEMA

REGISTRATION

↓

OBP-301 via Endoscopy
+
Carboplatin / Paclitaxel
+
Radiation

See full treatment details in Section 5.

1. OBJECTIVES

1.1 Primary Objective

To determine if the addition of OBP-301 to chemoradiation with carboplatin/paclitaxel is safe.

1.2 Secondary Objectives

1.2.1 To assess toxicities associated with the addition of OBP-301 to chemoradiation.

1.2.2 To assess the number of clinical complete responses (cCR).

1.2.3 To assess the number of patients alive/without progression (PFS) and the number of patients alive (OS) at 1 and 2 years.

1.3 Exploratory Objectives

To report correlate outcomes – cCR, PFS and OS – with immune and virus-based correlative assays

2. BACKGROUND

2.1 Definitive Chemoradiation (11-JAN-2022)

In the U.S., esophageal cancer is a rare but deadly malignancy. In 2020, it is estimated that 18,440 patients will be diagnosed, with 16,170 deaths (Siegel 2020). It ranks as the seventh cause of cancer deaths, despite its rarity, underlining the challenge of treating such patients. These poor outcomes are in part because approximately half of patients in the U.S. are diagnosed with metastatic disease.

For patients with localized disease, surgery remains the cornerstone of curative therapy. However, esophageal cancer is a disease of older patients, many of whom cannot tolerate the morbidity of an esophagectomy because of underlying cardiopulmonary comorbidities or because of a borderline performance status. Complications include respiratory problems in 11% to 20%, anastomotic leak in 3% to 7%, and wound infection in 5%. Operative mortality ranges from zero to 4%, even in high-volume institutions (Mathisen 1988, Griffin 2002, Nichols 2005).

As such, a standard-of-care that has emerged for patients with locally advanced esophageal and gastroesophageal junction (GEJ) cancer who are medically inoperable is definitive chemoradiation. This approach is based on the seminal Radiation Therapy Oncology Group (RTOG) 85-01 study, which revealed superior outcomes for 5-fluorouracil (FU)/cisplatin chemotherapy and radiation vs. radiation alone (Herskovic 1992); long-term survival was reported in both squamous cell cancer (SCC) patients and the small group of adenocarcinoma patients treated on this study (Cooper 1999).

Definitive chemoradiation is a particularly strong consideration for patients with esophageal SCC. Many of these patients have co-morbidities associated with alcohol and

tobacco use, and surgery carries with it a higher rate of post-operative mortality following neoadjuvant chemoradiation secondary to increased risks of cardiopulmonary complications. In addition, observation for patients with esophageal SCC who achieve a clinical complete response to chemoradiation is supported by the results of two randomized European trials, which did not show a clear improvement in overall survival for surgery following chemoradiation (Stahl 2005, Bedenne 2007).

2.2 Platinum/Taxane with Radiation (11-JAN-2022)

While the RTOG 85-01 study treated patients with 5-FU/cisplatin and radiation, the Dutch CROSS study established carboplatin/paclitaxel as a contemporary reference chemotherapy regimen in combination with radiation prior to surgery for operable tumors (van Hagen 2012). This regimen resulted in a 23% and 49% pathologic complete response rate (pCR) respectively in patients with esophageal adenocarcinomas and SCC. Compared to surgery alone, it also reduced the risk of distant metastases by 6% (Oppedijk 2014).

Of note, the widespread adoption of this regimen is in large part because it was very well-tolerated. For example, the rate of grade 3/4 esophagitis was only 1% and there was only 1 esophageal perforation in 171 patients. The grade 3/4 nausea rate was also only 1%. Unfortunately, the relatively low pCR rate with chemoradiation means that the majority of patients who do not undergo surgery will have locally persistent/recurrent disease, which is a source of significant morbidity, including dysphagia and bleeding. In addition, these patients are at significant risk for developing metastatic disease. As such, this population represents a high unmet medical need in what is already a highly malignant disease.

Relatively few studies have focused on this population as the majority of studies have mandated pre-operative therapy prior to surgery. A recent exception is the RTOG 0436 study, which randomized 344 patients (62% of whom had adenocarcinomas) to cisplatin/paclitaxel and radiation to 50.4 Gy (1.8 Gy/fraction ×28 fractions) with or without cetuximab, an antibody against epidermal growth factor receptor (Suntharalingam 2017). The study demonstrated there was no improvement in overall survival (OS), which was the primary endpoint; the 24-month OS rate was about 44%. Similarly, the clinical CR (cCR) rate – defined as endoscopic clearance of the primary tumor – was about 56% in both arms and the 24-month local failure rate for all patients was about 48%.

2.3 Oncolytic viruses

Oncolytic immunotherapy employs viruses that are designed to preferentially replicate in and lyse cancer cells and through this process trigger anti-tumor immunity. Following the first description of a virus engineered to replicate selectively in cancer cells over 20 years ago, the field of oncolytic immunotherapy has expanded dramatically. Over 10 different

viral species have now been assessed or are under assessment in human studies, including the H101 adenovirus product, which was approved for use in combination with chemotherapy as a treatment for head and neck cancer by the Chinese State Food and Drug Administration in November 2005.

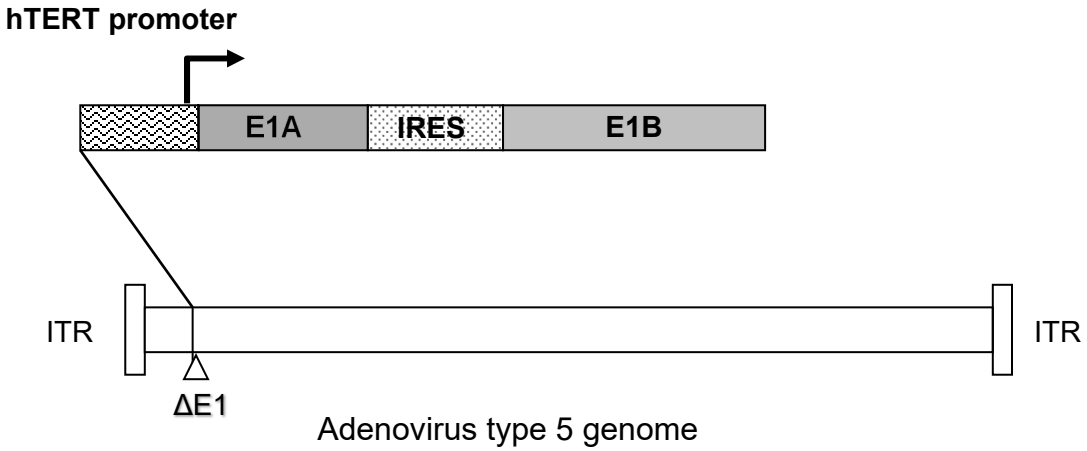
More recently, a phase III pivotal trial of talimogene laherparepvec, an oncolytic herpes virus expressing granulocyte-macrophage colony stimulating factor (GM-CSF) in patients with advanced melanoma met its primary endpoint, demonstrating a significant improvement in durable response rate versus GM-CSF alone (16% vs 2%, $p<0.0001$) in patients with advanced melanoma (Andtbacka 2015). Based on these results, in October 2015, it was granted approval as the first oncolytic viral therapy in the U.S. as local therapy for recurrent melanoma. The European Medicines Agency also approved talimogene laherparepvec for the treatment of adults with unresectable melanoma.

Conditionally replicable oncolytic viruses are engineered for selective replication in cancer cells that express certain oncogenic phenotypes (Hawkins 2001, Nemunaitis 2010). To this end, multiple viral backbones have been employed, although the most commonly utilized is derived from the adenovirus serotype 5 (Ad5).

2.4 OBP-301: Background

OBP-301 (Telomelysin^{TM}) is a novel, condition-restricted, replication-competent adenovirus serotype 5-based adenoviral construct that incorporates a human telomerase reverse transcriptase gene (hTERT) promoter. hTERT encodes the catalytic protein subunit of telomerase, a polymerase that acts to stabilize telomere lengths and is highly expressed in tumors but not in normal, differentiated adult cells. Thus infection with OBP-301 leads to viral cytolytic activity preferentially in cancer cells but not normal cells.

The adenoviral reproductive cycle is a highly orchestrated process. At the molecular level, the adenoviral genome contains 8 transcriptional units that are activated at different phases of infection. They are referred to as early (E1A), immediate early (E1B, E2, E3, E4), intermediate (IX and IVa2), and late genes. It has been reported previously that the transcriptional control of the E1A expression via the hTERT promoter could restrict adenoviral replication to telomeres-positive cells and efficiently lyse tumor cells (Fujiwara 2007). In the construct of OBP-301, the normal transcriptional regulatory element of the E1A gene is replaced by the hTERT promoter and additional modifications to enhance specificity include the replacement of the normal transcriptional element of viral E1B gene by an internal ribosomal entry site (IRES) sequence to minimize “leakiness”). Furthermore, OBP-301 is the first replication-competent adenovirus that retains a fully functional viral E3 region, which codes for proteins that regulate the immune response to the virally infected cell (Hawkins 2001).



ΔE1 = delta E1 adenovirus vector, E1A, E1B = transcriptional units, hTERT = human telomerase reversetranscriptase, IRES = internal ribosomal entry site, ITR = inverted terminal repeat

Figure 1. The construct of OBP-301

Telomerase activation is considered to be a critical step in carcinogenesis and its activity is closely correlated with hTERT expression. Thus, expression of hTERT is generally low in differentiated adult cells and active in cells with proliferative capacity such as tumors, gastrointestinal endothelium, or stem cells;(Shay 1997, Shay 2001) the majority of malignant tumors shown to demonstrate high telomerase activity. Previous studies have demonstrated control over the expression of exogenous genes to telomerase-positive cancer cells by the hTERT promoter (Kamradt 2003) and the hTERT promoter is an excellent candidate for generating cancer-specific oncolytic adenovirus.

There are several mechanisms by which adenoviruses in general, and OBP-301 in particular, can destroy tumor cells in vivo. Direct cell lysis occurs following viral replication and data from animal models have shown that non-replicating adenovirus in rodent tissue will induce both an acute (2-4 days) and chronic (14 days) inflammatory infiltration. During the acute phase, tumor necrosis factor (TNF-α) and interleukin (IL)-1 and IL-6 are produced in high concentrations locally within the tissue. These cytokines have both direct cytopathic effects and indirect effects through immune effector cell recruitment into the local tissue. Finally, adenovirus infection of a tumor cell may augment tumor antigen presentation and enhance tumor antigenicity, leading in turn to immunogenic cell death, which leads to release of danger-associated molecular patterns, thereby attracting innate immune cells, particularly dendritic cells to the tumor and leading to the recruitment and maturation of tumor specific T-cells in the tumor microenvironment, improved recognition and destruction of tumors by cytotoxic T cells (van Vloten 2018).

In vitro studies have validated the selective infectivity and direct cytolysis of OBP 301 in cancer cells and in vivo, intratumoral injection has demonstrated anti-tumor activity without significant toxicity. Additionally, distant viral uptake was observed following

intratumoral injection, evidenced by the presence of adenoviral protein in non-injected tumors following treatment of the contralateral tumor. These encouraging pre-clinical findings of safety and directed anti-tumor activity were supportive of the continuing clinical development of OBP 301 as an oncolytic therapeutic agent (Fujiwara 2007).

2.5 OBP-301: Clinical Experience

In a phase I study, OBP-301-001, (OBP-301), 16 patients were treated with single intra-tumoral injections of OBP-301(Nemunaitis 2010). No grade 3/4 toxicities were observed and common grade 1/2 toxicities included injection site reactions and systemic symptoms (fevers/chills in 6 patients). Viral DNA was transiently detected in 13 of the 16 patients. Of 11 patients with RECIST-evaluable lesions, 1 patient had a partial response, while 7 had stable disease.

In OBP-301-003-CV (OBP-301 + Radiation phase I/II study), OBP-301 was combined with radiation to 60 Gy in Japanese esophageal cancer patients who were assessed not to be candidates for surgery or chemoradiation. OBP-301 were administered at three dose levels; 1 mL of $1x10^{10}$ viral particles (vp)/mL, 1 mL of $1x10^{11}$ vp/mL, and 1mL of $1x10^{12}$ vp/mL, administered 3 times via endoscopic injection (Tanabe 2019). In total, 13 patients were treated (3 at the highest dose level of 1×10^{12} vp/ml). Toxicities were manageable. The only observed grade 3/4 toxicities were leukopenia (8%) and lymphopenia (77%). Grade 1/2 toxicities include transient fever without evidence of infection (62%), chills (15%), nausea (25%), esophagitis (31%) anorexia (39%) and pneumonitis (23%). Of the 13 patients treated, 8 achieved a local cCR to the combination of OBP-301 and radiation and 3 patients have achieved a partial response. The overall response rate was 91.7%. The clinical CR was 83.3 % in stage I and 60% in stages II and III. These results compare favorably with a historical cCR rate of about 37.2% for stages I-IV with radiation alone (Toh 2020, Fujiwara 2019).

Beyond augmenting the local effects of radiation, OBP-301 has been shown *in vitro* to inhibit DNA repair mechanisms, which may sensitize the infected tumor cells to radiation (Kuroda 2010), leading to synergistic effect. In addition to a local effect, OBP-301 may also potentiate immune responses. Viral replication is highly immunogenic and oncolysis induced by such viruses releases tumor epitopes and provides costimulatory danger signals (Endo 2008).

2.6 Combining OBP-301 with Chemoradiation (11-JAN-2022)

Given the excellent safety profile of both carboplatin/paclitaxel and radiation and OBP-301 with radiation (in an elderly population that was assessed not to be a eligible for even chemoradiation alone), there is a compelling rationale to combine OBP-301 with carboplatin/paclitaxel and radiation as definitive therapy for patients with esophageal/GEJ cancer who are not surgical candidates. In addition to improvement in locoregional control, there is also the potential to augment anti-tumor immunity, which may also impact positively on distant micrometastatses through an abscopal effect.

3. ELIGIBILITY AND INELIGIBILITY CRITERIA

Note: Per NCI guidelines, exceptions to inclusion and exclusion criteria are not permitted. For questions concerning eligibility, please contact the Biostatistical/Data Management Center (see protocol cover page). For radiation therapy-related eligibility questions, please contact RTQA (see protocol cover page).

3.1 Eligibility Criteria (19-MAY-2023)

A patient cannot be considered eligible for this study unless ALL of the following conditions are met.

3.1.1 Pathologically (histologically or cytologically) proven diagnosis of adenocarcinoma or squamous cell carcinoma (SCC) of the esophagus or gastroesophageal junction (GEJ) within 90 days prior to registration;

- Gastroesophageal junction tumors must be Siewert Type I/II;

3.1.2 Required diagnostic workup for study entry:

- History/physical examination prior to registration;
- CT of the chest/abdomen with intravenous contrast within 28 days prior to registration; If CT contrast is contraindicated MRI of the chest/abdomen without contrast is permitted;
- Bronchoscopy for squamous cell carcinoma (SCC) tumors that are adjacent to the airway to exclude a tracheoesophageal fistula within 42 days prior to registration;
- Endoscopic ultrasound (if technically feasible) within 90 days prior to registration;
- Whole body PET/CT scan within 42 days prior to registration: Note: scan will be used for radiation treatment planning, in addition to ruling out metastatic disease;

3.1.3 Age ≥ 18;

3.1.4 ECOG Performance Status of 0-2 within 14 days prior to registration;

3.1.5 Adequate hematologic function within 14 days prior to registration defined as follows:

- Absolute Neutrophil Count ≥1,500/mcL
- Hemoglobin ≥ 9 gm/dL
- Platelets ≥ 100,000/mcL

3.1.6 Adequate renal function within 14 days prior to registration defined as follows:

- Creatinine clearance of ≥50 ml/min (as calculated by Cockcroft-Gault equation)

3.1.7 Adequate hepatic function within 14 days prior to registration defined as follows:

- Total Bilirubin ≤ 1.5 x ULN (patients with known Gilbert Syndrome can have a Total Bilirubin <2.5 x ULN)
- AST/ALT ≤ 2.5 x ULN

3.1.8 Patients for whom non-operative management is a viable option in the opinion of a thoracic surgeon and/or multidisciplinary team and are candidates for chemoradiation; this does not preclude patients from receiving surgery after chemoradiation if felt to me medically indicated;

3.1.9 Patients must, in the opinion of a treating gastroenterologist, have a tumor that is amenable to intratumoral injection with at least 1 mL (1 x10^{12} vp/mL) of OBP-301 and be a candidate for 3 endoscopy procedures.

3.1.10 Female patients of child bearing potential must have a negative serum/urine pregnancy test within 14 days prior to study entry. A female not of childbearing potential is one who has undergone a hysterectomy, bilateral oophorectomy, tubal ligation, or who has had no menses for 12 consecutive months.

3.1.11 Patients of reproductive potential must agree to use effective contraception for the duration of study treatment as well as 6 months (for women) or 12 months (for men) after the last administered injection of OBP-301. Effective contraception includes oral contraceptives, implantable hormonal contraception, double-barrier method or intrauterine device.

3.1.12 The patient must provide study-specific informed consent prior to study entry.

3.1.13 Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.

3.1.14 Known acute or chronic hepatitis B or C infection (testing not required prior to study entry in patients with no known history of hepatitis B or C);

- For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.
- For patients with a history of hepatitis C virus (HCV) infection, they must (i) have been treated and cured, (ii) for patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load

3.1.15 HIV-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months prior to study entry are eligible for this trial.

3.2 Ineligibility Criteria (11-JAN-2022)
Patients with any of the following conditions are NOT eligible for this study.

3.2.1 Definitive clinical or radiologic evidence of metastatic disease including;

- Positive malignant cytology of the pleura, pericardium or peritoneum;
- Radiographic evidence of involvement of any adjacent mediastinal structure, e.g. aorta, trachea, which would increase the risk of repeated endoscopic interventions;
- Tracheoesophageal fistula
- Radiographic evidence of distant organ involvement;

- Non-regional lymph nodes that cannot be contained within a radiation field;

3.2.2 More than 1 esophageal lesion;

3.2.3 Prior systemic chemotherapy for the study cancer;

3.2.4 Prior radiotherapy to the region of the study cancer that would result in overlap of radiation therapy fields;

3.2.5 Biopsy-proven tumor invasion of the tracheobronchial tree or presence of tracheoesophageal fistula or recurrent laryngeal or phrenic nerve paralysis.

3.2.6 For patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, a New York Heart Association Functional Classification 2C or worse.

3.2.7 Uncontrolled diabetes;

3.2.8 Infection requiring IV antibiotics at the time of registration;

3.2.9 Patients requiring immunosuppressive medications including chronic suppressive steroid therapy (greater than the equivalent of 20mg/day of prednisone), methotrexate, azathioprine and TNF-α blockers within 7 days prior to study entry; See section 5.4.2 for further details;

3.2.10 Received live vaccine within 30 days prior to registration;

3.2.11 Received a blood transfusion, hematopoietic agent; granulocyte-colony stimu*l*ating factor (G-CSF), and/or oxygen supplementation within 7 days before the screening lab.

3.2.12 Breast feeding females.

4. REQUIREMENTS FOR STUDY ENTRY, TREATMENT, AND FOLLOW-UP
(19-MAY-2023)

PRE-TREATMENT ASSESSMENTS

Assessments	Prior to Registration (calendar days)	Prior to Initial OBP-301 Injection (calendar days)
Optional Patient History Form (Patient Reported)*		X
Endoscopy with biopsy	90	
Endoscopic Ultrasound (If technically feasible)	90	
Bronchoscopy (for SCC tumors that are adjacent to the airway)	42	
Whole body PET/CT scan	42	
CT/MRI chest/abdomen**	28	
CBC (to include absolute neutrophil count, Hgb and platelets)	14	
CMP (to include creatinine, AST/ALT, and total bilirubin)	14	
History and physical exam	X	
ECOG Performance status	14	
Concomitant Medications*	14	
Urine or Serum Pregnancy test	14 (for women of child bearing potential)	X
Optional collection of archival tumor tissue	(see Section 10)	
Optional Blood Collection (see Section 10)		≤2

*In person preferred; may be conducted by telehealth visit at the discretion of the site-identified qualified healthcare professional.

**CT of the chest/abdomen with intravenous contrast. If CT contrast is contraindicated, MRI of the chest/abdomen without contrast is permitted.

ASSESSMENTS DURING TREATMENT

Assessments	**Prior to OBP-301 injection**	**Weekly during concurrent chemotherapy and radiation**
Physical exam per Institutional Standard		X
ECOG PS assessment		X
Adverse Event Assessment		X
Concomitant Medications		X
CBC (to include WBC, absolute neutrophil count, Hgb and platelets)	X (see section 5.2)	X
CMP (to include creatinine, AST/ALT, and total bilirubin)		X
Optional Collection of tumor and blood for correlative assays	X(see Section 10)	
Urine or Serum Pregnancy test	Only prior to first OBP-301 treatment only (for women of child bearing potential)	

ASSESSMENTS IN FOLLOW UP

Assessment	1 week post chemoRT	6-8 weeks post RT completion	Q 3 mo from end RT X 2 YRS	Q 3-6 mo from end RT X 2 YRS	Q 6 mo from end RT X 2 YRS
Physical Exam	X				
CBC and CMP	X	X			
Whole Body PET/CT (if not covered by insurance CT/MRI is allowed and must include chest, abdomen, and pelvis)		X			
CT or MRI chest/Abdomen					X
Endoscopy with /without Biopsy (see definition of disease assessments below)		X		X *(only for clinical complete responders at 6-8 weeks post RT completion)*	
Physical Exam		X	X		
Adverse Event Evaluation		X	X		
ECOG PS		X	X		
Optional Collection of Tumor and Blood for Correlative Assays (See Section 10)		X			

Definition of Disease Assessments

The same method of imaging assessment and the same technique should be used to characterize baseline and post-treatment extent of disease. Imaging-based evaluation is preferred to evaluation by clinical exam when both methods have been used to assess the anti-tumor effect of therapy.

While it is recognized that it is not always possible to obtain pathologic proof of progressive disease, biopsy or autopsy material confirming recurrent cancer is preferred and every reasonable attempt to obtain such is encouraged.

Local Disease

6-8 weeks post RT completion for clinical complete response (cCR)

At the time of the 6-8 weeks post RT completion endoscopy, a visual inspection of the site of the original primary disease must be documented. Patients will be scored as follows:

- Those patients who are found to be grossly free of disease are NOT required to undergo biopsy and will be scored as achieving a **clinical complete response**
- Patients deemed to have residual disease or suspicion of residual disease must undergo a biopsy in order to pathologically confirm findings. Any patient with pathologically confirmed residual disease will be scored as a **local failure**. Patients who are pathologically proven to have no evidence of disease will be scored as achieving a **clinical complete response**

Follow-up after initial cCR assessment

- Clinical complete responders: If there is evidence or suspicion of disease on follow-up CT/MRI scans, biopsy is required to confirm recurrence of disease. In the absence of clinical symptoms or radiographic findings, patients should undergo a surveillance EGD every 6 months for up to 2 years from the start of protocol therapy.
- Patients with residual disease: These patients should be followed for local disease per institution's standard of care.

Distant Disease

In the absence of histologic or cytologic proof of recurrence, clinical evidence (including e.g. new masses on CT scan, new lesions on bone scan, ascites not explained by other causes or enlarging mass by endoscopic ultrasound) should be considered highly suspicious of recurrent disease. It is strongly suggested that these findings lead to a biopsy to confirm progression. However, if the patient's clinical condition does not allow biopsy to be performed and these findings are unequivocal, they may be acceptable as progression of disease.

5. TREATMENT PLAN/REGIMEN DESCRIPTION

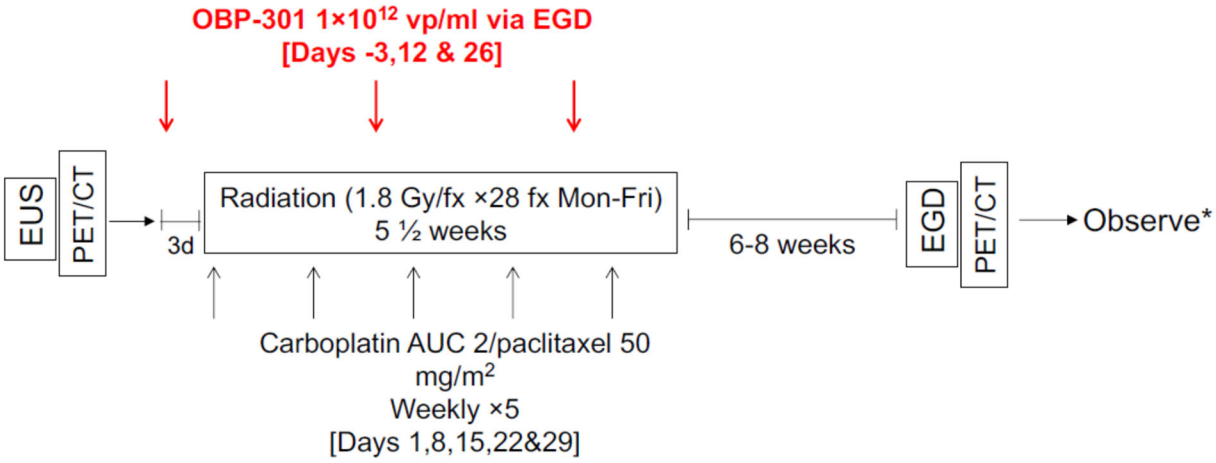
Protocol treatment must begin within 2 weeks after protocol registration.

5.1 Treatment Summary (19-MAY-2023)

Patients undergo an intra-tumoral injection via endoscopy (EGD) with OBP-301 (1×10^{12} vp/mL).

Patients will then receive chemoradiation with carboplatin and paclitaxel administered on a Monday or Tuesday and radiation administered Monday through Friday.

TREATMENT SCHEMA FOR INTIAL COHORT



*Surgery is permitted post-chemoradiation if medically indicated.

Treatment Regimen with Initial OBP-301 Dose Level:OBP-301 injection must start Thursday or Friday. Chemoradiation must start Monday or Tuesday.

		Week 1	Week 2		Week 3	Week 4		Week 5
	Day -3 (+/- 1 day)	Day 1 (+/- 1 day)	Day 8 (+/- 2 day)	Day 12 (+/- 1 day)	Day 15 (+/- 2 day)	Day 22 (+/- 2 day)	Day 26 (+/- 1 day)	Day 29 (+/- 2 day)
OBP-301 by EGD 1 x10^{12} vp/mL (q14d ±1 day)	×			×			×	
Carboplatin AUC 2		×	×		×	×		×
Paclitaxel 50mg/m^2		×	×		×	×		×
Radiation 1.8 Gy/fraction		Daily Monday-Friday for 5 ½ weeks (28 fractions)						

IF NEEDED; Treatment Regimen with DE-ESCALATED OBP-301 Dose Level :

		Week 1	Week 2		Week 3	Week 4		Week 5
	Day -3 (+/- 1 day)	Day 1 (+/- 1 day)	Day 8 (+/- 2day)	Day 12 (+/- 1 day)	Day 15 (+/- 2 day)	Day 22 (+/- 2 day)	Day 26 (+/- 1 day)	Day 29 (+/- 2 day)
OBP-301 by EGD 1 x10^{11} vp/mL (q14d ±1 day)	×			×			×	
Carboplatin AUC 2		×	×		×	×		×
Paclitaxel 50mg/m^2		×	×		×	×		×
Radiation 1.8 Gy/fraction		Daily Monday-Friday for 5 ½ weeks (28 fractions)						

5.2 Systemic Therapy (26-JUL-2021)

5.2.1 OBP-301 Treatment

OBP-301 (1×10^{12} vp/mL) is administered as intratumoral injection into a primary esophageal tumor mass that is suitable for injection, and in a location amenable for endoscopic injection. Irrespective of the size of the tumor, every effort should be made to inject 1.0 to 2 ml of OBP-301; the maximal volume of 2 ml is preferred and should be injected as long as this is technically possible.

Pre-injection planning is the key to success, therefore, a sketch of the tumor and the planned injection sites should be made. The sketch will be helpful in determining the total quantity of study drug to inject. Please refer to Appendix I.

The optimum technique for injection of a primary tumor is to inject the viral particles into the lesion. This will ensure that the viral particle will maximally penetrate into the lesion. Endoscopists who perform a polypectomy will be eligible to perform the procedure. A training video (prepared by Oncolys Biopharma) is available as well.

The procedure will be performed in the Endoscopy Unit with standard sterilization procedures. A standard gastroscope and needle (e.g. GIF-HQ190 Olympus gastroscope with 23 or 25 gauge injection needle made by Boston Scientific/ or Olympus (NeedleMaster) is used for the procedure.
Guidelines for each injection procedure are provided below:

Pre-Injection

- Review of medications
- Abbreviated physical exam and performance status

- Vital signs

Post-Injection

- Monitor patient for 60-90 minutes for any symptoms of injection site pain, bleeding, nausea.
- Vital signs (except weight)

Standard sedation procedures used for endoscopy will be used, and consists of monitored anesthesia care with an anesthesiologist and the patient monitored at all times.

For patients who consent to the optional collection of tumor (see Section 10), biopsy of the primary tumor should be performed prior to OBP-301 injection. Up to 3 biopsies should be performed and processed by Pathology per standard procedure. If <3 core biopsies are performed, the reason should be documented in the procedure note.

The tumor area to be injected will be ≥1 cm and can be mapped into (preferably at least) 5 sections. The dose (volume) delivered to the lesion should be within the range of 1.0 to 2.0 mL.

Refer to the OBP-301 Pharmacy Manual (Appendix I) for details to complete the endoscopic injection of OBP-301.

Parameters for Treatment

- A CBC is required within 3 days prior to OBP-301 administration.

Parameters for treatment on Day -3 are as follows:

- WBC ≥3,000/µL
- ANC ≥1,500/µL
- Platelets ≥100,000/µL
- Hgb ≥9 g/dL

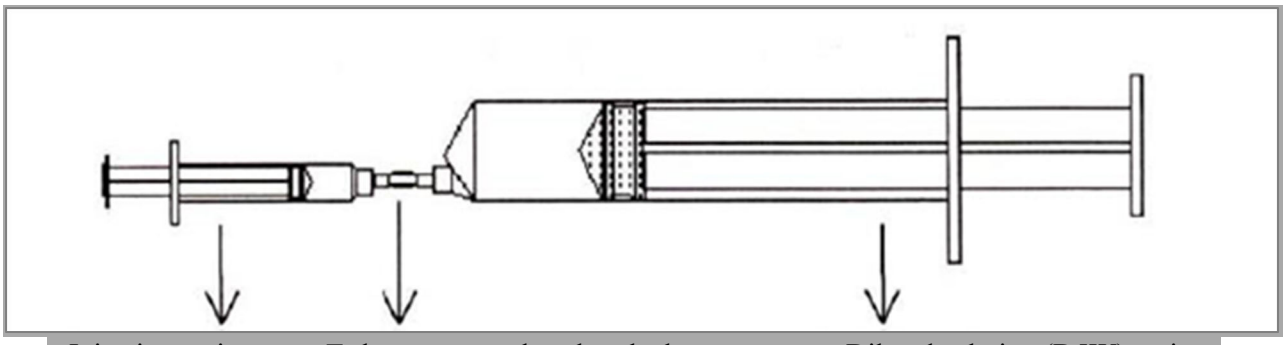
Parameters for treatment on any other subsequent day are as follows and based on a CBC obtained at any time during that week prior to OBP-301 administration.

- ANC ≥1,000/µL
- Platelets ≥75,000/µL

FOR THE DE-ESCALATED COHORT:

Preparation Step: Use injection syringe to suck the 1 ml from $1x10^{12}$ vp/mL vial and use diluted solution syringe to suck the 9 mL D5W.

Dilution Step: Slowly push 1 mL OBP-301 to Diluted solution syringe, and mix (The concentration of diluted solution syringe would be 1 x 10^{11} vp/mL).



Injection syringe 1 mL | T shape stop cock or luer lock connector | Diluted solution (D5W) syringe 9 mL

Dose Levels

Dose Level for DLT Assessment	Number of OBP-301 Doses	Details
Initial COHORT	3	▪ OBP-301 1×10^{12} viral particles (vp)/mL (up to 2mL of OBP-301 should be injected based on the tumor volume.) will be given by an intra-tumoral injection via EGD every 14 days on Days -3, 12 and 26 (±1 day).
IF NEEDED; DE-ESCALATED COHORT	3	▪ OBP-301 1×10^{11} viral particles (vp)/mL (up to 2mL of OBP-301 should be injected based on the tumor volume)) will be given by an intra-tumoral injection via EGD every 14 days on Days -3,12 and 26 days (±1 day).

5.2.2 Paclitaxel/Carboplatin Chemotherapy

Paclitaxel

Paclitaxel will be administered at a fixed dose of 50 mg/m^2 over 60 minutes weekly; rounding dose down to the nearest 10 mg is permitted as long as within 10% of the calculated dose. This is the recommended dose administration guidance but sites may follow institutional standards.

The paclitaxel infusion is administered prior to the carboplatin infusion.

The first dose will be given on Monday or Tuesday during the first week of radiation. Subsequent doses will be given every 7 days +/-1 day for a total of 5 doses.

Recommended pre-medications or per institutional standards:

- Dexamethasone 10mg IVP, completed 30 minutes prior
- Diphenhydramine 50mg IVP or equivalent
- H-2 blocker of choice (Cimetidine 300mg IV or ranitidine 50mg IV or famotidine 20mg IV) 30 minutes prior

Carboplatin

See Carboplatin Dose Calculation Instruction in Appendix IV
Carboplatin will be administered at a fixed dose AUC 2 over 30 minutes weekly. The first dose will be given on either Monday or Tuesday during the first week of radiation. Subsequent doses will be given every 7 days +/-1 day for a total of 5 doses.
The maximum dose of carboplatin will be capped at 300 mg.

Recommended pre-medications or per institutional standards:

- Antiemetic regimen including a $5HT_3$-antagonist and steroid

5.3 Radiation Therapy (10-JUL-2023)
Radiation must begin on a Monday or Tuesday following the first intratumoral injection with OBP-301.

Radiation Therapy Schema
Radiation will be given in 1.8 Gy/fraction ×28 fractions (50.4 Gy total)- 5 fractions/week Monday-Friday starting on Day 1. Radiation can either precede or follow chemotherapy on days when they are given concurrently.

5.3.1 Treatment Technology
Proton treatments are not allowed, IMRT only.

Photon beam intensity modulated radiation therapy (IMRT) /VMAT with effective energies between 6 and 10 MV is required. IMRT may be delivered using multiple fixed fields employing dynamic multi-leaf collimator, helical arc therapy or volumetric modulated arc therapy using any of the commercially available delivery systems. Daily image-guidance (IGRT) is also required for all patients.

5.3.2 Immobilization and Simulation
Proper immobilization is important for this protocol. Patient setup reproducibility must be achieved using appropriate clinical devices.

Assessment and Management of Internal Organ Motion
Special considerations must be made to account for the effect of internal organ motion (e.g., primarily breathing associated motion) on target positioning and reproducibility. As a first step, it is required that the treatment team quantify the specific motion of a target so as to determine if management strategies are needed. The patient should be in normal free breathing at the time of initial tumor motion assessment. Deep inspiration or expiration breath hold is not allowed for initial tumor motion assessment as such assessment generally overestimates free breathing tumor motion. Options for motion

assessment include real time fluoroscopy and 4D CT scanning. Any strategy, including 4D CT should incorporate appropriate image review and quality assurance to ensure suitability for treatment planning and target delineation.

In some tumor locations, tumor motion measurement may demonstrate motion exceeding the required small tumor expansions per this protocol (resulting in marginal miss or excessive volume of irradiation) unless a motion management strategy is employed. Acceptable maneuvers for motion management include reliable abdominal compression, accelerator beam gating with the respiratory cycle, tumor tracking, and active breath-holding techniques or other methods approved by the study committee. These measures are recommended in cases where the extent of motion quantified on motion assessment exceeds 1 cm. Internal organ management maneuvers must be reliable enough to insure that the GTV does not deviate beyond the confines of the PTV with any significant probability (i.e., < 5%).

Table 5.3.2 highlights the recommended and minimum requirements for motion assessment and treatment planning imaging.

Table 5.3.2 Motion Assessment/Management Guidelines for Simulation

Treatment Technique	Recommended Method for Motion Assessment During Simulation	Minimum Method for Motion Assessment During Simulation	Scan(s) Recommended for Treatment Planning
Free breathing treatment using an internal gross target volume (IGTV) approach, including abdominal compression	4D CT or fluoroscopy as long as tumor can be directly visualized	Repeated slow acquisition CT scanning through the target (to sample motion) fused to the planning CT dataset	Average intensity projection (AveIP) scan from a full field of view 4D CT for dose calculations; the maximum intensity projection (MIP) scan may be desirable to aid IGTV definition; Free-breathing scans are not recommended for treatment planning.
Gating with a gating window	4D CT	Exhale CT plus fluoroscopy (free-breathing + fluoroscopy strongly discouraged due to baseline shift)	Reconstructed average of gating window scans from 4D CT.
Breath hold (i.e. ABC)	Reproducibility of breath hold confirmed (examples: multiple low dose scans over tumor,	N/A	Scan in breath hold position (inhale recommended since it maximizes lung volume).

	repeat fluoroscopy or scout images)		
Tracking	4D CT or breath hold CT	N/A	4D CT or breath hold CT

Simulation Imaging

Motion assessment during simulation should be performed on all patients to account for the tumor and nodal excursion with respiration and the appropriate type of treatment planning CT required. Respiratory motion may be significant for esophageal (particularly distal) and GE junction lesions. When 4D-CT planning or other motion management techniques are used, margins may be modified to account for observed motion and may also be reduced if justified. The 4D-CT data may also be used to create an internal target volume (IGTV) from which subsequent clinical target volume (CTV) and planning target volume (PTV) expansions can be made.

A motion management technique-specific treatment planning CT (e.g., 4D-CT, breath-hold, gated CT, etc.) should be used during simulation to define gross tumor volume (GTV*)*,internal gross tumor volume (IGTV), clinical target volume (CTV), and planning target volume (PTV) (see definitions in Section 5.3.4). Contiguous CT slices, having no more than 3 mm thickness are to be obtained starting from the level of the cricoid cartilage and extending inferiorly through the entire liver and kidney(s) volume. The field of view must be large enough so that none of the patient's anatomy along the path of the treatment beams is cut off.

A treatment planning FDG PET/CT scan (or FDG-PET alone) (if available for simulation) with the patient in the treatment position is encouraged for treatment planning. In the case where the PET/CT is obtained in the treatment position as part of radiation simulation, the CT from this study may be used as the planning CT scan, but a motion management technique-specific treatment planning CT should still be done.

Intravenous contrast during the planning CT is optional provided a diagnostic chest CT was done with contrast to delineate the major blood vessels. If not, intravenous contrast should be given during the planning CT. If contrast is used, the densities or RLSPs should be over-ridden or the contrast scan must be registered to a non-contrast scan for planning purposes.

5.3.3 Imaging for Structure Definition, Image Registration/Fusion

Limited extent FDG-PET/CT +/- contrast (unless contraindicated) imaging will be preferred as part of staging and to assist in volume delineation in all eligible patients. CT + contrast (unless contraindicated) will be allowed as a substitute if for some reason FDG-PET is not able to be acquired.

5.3.4 Definition of Target Volumes and Margins

Note: All structures must be named for digital RT data submission as listed in the table below. The structures marked as "Required" in the table must be contoured and

submitted with the treatment plan. Structures marked as "Required when applicable" must be contoured and submitted when applicable.
Resubmission of data may be required if labeling of structures does not conform to the standard DICOM name listed. Capital letters, spacing and use of underscores must be applied exactly as indicated.

Standard Name	**Description**	**Validation Required/Required when applicable/Optional**
GTVp_5040	Primary disease GTV to receive 5040 cGy	Required
GTVn_5040	Nodal disease GTV (if applicable) as confirmed by PET/CT or EUS FNA to receive 5040 cGy. Peritumoral nodal disease are included with the GTVp.	Required
IGTVp_5040	Primary disease GTV that accounts for motion over the respiratory cycle to receive 5040 cGy.	Required if 4D-CT used to assess and account for GTV motion. Not required if 4D-CT is not obtained or motion is minimal on 4D-CT (<10mm). If treatment is to be delivered with breath hold or gated techniques, this is not required.
IGTVn_5040	Nodal disease GTV (if applicable) as confirmed by PET/CT or EUS FNA that accounts for over the respiratory cycle to receive 5040 cGy. Peritumoral nodal disease are included with the IGTVp_5040.	Required if 4D-CT used to assess and account for GTV motion. Not required if 4D-CT is not obtained or motion is minimal on 4D-CT (<10mm). If treatment is to be delivered with breath hold or gated techniques, this is not required.
GTV_5040 and IGTV_5040	Sum of GTVp/IGTVp_5040 and GTVn/IGTVn_5040, which is the volume enveloping all GTV(s) or IGTV(s).	Required for GTV_5040. Required for IGTV_5040, unless IGTVs not made.
CTV_5040	CTV to receive 5040 cGy	Required

PTV_5040	PTV to receive 5040 cGy	Required

Detailed Specifications
Target volumes: The definitions of volumes will be in accordance with the 1999 ICRUReport#62.

GTVp_5040 and IGTVp_5040: The GTVp includes the GTV defined as the primary tumor in the esophagus as delineated on planning scan and other pre-treatment diagnostic studies (e.g. PET/CT, EUS). GTVp should be contoured on the primary CT dataset (see Section 5.3.8 for definition). When 4DCT simulation is performed for non-gated free-breathing delivery, the phases of the breath cycle could be used to construct the composite primary GTV volume (IGTVp). Ideally, targets should be contoured on all phase images in order to construct IGTVp. Some commercial software may be used to populate the physician's GTVp contour on one phase images to the other phase images. If such software is unavailable, at least 4 phase images (end of inspiration, mid-inspiration, end of expiration and mid-expiration) should be used to assure IGTVp accuracy. Maximum Intensity Projected (MIP) images should not be used to generate IGTV (see Chen X, Lu H, Tai A, et al. Determination of internal target volume for radiation treatment planning of esophageal cancer by using 4-dimensional computed tomography (4DCT). Int J Radiat Oncol Biol Phys. 2014;90(1):102-9).

GTVn_5040 and IGTVn_5040: The GTVn defined as any grossly involved regional lymph nodes, either considered suspicious on PET/CT or proven by EUS biopsy. GTVn should be contoured on the primary CT dataset (see 5.3.8 for definition). When 4DCT simulation is performed for non-gated free-breathing delivery, the phases of the breath cycle could be used to construct the composite nodal GTV volume (IGTVn). Ideally, targets should be contoured on all phase images in order to construct IGTVn. Some commercial software may be used to populate the physician's GTVn contour on one phase images to the other phase images. If such software is unavailable, at least 4 phase images (end of inspiration, mid-inspiration, end of expiration and mid-expiration) should be used to assure IGTVn accuracy. Maximum Intensity Projected (MIP) images should not be used to generate IGTVn (see Chen X, Lu H, Tai A, et al. Determination of internal target volume for radiation treatment planning of esophageal cancer by using 4-dimensional computed tomography (4DCT). Int J Radiat Oncol Biol Phys. 2014;90(1):102-9).

CTV_5040: The CTV is defined as the GTVp with a 3.5-4.0 cm expansion superiorly and inferiorly along the length of the esophagus and cardia and a 1.0-1.5 cm radial expansion. It should also include GTVn with a 1.0-1.5 cm expansion in all dimensions. This CTV expansion needs to be trimmed away from the edges of anatomic boundaries of microscopic disease spread, including major blood vessels, vertebral body, pericardial lining/heart, pleura, liver, pancreas, kidneys and spleen. This volume should be expanded if needed to cover the paraesophageal, celiac and supraclavicular lymph node regions. If not involved, the celiac nodes (for distal and gastroesophageal junction

tumors) or the bilateral supraclavicular nodes (for upper thoracic tumors) are recommended to be included for N0 status, but required to be covered for N+ status. Such rules also apply to any large mid esophageal tumors with extension to either upper thoracic or distal esophagus. The 3.5-4 cm superior and inferior expansion should follow the contour of the esophagus and proximal stomach. The intent is to extend the margin along the length of the esophagus and proximal stomach to provide a margin for coverage of submucosal extension of tumor and lymphatics. For cervical esophageal cancer, nodal coverage of the cervical lymph node regions in the head and neck (e.g. neck levels 3, 4, etc.) is at the discretion of the treating physician, but recommended for N+ status.

PTV_5040: Additional margin shall be added to the CTV for set up error and movement. This expansion should be 0.5 to 1.0 cm and does not need to be uniform in all dimensions. 4DCT data is allowed to customize PTV expansion.

5.3.5 Definition of Critical Structures and Margins

Note: All structures must be named for digital RT data submission as listed in the table below. The structures marked as "Required" in the table must be contoured and submitted with the treatment plan. Structures marked as "Required when applicable" must be contoured and submitted when applicable.
Resubmission of data may be required if labeling of structures does not conform to the standard DICOM name listed. Capital letters, spacing and use of underscores must be applied exactly as indicated.

Standard Name	**Description**	**Validation Required/Required when applicable/Optional**
SpineCanal	Spinal Canal	Required
Lungs	Right + Left Lung	Required
Lung_R	Right Lung	Required
Lung_L	Left Lung	Required
Esophagus-GTV	Esophagus minus GTV	Required
Esophagus-IGTV	Esophagus minus IGTV	Required if IGTV made
Heart	Heart	Required
Liver	Liver	Required for all mid/distal tumors
Kidney_R	Right Kidney	Required for all mid/distal tumors

Kidney_L	Left Kidney	Required for all mid/distal tumors
Spleen	Spleen	Required for all mid/distal tumors
Stomach-GTV/IGTV	Stomach minus GTV/IGTV	Stomach-GTV required for all mid/distal tumors. Stomach-IGTV required if IGTV made.
Bowel_Small	Small bowel	Required for all mid/distal tumors
Bowel_Large	Large bowel	Required for all mid/distal tumors

Detailed Specifications
SpineCanal: Boundaries: Cranial:1st slice of CT; Caudal: last slice of CT;
Lungs: Boundaries: Cranial: From apex bilaterally; Caudal: to bottom of L2
Esophagus-GTV/IGTV: Boundaries: Cranial: Bottom of cricoid; Caudal: GE junction; subtract IGTV from structure
Heart: Boundaries: Base: Bottom of the aortic arch; Inferior: Apical most of the ventricle
Liver: Boundaries: Cranial: From dome; Caudal: Inferior tip
Kidneys: Entire kidneys contoured separately as Kidney_R and Kidney_L
Spleen: Entire spleen
Stomach-GTV/IGTV: Boundaries: whole stomach minus GTV/IGTV
Bowel_Small: Boundaries: Level of PTV
Bowel_Large: Boundaries: Level of PTV

5.3.6 Dose Prescription

Note: The information provided in this section can be used for adjusting the dose constraints for treatment planning purposes. This table together with the planning priority table should be used during dose optimization. It is important to remember that ideal plans might not be achievable in all cases. Thus, the Compliance Criteria table could be different than the information given here. Cases will be scored using the Compliance Criteria table.

Target Standard Name	Dose (Gy)	Fraction Size (Gy)	# of fractions	Frequency	Dose specification technique
PTV_5040	50.4	1.8	28	Daily	Covering 95% of PTV*
CTV_5040	50.4	1.8	28	Daily	Covering 95-99% of CTV
IGTVp_5040 IGTVn_5040 IGTV_5040	50.4	1.8	28	Daily	Covering >=99% of IGTV

GTVp_5040 GTVn_5040 GTV_5040	50.4	1.8	28	Daily	Covering >=99% of GTV

**Prescribe to the isodose line that covers 95% of the PTV, or inverse plan to cover 95% of the PTV with the prescription dose*

5.3.7 Compliance Criteria

The compliance criteria listed here will be used to score each case. Given the limitations inherent in the treatment planning process, the numbers given in this section can be different than the prescription table. The Per Protocol and Variation Acceptable categories are both considered to be acceptable. The Per Protocol cases can be viewed as ideal plans, and the Variation Acceptable category can include more challenging plans that do not fall at or near the ideal results. A final category, called Deviation Unacceptable, results when cases do not meet the requirements for either Per Protocol or Variation Acceptable. Plans falling in this category are considered to be suboptimal and additional treatment planning optimization is recommended.

Normalization of Dose: The plan is normalized such that 95% of the PTV_5040 volume receives prescription dose of 50.4 Gy.

Note: Deviation Unacceptable occurs when dose limits for Variation Acceptable are not met

Target Volume Constraints and Compliance Criteria

Name of Structure	Dosimetric parameter	Per Protocol	Variation Acceptable*
PTV_5040	D0.03cc[%]	<= 110% Rx Dose	<= 113% Rx Dose
	V50.4Gy[%]	>=95%	>90%

*Per Protocol range is excluded from Variation Acceptable range.

Normal Structure Constraints and Compliance Criteria

Name of Structure	Dosimetric parameter	Per Protocol	Variation Acceptable*
Lungs	D0.03cc[%]	<= 110% Rx Dose	<= 113% Rx Dose
	Mean[Gy]	<= 20 Gy	<= 21 Gy
	V30Gy[%]	<= 20%	<= 25%
	V20Gy[%]	<= 30%	<= 35%
	V10Gy[%]	<= 50%	<= 55%
	V5Gy[%]	<= 65%	<= 75%
Heart	D0.03cc[Gy]	<= 52Gy	<= 54 Gy
	Mean[Gy]	<= 38 Gy	<= 40 Gy
	V40Gy[%]	<= 50%	<= 55%
Kidney_L	D0.03cc[Gy]	<= 45 Gy	<= 50 Gy

	Mean[Gy]	<= 18 Gy	<= 20 Gy
	V20Gy[%]	<= 30%	<= 40%
Kidney_R	D0.03cc[Gy]	<= 45 Gy	<= 50 Gy
	Mean[Gy]	<= 18 Gy	<= 20 Gy
	V20Gy[%]	<= 30%	<= 40%
SpineCanal	D0.03cc[Gy]	<= 45 Gy	<= 50 Gy
Liver	Mean[Gy]	<= 21 Gy	<= 25 Gy
	V30Gy[%]	<= 30%	<= 40%
Bowel_Small	D0.03cc[Gy]	<= 52Gy	<= 54 Gy
Bowel_Large	D0.03cc[Gy]	<= 52Gy	<= 54 Gy
Stomach-GTV/IGTV (parameters apply to Stomach-IGTV if made, otherwise they apply to Stomach-GTV)	Mean[Gy]	<= 40 Gy	<= 45 Gy
Spleen	Mean[Gy]	<= 45 Gy	<= 50 Gy

*Per Protocol range is excluded from Variation Acceptable range.

Recommended dose acceptance criteria for other normal tissue, but not to be used for plan score.

Heart	Mean [Gy] <=30 Gy
Lungs	Mean [Gy] <=18 Gy

Delivery Compliance criteria

	Per Protocol	Variation Acceptable	Deviation Unacceptable
Radiation Start date	Day 1	2-3 days	>3 days
Overall Treatment time	≤40 days	41-52 days	>52 days
Interruptions	None	≤1 week	>1 week

5.3.8 Treatment Planning Priorities and Instructions

Critical Structure and Target priorities are listed in order of decreasing importance. Use these priorities when not all planning constraints can be met:

1. Spinal Cord
2. PTV
3. Lungs
4. Heart

- Required algorithms

The dose calculation algorithm in treatment planning system (TPS) should be one of those

approved by IROC, which includes Convolution Superposition, Collapsed Cone Convolution, AAA and Monte Carlo (see http://rpc.mdanderson.org/RPC/home.htm for details). These algorithms have been tested using the IROC lung phantom to provide acceptable dose calculation accuracy within a heterogeneous medium. The dose calculation algorithms like Clarkson or pencil beam should not be used for photon treatment planning.

For Convolution/Superposition type algorithms, dose should be reported as computed inherently by the given algorithm. For Monte Carlo or Grid Based Boltzmann Solver algorithms, conversion of Dm (dose-to-medium) to Dw (dose-to-water) should be avoided. Dm, computed inherently by these algorithms, should be reported.

- Primary dataset for dose calculation
The primary dataset for dose calculation must be a free-breathing CT that is generated from 4DCT, such as an average intensity pixel CT (AveIP), mid-ventilation CT or, the breath-hold/gated CT, or the free-breathing CT acquired with no other motion management. Maximum Intensity Pixel (MIP) generated images from 4DCTs may not be used as the primary dose calculation dataset. In the case in which contrast is present during the treatment planning CT, the density of the contrast should be overridden to a representative background electron density or RSLP.

-Dose matrix resolution
Dose grid size should be ≤ 3 mm in all directions.

Planning Procedures

- Using a motion management technique-specific treatment planning CT from the simulation, primary tumor motion should be evaluated.
- For tumor motion > 1.0 cm, motion management techniques such as breathhold or respiratory gating techniques should be considered for planning and treatment but not required, as long as the primary tumor motion is accounted for in the planning for both modalities and that daily image guidance and weekly anatomic imaging verification are done to ensure proper set up and targeting.

Planning Procedures

- For IMRT (multiple fixed fields or arc therapy) the PTV will be treated with any combination of coplanar, non-coplanar, or dynamically arcing fields. Please refer to Section 8.4 for credentialing requirements. Margins to be used are stated in Section 5.3.4.

5.3.9 Patient-Specific QA
Any patient-specific QA that needs to be acquired should follow institutional guidelines and AAPM task group report recommendations. Patient specific QA is highly recommended. The recommended patient specific QA criteria is for ≥90% of the comparison points to pass a ±3%/3mm Gamma Index analysis.

5.3.10 Daily Treatment Localization/IGRT

Image-guided radiation therapy (IGRT) is radiation therapy using imaging to facilitate accuracy and precision throughout its entire process. In this section we use the terminology IGRT to focus on image-guidance at the time of radiation delivery to ensure its adherence to the planned treatment, with computer assisted process, i.e. image handling together with calculation of shift and rotations (if available) must be determined with computer assistance.

- Daily IGRT is required for all treatments on this protocol and may be achieved using any one or more of the following techniques:
  - Orthogonal kilovoltage (KV) images, e.g. ExacTrac; on-board imagers (OBI) or similar systems;
  - Linear-accelerator mounted kV and MV conebeam CT images;
  - Linear-accelerator mounted MV CT images (e.g., Tomotherapy);
  - MRI-on-rails, ViewRay and MR-Linac.

- The institution's procedure to register treatment day image dataset with the reference dataset should comply with the following recommendations:
  - Region-of-Interest (ROI) or "clip box" for fusion should be set to encompass the high dose PTV and adjacent vertebral bodies. (**Note**: The same strategy should be used for repeat CT scans required for verification, QA or replanning.)
  - If the fusion software allows the user to create an irregular ROI (e.g., ExacTrac), treatment room objects seen on in-room X-rays should be excluded from the registration.
  - Automatic (e.g., based on mutual information bone or soft tissue fusion) types of registration should be used; the result of the fusion must be visually checked for alignment of the target or bony structures, such as vertebral bodies when appropriate. Manual adjustments (using drag-and-drop capabilities) should be made when necessary.

- Following the registration, the translational and (if the appropriate technology is available) rotational corrections should be applied to the treatment couch. If all the variances are less than 3 mm, the treatment can proceed without correction. If one or more corrections are 3-5 mm, adjustment is necessary prior to treatment; however, re-imaging is not recommended. If one or more of the corrections are larger than 5 mm, the imaging can be repeated in addition to performing table/positioning adjustments.

- If orthogonal projection imaging is used for setup, this fact should be communicated to the therapists (i.e. bony anatomy or fiducial). The relationship between the image surrogate of

internal anatomy and the soft tissue targets shall be verified, at a minimum, as part of the repeat CTs done for QA, verification or replanning.

- If in-room CT is available but orthogonal projection X-ray imaging is used for daily setup, weekly verification of soft tissue with setup surrogate is recommended.
- If in-room CT is used for daily setup, the setup surrogate needs to be communicated to the therapists (i.e. bony anatomy, IGTV, or other). If, due to changing anatomy, a compromise must be made between multiple target structures, the therapists shall be guided by the treating physician as to the best compromise.

5.4 General Concomitant Medication and Supportive Care Guidelines (19-MAY-2023)

5.4.1 Permitted Supportive/Ancillary Care, Concomitant Medications, and Post-Chemoradiation Resection

All supportive therapy for optimal medical care will be given during the study period at the discretion of the attending physician(s) within the parameters of the protocol and documented on each site's source documents as concomitant medication.

Concurrent use of hormones for non-cancer-related conditions (e.g., insulin for diabetes and hormone replacement therapy) is acceptable.

Surgery is permitted post-chemoradiation if medically indicated.

5.4.2 Prohibited Therapies

- Any other investigational anti-cancer therapy
- Any other concurrent chemotherapy, radiotherapy, immunotherapy, biologic or hormonal therapy for cancer treatment other than the treatments stated in this protocol.
- Immunosuppressive medications including, but not limited to systemic corticosteroids at doses exceeding 20 mg/day of prednisone or equivalent, methotrexate, azathioprine, and TNF-α blockers. Use of immunosuppressive medications in subjects with contrast allergies is acceptable. In addition, use of inhaled and intranasal corticosteroids is permitted. The shortest possible duration of steroids will be allowed for different indications, at the discretion of the principal investigator (e.g., chronic obstructive pulmonary disease, radiation, nausea, etc.)
- Live attenuated vaccines within 30 days of OBP-301 dosing, i.e., 30 days prior to the first dose, during treatment with OBP-301 and for 30 days post discontinuation of OBP-301. Inactivated vaccines, such as the injectable influenza vaccine, are permitted

5.4.3 Nutritional and Herbal Supplements

The concomitant use of herbal therapies is not recommended, as their pharmacokinetics, safety profiles, and potential drug-drug interactions are generally unknown. However the use of general nutritional foundation supplements will be allowed including: calcium with

vitamin D and/or minerals, Omega3s (fish oil), Vitamin B6, Vitamin B12, a basic multivitamin, L-glutamine, or probiotics oral supplements will be permitted as long as at or below recommended dosing by a healthcare provider. Herbal-based multivitamins are not allowed.

5.4.4 Participation in Other Trials

Patients are not to participate in other therapeutic trials during this study. However, trials that do not add experimental agents are allowed (e.g. imaging trials, quality of life, etc).

5.5 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue as specified in the above treatment modality sections or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment;
- Unacceptable adverse event(s), as described in Section 6;
- Patient decides to withdraw consent for participation in the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator;

6. TREATMENT MODIFICATIONS/MANAGEMENT

6.1 General Guidelines (26-JUL-2021)

The following side effects are anticipated: nausea, vomiting, myelosuppression (including lymphopenia), esophagitis, dysphagia, mucositis and fatigue.

- If doses of chemotherapy are missed, they should not be made up.
- Any delay of > 3 weeks requires discontinuing paclitaxel and carboplatin chemotherapy. If a dose is missed it will not be made up once RT is completed;
- If chemotherapy needs to be permanently discontinued, patients may continue with radiation and OBP-301 alone if it is considered to be in the patient's best interests.

Use of filgrastim is recommended, at the discretion of the treating investigator if chemoradiation is held for neutropenia. All dose reductions are permanent, i.e. drug doses will not be re-escalated after resolution of toxicity.

Dose levels for carboplatin and paclitaxel are as follows:

Dose level	**Weekly dose**	
	Carboplatin	Paclitaxel
0	AUC 2	50 mg/m^2
-1	AUC 1.5	40 mg/m^2

There will not be any additional dose reductions.

6.1.1 Hematologic toxicity

Week 2-5 blood counts during chemoradiation	Dosage

ANC		Platelet count	
≥1,000/μL	AND	≥75,000/μL	Chemotherapy at full dose
<1,000/μL	OR	<75,000/μL	First occurrence Hold all treatments (chemo,OBP-301 and RT). Re-check CBC. Consider filgrastim. When ANC ≥1,000/μL and platelets ≥75,000/μL, resume chemotherapy at dose level -1 Second occurrence Hold all treatments and resume when ANC≥1,000 and platelets ≥75,000. Continue chemo at the -1 dose level, i.e. no further dose reductions are permitted.

6.1.2 Non-hematologic toxicity

Toxicity	CTCAE version 5 grade	Modality	Modification
Nausea/vomiting, dehydration (despite maximum medical support)	≥3	Carboplatin Paclitaxel OBP-301 Radiation Therapy	First occurrence Hold all treatment until toxicities improve to grade ≤2. Resume chemo at dose level -1. OBP-301 injections will resume at the next previously scheduled time-point. Second and further occurrence Hold all treatment until toxicities improve to grade ≤2. Continue chemo at the -1 dose level, i.e. no further dose reductions are permitted. OBP-301 injections will resume at the next previously scheduled time-point.
Esophagitis	≥3	Carboplatin Paclitaxel OBP-301 Radiation Therapy	First occurrence Hold all treatment until toxicities improve to grade ≤2. Resume chemo at dose level -1. OBP-301 injections will resume at the next previously scheduled time-point. Second and further occurrence Hold all treatment until toxicities improve to grade ≤2.

			Continue chemo at the -1 dose level, i.e. no further dose reductions are permitted. OBP-301 injections will resume at the next previously scheduled time-point. If grade ≥3 in last week of treatment-Hold chemo but continue the RT at the discretion of the treating physician.
Mucositis (despite maximum supportive care	≥ 3	Carboplatin Paclitaxel OBP-301 Radiation Therapy	First occurrence Hold all treatment until toxicities improve to grade ≤2. Resume Carboplatin and Paclitaxel at dose level -1. OBP-301 injections will resume at the next previously scheduled time-point. Second and further occurrence Hold all treatment until toxicities improve to grade ≤2. Continue Carboplatin and Paclitaxel at the -1 dose level, i.e. no further dose reductions are permitted. OBP-301 injections will resume at the next previously scheduled time-point.
Fatigue	≥3	Carboplatin Paclitaxel OBP-301 Radiation Therapy	First occurrence Hold all treatment until toxicities improve to grade ≤2. Resume Carboplatin and Paclitaxel chemo at dose level -1. OBP-301 injections will resume at the next previously scheduled time-point. Second and further occurrence Hold all treatment until toxicities improve to grade ≤2. Continue Carboplatin and Paclitaxel at the -1 dose level, i.e. no further dose reductions are permitted. OBP-301 injections will

			resume at the next previously scheduled time-point.
Any other toxicity	≥3	Carboplatin Paclitaxel OBP-301 Radiation Therapy	<u>First occurrence</u> Hold all treatment until toxicities improve to grade ≤2. Resume Carboplatin and Paclitaxel at dose level -1. OBP-301 injections will resume at the next previously scheduled time-point. <u>Second and further occurrence</u> Hold all treatment until toxicities improve to grade ≤2. Continue Carboplatin and Paclitaxel at the -1 dose level, i.e. no further dose reductions are permitted. OBP-301 injections will resume at the next previously scheduled time-point.
Diarrhea (despite maximum medical support)	≥3	Paclitaxel Only	<u>First occurrence</u> Hold Paclitaxel until diarrhea improves to grade ≤2. Dose reduce Paclitaxel by 1 dose level. <u>Second occurrence</u> Hold all Paclitaxel until toxicities improve to grade ≤2. Continue Paclitaxel at the -1 dose level, i.e. no further dose reductions are permitted
Hepatotoxicity (ALT, AST, blood bilirubin)	≥1	Paclitaxel Only	Decrease paclitaxel by 1 dose level. Resume paclitaxel if Total Bili ≤1.5× ULN OR AST/ALT ≤2.5× UL N. Carboplatin dose will remain at current dose level.
Sensory neuropathy	2	Paclitaxel Only	Hold Paclitaxel. When toxicity is grade ≤1, resume Paclitaxel at -1 dose level for all subsequent cycles. Carboplatin dose will remain at current dose level.
	≥3		Patients will not receive any more paclitaxel.

Infusion related reaction	≥3	Carboplatin Paclitaxel	Permanently discontinue Paclitaxel and Carboplatin.

Hypersensitivity Reaction Management per Institutional Standard

Hypersensitivity reactions include anaphylactic-like reactions. Tachycardia, bronchoconstriction, hypotension, facial edema and erythema may occur and should be treated with antihistamine, corticosteroids, and epinephrine as per institutional hypersensitivity management protocol. If the treating Physician feels that it is appropriate to continue with protocol therapy, options include pre-medication with steroids and diphenhydramine the night before and at the time of carboplatin or paclitaxel infusion. For patients who develop a grade 1/2 carboplatin reaction, a desensitization protocol can be considered, after consultation with the Principal Investigator

6.2 OBP-301

OBP-301 injection will be delayed for any ongoing non-hematologic Grade 3 toxicity or hematologic Grade 4 toxicity (except for Grade 4 Lymphopenia) at the time of the next scheduled injection. Patients who require a delay of OBP-301 injection of > 4 weeks (i.e., approximately 6 weeks from the previous injection) due to lack of resolution to Grade ≤ 2 toxicity will not receive subsequent OBP-301 treatments.

Any patient who experiences any of the following grade ≥3 toxicities directly attributable to OBP-301 and/or endoscopic injections will not receive further OBP-301 treatments:

- Perforation
- Bleeding
- Infection
- Pain lasting >3 days despite maximal medical management

If a dose of OBP-301 is missed within the 2 day window, unless there is a specific reason that OBP-301 administration via EGD is contraindicated, administration during the same calendar week is permitted but **must not be on the same day as chemotherapy administration**.

Injection site pain is the one of the more common adverse events in connection with intratumoral injections. Therefore, the following pain control measures are suggested to improve the comfort of the patient.

1. Review the analgesics already prescribed for the patient and determine the level of pain control.
2. Additional pain medication will with all likelihood be needed during the day of the intratumoral injections and for several days afterwards. Local anesthesia may be required just before or during the intratumoral injection at the discretion of the treating gastroenterologist.

6.3 Radiation Therapy (26-JUL-2021)

Radiation therapy will typically continue as prescribed without modifications while these drug-related adverse events are being managed.

Fatigue, esophagitis, cough, nausea and vomiting can be common acute side effects of radiation to the esophagus.

For nausea and vomiting, anti-emetics should be initiated at the onset of symptoms and continued as directed by the treating physician until resolution of symptoms to grade 0-1. Additional supportive care measures, e.g. oral or intravenous rehydration, etc., should be instituted as required by the patient's clinical condition. Additional medical evaluation is recommended for those patients with continued ≥ grade 2 nausea/vomiting, lasting ≥ 48 hours despite institution of optimal supportive care measures. Primary prophylaxis should be initiated once nausea or vomiting has occurred with the prior treatment. Admit to hospital and administer IV fluids and all supportive measures for symptoms unresponsive to medication and signs of dehydration.

For cough, dextromethorphan with or without guaifenesin, benzonatate, or mild narcotic medications (e.g. codeine syrup) can be used.

Esophagitis can be managed by the preference of the treating physician and may commonly include medications such as sucralfate, Magic Mouthwash (e.g. custom mix of Benadryl, Maalox, lidocaine/xylocaine), proton pump inhibitors, or narcotics for pain.

In the unlikely event that radiation must be permanently discontinued, patients will discontinue all treatment.

7. ADVERSE EVENTS REPORTING REQUIREMENTS

7.1 Protocol Agents

Investigational Agent

The investigational agent OBP-301 administered in NRG-GI007 is being made available under an IND sponsored by NRG Oncology. For OBP-301, determination of whether an adverse event meets expedited reporting criteria, see the reporting table in section 7.5 of the protocol.

Commercial Agents

The commercial agents in NRG-GI007 are carboplatin and paclitaxel.

7.2 Adverse Events and Serious Adverse Events

7.2.1 This study will utilize the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 for CTEP-AERS (CTEP Adverse Event Reporting System) CAERs reporting of adverse events (Aes), located on the CTEP web site, http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0.

7.2.2 Definition of an Adverse Event (AE)

Any untoward medical occurrence associated with the use of a drug in humans, whether

or not considered drug related. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of unrelated, unlikely, possible, probable, or definite). (International Conference on Harmonisation [ICH], E2A, E6).

For multi-modality trials, adverse event reporting encompasses all aspects of protocol treatment including radiation therapy, surgery, device, and drug.

Due to the risk of intrauterine exposure of a fetus to potentially teratogenic agents, the pregnancy of a study participant must be reported via CTEP-AERS in an expedited manner.

7.3 Adverse Events for Investigational Study Agent: OBP-301 (07-DEC-2020)

Identified Risks of OBP-301

- Administration site reaction
- Lymphocyte count decrease

Potential Risks of OBP-301

- Accidental exposure of a third party to OBP-301
- Spread of OBP-301 to close contacts or healthcare providers after direct contact with patients
- Adenovirus infection affecting the entire body in patients with impaired immune function
- Inflammatory lesions in virus-infected organs (liver, kidney, lung)
- Influenza-like symptoms

For more details see Investigator's Brochure.

7.4 Adverse Events for Commercial Study Agents: Paclitaxel and Carboplatin

Refer to the package insert for detailed pharmacologic and safety information

7.5 Expedited Reporting of Adverse Events (11-JAN-2022)

All serious adverse events that meet expedited reporting criteria defined in the reporting table below will be reported via the CTEP Adverse Event Reporting System, CTEP-AERS, accessed via the CTEP web site, https://eapps-ctep.nci.nih.gov/ctepaers/pages/task?rand=1390853489613

Submitting a report via CTEP-AERS serves as notification to the NRG Biostatistical/Data Management Center and satisfies NRG requirements for expedited adverse event reporting.

In the rare event when Internet connectivity is disrupted, a 24-hour notification must be

made to the NRG Oncology by phone at: 1-215-574-3191. An electronic report must be submitted immediately upon re-establishment of the Internet connection.

7.5.1 Expedited Reporting Methods

- Per CTEP NCI Guidelines for Adverse Events Reporting, a CTEP-AERS 24-hour notification must be submitted within 24 hours of learning of the adverse event. Each CTEP-AERS-24-Hour Notification must be followed by a complete report within 1 day.
- Supporting source documentation is requested by NRG as needed to complete adverse event review. Deidentified supporting source documentation is uploaded to the source document portal via CTEP-AERS.
- A serious adverse event that meets expedited reporting criteria outlined in the AE Reporting Tables but is assessed by the CTEP-AERS as "an action *not* recommended" must still be reported to fulfill NRG safety reporting obligations. Sites must bypass the "NOT recommended" assessment; the CTEP-AERS allows submission of all reports regardless of the results of the assessment.
- NRG Oncology reports serious adverse events to Oncolys within 24 hours of report completion. Additionally, NRG Oncology reports applicable adverse events to FDA per 21 CFR 312.32 and submits the FDA report to Oncolys within 3 days for life threatening or fatal adverse events and within 7 days for all other FDA reportable adverse events following Oncolys' initial receipt of the information.

7.5.2 Expedited Reporting Requirements for Adverse Events

Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes

Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" – The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 3, 4, and Grade 5 Aes

Expedited 10 calendar day reports for:

- Grade 2 Aes resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

Additional Protocol-Specific Instructions or Exceptions to Expedited Reporting Requirements

Not applicable

7.5.3 Reporting to the Site IRB/REB

Investigators will report serious adverse events to the local Institutional Review Board (IRB) or Research Ethics Board (REB) responsible for oversight of the patient according to institutional policy.

7.5.4 Secondary Malignancy

A secondary malignancy is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur during or subsequent to treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. In addition, secondary malignancies following radiation therapy must be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

Second Malignancy:
A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

7.6 Routine Reporting Requirements for Adverse Events

All Adverse Events **must** be reported in routine study data submissions. **Aes reported expeditiously through CTEP-AERS must also be reported in routine study data submissions.**

7.7 Pregnancy

Any pregnancy occurring in a patient or patient's partner from start of protocol treatment to 90 days after the last study treatment must be reported expeditiously as a grade 3 SAE coded in the CTCAE as "pregnancy, puerperium and perinatal conditions, other—pregnancy" in CTEP-AERS and also submit the Pregnancy Information Form found at this link:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportFormUpdated.pdf

The form should be submitted within 14 days of notification **AND** the form should also be uploaded to the patient case file in RAVE in the source documentation upload folder. NRG Oncology will report the pregnancy to Oncolys within 1 business day of notification of the event. Newborn infants should be followed until 8 weeks old and the pregnancy outcome for patients on study should be reported to NRG Oncology. NRG Oncology will report the status to Oncolys.

8. REGISTRATION AND STUDY ENTRY PROCEDURES (19-MAY-2023)

Food and Drug Administration (FDA) regulations require sponsors to select qualified investigators. National Cancer Institute (NCI) policy requires all individuals contributing to NCI-sponsored trials to register with their qualifications and credentials and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. Investigators and clinical site staff who are significant contributors to research must register in the Registration and Credential Repository (RCR). The RCR is a self-service online person registration application with electronic signature and document submission capability. RCR utilizes five person registration types.

- Investigator (IVR) — MD, DO, or international equivalent;
- Non Physician Investigator (NPIVR) — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- Associate Plus (AP) — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System [RUMS], OPEN, Rave, acting as a primary site contact, or with consenting privileges;

- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials; and
- Associate Basic (AB) — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✓	✓			
Financial Disclosure Form	✓	✓	✓		
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓		
GCP training	✓	✓	✓		
Agent Shipment Form (if applicable)	✓				
CV (optional)	✓	✓	✓		

IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster;
- Selection as the treating, credit, or drug shipment investigator or consenting person in OPEN;
- Ability to be named as the site-protocol Principal Investigator (PI) on the IRB approval.

In addition, all investigators acting as the Site-Protocol PI (investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, must be rostered at the enrolling site with a participating organization.

Refer to the NCI RCR page on the CTEP website for additional information. For questions, please contact the **RCR Help Desk** by email at RCRHelpDesk@nih.gov.

8.1 Cancer Trials Support Unit Registration Procedures (19-MAY-2023)

Permission to view and download this protocol and its supporting documents is restricted and is based on the person and site roster assignment housed in the Roster Maintenance application and in most cases viewable and manageable via the Roster Update Management System (RUMS) on the Cancer Trials Support System Unit (CTSU) members' website.

This study is supported by the NCI CTSU.

IRB Approval

As of March 1, 2019, all U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB) in order to participate in Cancer Therapy Evaluation Program (CTEP) and Division of Cancer Prevention (DCP) studies open to the National Clinical Trials Network (NCTN) and NCI Community Oncology Research Program (NCORP)

Research Bases. In addition, U.S.-based sites must accept the NCI CIRB review to activate new studies at the site after March 1, 2019. Local IRB review will continue to be accepted for studies that are not reviewed by the CIRB, or if the study was previously open at the site under the local IRB. International sites should continue to submit Research Ethics Board (REB) approval to the CTSU Regulatory Office following country-specific regulations.

Sites participating with the NCI CIRB must submit the Study Specific Worksheet for Local Context (SSW) to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by email or calling 1-888-651-CTSU (2878).

Sites using their local IRB or REB must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation;
- IRB-signed CTSU IRB Certification Form; and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol Principal Investigator (PI) (i.e. the investigator on the IRB/REB approval) must meet the following criteria for the site to be able to have an Approved status following processing of the IRB/REB approval record:

- Have an active CTEP status;
- Have an active status at the site(s) on the IRB/REB approval on at least one participating organization's roster;
- If using NCI CIRB, be active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record;
- Include the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile;
- List all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
- Have the appropriate CTEP registration type for the protocol.

Additional Requirements for Protocol NRG-GI007 Site Registration
Additional requirements to obtain an approved site registration status include:

- An active Federal Wide Assurance (FWA) number;

- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO);
- Training for Gastroenterologists, who will perform intra-tumoral injection of OBP-301, are required to view the video produced by Oncolys BioPharma Inc. They are also required to have direct communication via email or telephone call with the Gastroenterology Co-Chair, Dr. Makoto Nishimura, to ensure there is clear understanding of the procedure for intra-tumoral injection. Documentation that they have fulfilled both of these criteria must be submitted to Dr. Nishimura at nishimum@mskcc.org who will review, contact the Gastroenterologist and correct any procedural errors with them. Gastroenterologist certification documents can be found on the NRG Oncology website; Documents & Materials tab of the NRG-GI007 homepage. If the gastroenterologist demonstrates competency he/she will be notified of the certification approval to perform the procedure. The certification form will be signed by Dr. Nishimura and provided to NRG Oncology. NRG Oncology will submit the required documentation to CTSU. Only gastroenterologists who are credentialed in this manner are permitted to administer OBP-301. The video and further credentialing details are posted on the NRG protocol website.
- Per "NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules" this trial falls under a Risk Group 2 category given OBP-301 is a virus. Therefore, IBC approval is needed prior to patient enrollment. Upon receipt of Institutional Biosafety Committee (IBC) approval documentation should be submitted to CTSU (see below for submission details).
- An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and

- Compliance with all applicable protocol-specific requirements (PSRs).

- Requirements for the Initial Shipment of OBP-301 for ALL sites
  Institutions must electronically complete (versus hand write) and submit a Drug Request form to the Oncolys contact listed in the form. The Oncolys contact will work directly with the drug distributor to arrange OBP-301 shipment to sites. The Drug Request form is available on the NRG protocol website.

  The following items must be submitted to NRG Regulatory (Regulatory-PHL@nrgoncology.org)
  - 1572 for each investigator conducting the study
  - CV for each investigator conducting the study

- This is a study with a radiation and/or imaging (RTI) component and the enrolling site must be aligned to an RTI provider. To manage provider associations or to add or remove associated providers, access the Provider Association page from the

Regulatory section on the CTSU members' website at https://www.ctsu.org/RSS/RTFProviderAssociation. Sites must be linked to at least one Imaging and Radiation Oncology Core (IROC) credentialed provider to participate on trials with an RTI component. Enrolling sites are responsible for ensuring that the appropriate agreements and IRB approvals are in place with their RTI provider. An individual with a primary role on any roster is required to update provider associations, though all individuals at a site may view provider associations. To find who holds primary roles at your site, please view the Person Roster Browser under the RUMS section on the CTSU members' website.

IROC Credentialing Status Inquiry (CSI) Form – this form is submitted to IROC to begin the modality credentialing process.

To complete protocol-specific credentialing the RTI provider or enrolling site should follow instructions in the protocol to submit documentation or other materials to the designated IROC Quality Assurance (QA) center. Upon the IROC QA center approving the RTI provider for the study modality, IROC will automatically send the approval to the Regulatory and Roster Maintenance applications to comply with the protocol-specific requirement, unless otherwise noted at the bottom of the IROC Credentialing Approval notification. IROC will continue to copy the provider and/or enrolling site on modality approvals.

Upon site registration approval in the Regulatory application, the enrolling site may access Oncology Patient Enrollment Network (OPEN) to complete enrollments. If the study is using the IROC integration suite, the enrolling site will select their credentialed provider treating the subject in the OPEN credentialing screen and may need to answer additional questions related to treatment in the eligibility checklist.

Downloading Site Registration Documents:

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted to institutions and their associated investigators and staff on a participating roster. To view/download site registration forms:

- Log on to the CTSU members' website (https://www.ctsu.org);
- Click on *Protocols* in the upper left of the screen
    - Enter the protocol number in the search field at the top of the protocol tree, or
    - Click on the By Lead Organization folder to expand, then select *NRG* and protocol number *NRG-GI007*.
- Click on *Documents*, *Protocol Related Documents, and* use the *Document Type* filter and select *Site Registration* to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

Submitting Regulatory Documents:

Submit required forms and documents to the CTSU Regulatory Office via the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal log in to the CTSU members' website, go to the Regulatory section and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support.

Checking Your Site's Registration Status:

Site registration status may be verified on the CTSU members' website.

- Click on *Regulatory* at the top of your screen;
- Click on *Site Registration; and*
- Enter the sites 5-character CTEP Institution Code and click on Go.
    - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

8.2 RT-Specific Pre-Registration Requirements (19-MAY-2023)

For detailed information on the specific technology requirement required for this study, please refer to the table below and utilize the web link provided for detailed instructions. The check marks under the treatment modality columns indicate whether that specific credentialing requirement is required for this study. Specific credentialing components may require you to work with various QA centers; however, IROC Houston will notify your institution when all credentialing requirements have been met and the institution is RT credentialed to enter patients onto this study. The credentialing notification document (email) must be uploaded by the site to the CTSU Regulatory Submission Portal for RSS to be updated.

RT Credentialing Requirements	**Web Link for Procedures and Instructions: http://irochouston.mdanderson.org**	
	Treatment Modality **Photons**	**Key Information**
Credentialing Status Inquiry	**x**	To determine if your institution has completed the requirements above, please complete a "Credentialing Status Inquiry Form" found under Credentialing on the

Form		IROC Houston QA Center website (**http://irochouston.mdanderson.org**).
Facility Questionnaire	**x**	The IROC Houston electronic facility questionnaire (FQ) should be completed or updated with the most recent information about your institution. To access this FQ, email irochouston@mdanderson.org to receive your FQ link.
Phantom Irradiation	**x**	An IMRT thorax phantom study provided by the IROC Houston QA Center must be successfully completed. Instructions for requesting and irradiating the phantom are found on the IROC Houston web site (**http://irochouston.mdanderson.org**).
Credentialing Issued to:		
Institution	**x**	Institution will be credentialed for the treatment modality that they intend to use on all patients. IROC Houston QA Center will notify the institution and NRG Headquarters that all desired credentialing requirements have been met.

8.2.1 Digital RT Data Submission to NRG Using TRIAD

Transfer of Images and Data (TRIAD) is the American College of Radiology's (ACR) image exchange application. TRIAD provides sites participating in clinical trials a secure method to transmit images. TRIAD anonymizes and validates the images as they are transferred.

TRIAD Access Requirements:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems;
- Registration type of: Associate (A), Associate Plus (AP), Non-Physician Investigator (NPIVR), or Investigator (IVR). Refer to the CTEP Registration Procedures section for instructions on how to request a CTEP-IAM account and complete registration in RCR; and
- TRIAD Site User role on an NCTN, ETCTN, or other relevant roster.

All individuals on the Imaging and Radiation Oncology Core provider roster have access to TRIAD, and may submit images for credentialing purposes, or for enrollments to which the provider is linked in OPEN.

TRIAD Installation:

To submit images, the individual holding the TRIAD Site User role will need to install the TRIAD application on their workstation. TRIAD installation documentation is available at https://triadinstall.acr.org/triadclient/.

This process can be done in parallel to obtaining your CTEP-IAM account and RCR registration.

For questions, contact TRIAD Technical Support staff via email TRIAD-Support@acr.org or 1-703-390-9858.

8.3 **Patient Enrollment** (19-MAY-2023)

Patient registration can occur only after evaluation for eligibility is complete, eligibility

criteria have been met, informed consent is obtained, and the study site is listed as 'approved' in the CTSU RSS.
Patients must have signed and dated all applicable consents and authorization forms.

Informed Consent: Patients must be aware of the neoplastic nature of their disease and informed of the procedure(s) to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts prior to signing the informed consent in accordance with institutional and federal guidelines. Current IRB/RE/REC approval of this protocol and a consent form is required prior to patient consent and registration. The model consent form created for this study adheres to the NCI informed consent template requirements.

8.3.1 Oncology Patient Enrollment Network (OPEN)

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the LPOs' registration/randomization systems for retrieval of patient registration/randomization assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems;
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or participating organization roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type;
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes; and
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. You may print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the

CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with the patient enrollment in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

9.0 DRUG INFORMATION

9.1 Investigational Study Agent: OBP-301 (19-MAY-2023)

To supplement the toxicity information contained in this document, investigators must obtain the current version of the Investigator Brochure for comprehensive pharmacologic and safety information

The Investigator Brochure can be obtained from the protocol specific page of the NRG website.

9.1.1 Adverse Events

See Section 7.3 and consult the Investigator Brochure for comprehensive information.

The OBP-301 clinical study material and product is generated according to current Good Manufacturing Practice (cGMP). Below shows an overview of OBP-301. OBP-301 will be provided by Oncolys BioPharma Inc.

Investigational product code:	OBP-301
Content:	2 mL/vial (1×10^{12} Viral Particles/mL)
Dosage form:	Solution; formulated in 20 mM Tris pH 8.0, 25 mM NaCl with 2.5% glycerin, USP by volume, filled in each 5 mL glass vial
Storage method for the investigational product:	Store at ≤ -60°C

9.1.2 Supply, Packaging, Accountability, and Drug Ordering

This study will be conducted under IND# 19404 to be held by NRG and will require FDA submission and approval as part of the IND.

Study Supplies

Oncolys BioPharma Inc. will be responsible for the preparation and labelling of the Investigational Product (IP) in accordance with local regulatory requirements. Oncolys BioPharma Inc. will also provide details of batch numbers and all associated safety and stability data.

Oncolys BioPharma Inc. in coordination with the drug distributor, Catalant, is responsible for packaging and shipment of the IP to the site(s). Due to product specific requirements, shipments of IP will be made on dry ice using a validated container and temperature

monitoring. An official shipping form to be completed by the study Investigator or designee (e.g., pharmacist) will be included in each shipment and returned to the drug distributor per instructions on the form. (See Appendix I: Pharmacy Manual, for receipt procedures).

Storage Conditions

The study Investigator or designee is responsible for receiving each shipment of IP. Upon receipt of the IPs at the investigational site, the receipt procedures should be completed and IPs stored appropriately at ≤ -60°C. A record to account for all dispensing and return of unused IPs will be maintained by the investigational sites. At the end of the study the IPs will be reconciled and a copy of the record submitted to Oncolys BioPharm, Inc. at t.biran@oncolys.com. The Investigator will be fully responsible for the security, accessibility, and storage of the IPs while they are at the investigational facility.

Vials must be stored in the original vial storage boxes to facilitate supply tracking. It is recommended to leave the vial storage boxes in the original leak-proof bag or in a substitute leak-proof container, particularly if the supplies are not segregated (i.e., in a separate section of the freezer) from other products or biological samples.

The freezer should be in a locked or secured area, with limited access. Additionally, the freezer should have a temperature recorder and an alarm system. The temperature plot should be properly maintained and checked. Documentation for calibration of the freezer at installation and at each maintenance inspection should be available (at least annually or as per manufacturer's recommendations). A biohazard symbol should be affixed to the outside of the storage compartment.

Accountability and Compliance

The Investigator, pharmacist, or designee may only dispense clinical supplies in accordance with this protocol. The NCI's Investigational Agent Accountability Record (located on CTEP/PMB website) must be used for accountability.

The Investigator, pharmacist, or designee, is responsible for maintaining an accurate and current record of all clinical supplies received from the drug distributor, dispensed to the Investigator or destroyed. Accountability records must be maintained throughout the course of the study, showing receipt and disposition of the clinical supplies.

Refer to the pharmacy manual (Appendix I) for specific disposal and destruction information.

Drug Ordering

A standard amount of study material will be provided to each site upon request at the beginning of the study. Sites must submit a drug request form to the contact listed in the form with a copy to the NRG-GI007 study mailbox (NRG-GI007@NRGOncology.org). Catalent will be the drug distributor and will ship OBP-301 to each site. Please allow 4 weeks for receipt of initial supply. The Drug Request form is available on the NRG website.

The site may request additional OBP-301 study material when the supply is low (i.e. study material on site is only enough for 1 participant). Please allow four weeks for preparation and delivery to site. To request additional study material supplies, the site will contact the assigned supplier using the Drug Request Form (located on the NRG website).

9.1.3 *Agent Specific Information: OBP-301*

Refer to the Pharmacy Manual (Appendix I) and the Investigator Brochure for detailed information

Other Names: Suratadenoturev (pending trade name Telomelysin ^{TM}), hereafter referred to as OBP-301

Classification: Adenovirus

Description: Adenoviral vector containing the adenoviral E1A and E1B gene linked with an internal ribosome entry site (IRES) under the control of the human Telomerase Reverse Transcriptase (hTERT) promoter.

Mode of Action: The normal transcriptional regulatory element of the Ad5 E1A gene is replaced by the human Telomerase Reverse Transcriptase gene (hTERT) promoter, and the normal transcriptional element of the E1B gene is replaced by an IRES (Internal Ribosomal Entry Site) sequence.

How Supplied: OBP-301 will be provided as a frozen viral suspension formulated in 20 mM Tris pH 8.0, 25 mM NaCl with 2.5% Glycerin, USP by volume. The product is packaged using a 5 mL glass vial, siliconized gray butyl-rubber stoppered vials, an aluminum seal, and yellow flip-top plastic cap. Each vial contains a volume of 2.0 mL at a concentration of 1 x 10^{12} viral particles (vp)/mL. The product in this configuration is stored at ≤ -60° C prior to administration.

Preparation: OBP-301 should be prepared according to Biosafety Level 2 Guidelines, unless local regulations require a more rigorous containment level. See Appendix I and Appendix II.

Storage: See Appendix I and section 9.1.2.

Route of Administration: Injection into the tumor

Method of Administration: OBP-301 is administered as intratumoral injection into a primary esophageal tumor mass that is suitable for injection, e.g. greater than 1 cm^2 in size and in a location amenable for endoscopic injection.

Administration Instructions can be found in the pharmacy manual (Appendix I)

9.1.4 Destruction of Used and Unused Investigational Product

Immediately after use, the used vials should be placed in a biohazardous waste container.

Empty or partially used vials should be destroyed, by the study pharmacy, in accordance with institutional biosafety policy and procedures for biohazardous waste management. Proper accounting of the vials must be performed immediately using the NCI Investigational Agent Accoountability Record.

Written authorization must be obtained from Oncolys BioPharma Inc. before any destruction of unused clinical material supplies. This will occur at the completion of the clinical study or when the expiration date has been reached and could not be prolonged by Oncolys BioPharma Inc. based on updated stability information. In addition, destruction of unused supplies may only be performed after product reconciliation by the monitor.

The unused vials must be destroyed by the study pharmacy, in accordance with institutional Biosafety policy and procedures for biohazardous waste management or returned to Oncolys BioPharma Inc. Unless approved by Oncolys BioPharma Inc., unused drug cannot be saved for future use.

The Investigator or designee must fill in, sign, and date the appropriate section of Clinical Trial Material Destruction Certificate. Discrepancies in vial accountability must be explained in writing by the Investigator or designee. A copy will be retained for the site study file.

9.2 Commercial Agent: Paclitaxel

Sites must refer to the package insert for detailed pharmacologic and safety information.

9.2.1 Product Description: Paclitaxel injection is a clear colorless to slightly yellow viscous solution.

9.2.2 Solution Preparation: Paclitaxel injection should be diluted in 0.9% Sodium Chloride Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP; or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL.

9.2.3 Route of Administration: Intravenous

9.2.4 Agent Ordering/Availability: Commercially available

Please see Section 5.2.2 for administration instructions. Please refer to the current FDA-approved package insert provided with each drug and the site-specific pharmacy for toxicity information and instructions for drug preparation, handling, and storage.

9.2.5 Adverse Events

Please refer to the package insert.

9.3 Commercial Agent: Carboplatin

Sites must refer to the package insert for detailed pharmacologic and safety information.

9.3.1 Product Description: Carboplatin injection is a premixed aqueous solution of 10 mg/mL carboplatin.

9.3.2 Solution Preparation: Carboplatin aqueous solution can be further diluted to concentrations as low as 0.5 mg/mL with 5% Dextrose in Water (D 5W) or 0.9% Sodium Chloride Injection.

9.3.3 Route of Administration; Intravenous

9.3.4 Agent Ordering/Availability; Commercially available

Please see Section 5.2.2 for administration instructions. Please refer to the current FDA-approved package insert provided with each drug and the site-specific pharmacy for toxicity information and instructions for drug preparation, handling, and storage.

9.3.5 Adverse Events

Please refer to the package insert.

10. PATHOLOGY/BIOSPECIMEN

10.1 Biospecimen Submission Tables (07-DEC-2020)

10.1.1 Optional Specimen Submissions

Optional Study Description #1 FFPE Tumor tissue for Immunofluorescence/IHC Assays (See Appendix VI for details)

Why specimen is being collected: To identify potential biomarkers of response.
Specimens and Processing: Tumor specimens will be collected and prepared following standard pathology practices and sent to NRG Biospecimen Bank – San Francisco
Required Forms: Specimen Transmittal Form and copy of pathology report with accession number and date of procedure visible. All other PHI information should be redacted.
Kits and Shipping costs: Sites are responsible for all FFPE shipping costs
Shipping Days: Sites can ship FFPE samples Monday-Friday

Residual Material: Sites can request blocks and any un-used slides be returned once study is completed and the pre-planned analyses are complete. Sites will be responsible for return FFPE shipping costs. Sites must check with their pathology department to ensure they can release the FFPE material for all patients that consent. FFPE Blocks will be returned to sites after the end of study testing is completed.

Ship FFPE samples to:
Attn: NRG Oncology Biospecimen Bank – San Francisco
2340 Sutter Street- Room S341
San Francisco, CA 94115
415-476-7864/Fax 415-476-5271
Email: NRGBB@ucsf.edu

For questions, please contact the San Francisco Bank at:

Email: NRGBB@ucsf.edu
415-476-7864/Fax 415-476-5271

Specimen Type	Collection Time Points	Collection Information and Requirements/Instructions for Site	Shipping
H&E Slide and FFPE Tumor block	Baseline: From diagnostic biopsy. Pre-treatment: Biopsy of the primary tumor prior to first OBP-301 injection. During Treatment: Biopsy of the primary tumor prior to each OBP-301 injection on Days 12 and 26 (up to 1 day before) Post Treatment: 6-8 weeks post RT if applicable per disease assessment	Paraffin-embedded block. If site is unable to send block site must send 15-20 five micron unstained slides at the end of treatment to ensure they are cut immediately prior to shipment. Sites must submit an ST form to the biobank stating they will be submitting unstained slides for all timepoints.	Store all samples and batch ship ambient to NRGBB_SF at end of treatment.

Optional Study Description #2 Whole Blood for PBMC isolation and Plasma Assays. (See Appendix VI for details)

Why specimen is being collected: To identify potential biomarkers of response.
Required Forms: Specimen Transmittal Form
Kits: CPT tubes and shipping supplies can be requested from the NRG Biospecimen Bank- San Francisco. Allow 5-10 business days for receipt of kits from California by Fed Ex Ground. If sites need kits expedited they must provide a shipping label or Fed Ex account number.
Shipping costs: Priority overnight Fed Ex labels for shipment to MSK lab can be requested for all timepoints from the NRG Biobank (NRGBB@ucsf.edu) once a patient is screened.
Shipping Days: Fresh Blood must be shipped ambient overnight courier Monday-Thursday.
Notice of anticipated shipment must be provided **at least 24 hours** and tracking information must be sent day of shipment to the Immune Monitoring Facility at wongp@mskcc.org.
Residual Material: Blood samples will be destroyed once the study is completed and the pre-planned analyses are complete.

Ship Samples to:
Phil Wong
Immune Monitoring Facility, Memorial Sloan Kettering Cancer Center
Zuckerman Research Building, Z-1513
408 E. 69th St.
New York, NY 10065
Phone: 646-888-3514
Email: wongp@mskcc.org

For questions, contact the Immune Monitoring Facility at : wongp@mskcc.org

Specimen Type	Collection Time Points	Collection Information and Requirements/Instructions	Shipping

		for Site	
5-10 mL Whole Blood drawn into 4 CPT tubes.	Pre Treatment: Prior to first OBP-301 injection on Day -3 During Treatment: Prior to OBP-301 injection on Days 12 and 26 (up to 1 day before) Post Treatment: 6-8 weeks post RT	Collect whole blood, label with identifier and time of collection, and place in insulated tube box inside biohazard specimen transport bag. Store at ambient temperature until ready to ship. Samples must be shipped on day of collection.	Ship ambient via overnight courier to Immune Monitoring Facility, MSKCC

11. SPECIAL STUDIES (NON-TISSUE)

Not Applicable

12. MODALITY REVIEWS

12.1 Medical Oncology Modality Quality Assurance Reviews

The Medical Oncology Chair, Geoffrey Ku, MD will perform a Systemic Therapy Assurance Review for OBP-301(Telomelysin™),Carboplatin and Paclitaxel for all patients who receive systemic therapy in this trial. The goal of the review is to evaluate protocol compliance. The review process is contingent on timely submission of systemic therapy treatment data. The scoring mechanism is: **1) Per Protocol, 2) Acceptable Variation, 3) Unacceptable Deviation, and 4) Not Evaluable**.

Dr. Ku will perform a Quality Assurance Review after NRG Data Management Center has received complete data for cases enrolled. The reviews will be ongoing and performed remotely. The final cases will be reviewed within 3 months after this study has reached the target accrual or as soon as NRG Data Management Center has received complete data for all cases enrolled, whichever occurs first.

12.2 Radiation Therapy Quality Assurance Reviews

The Radiation Oncology Co-Chair will perform an RT Quality Assurance Review after IROC Philadelphia-RT has received complete data in TRIAD. The RT reviews will be ongoing and performed remotely. The scoring mechanism is: **Per Protocol, Variation Acceptable, and Deviation Unacceptable.**

13. DATA AND RECORDS

13.1 Data Management/Collection (19-MAY-2023)

Medidata Rave is the clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments. Requirements to access Rave via iMedidata:

- Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems; and

- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.

Rave role requirements:

- Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type;
- Rave Investigator role must be registered as a Non-Physician Investigator (NPIVR) or Investigator (IVR); and
- Rave Read Only or Rave SLA role must have at a minimum an Associate (A) registration type.

Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

Upon initial site registration approval for the study in the Regulatory application, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

Site staff who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in the Regulatory application will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Data Management section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

13.2 Summary of Data Submission

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during the trial using Medidata Rave®. Additionally, certain adverse events must be reported in an expedited manner for more timely monitoring of patient safety and care. See Sections 7.5 and 7.6 for information about expedited and routine reporting.

Summary of All Data Submission: Refer to the NRG Protocol website

See Section 8 for TRIAD account access and installation instructions. See data submission table for TRIAD below.

<table>
<tr><td rowspan="4">DICOM Items</td><td>DICOM CT Image</td><td rowspan="5">Due Within 1 week of start of RT. Triad Time Point:

RT Digital Plan</td></tr>
<tr><td>DICOM Structure</td></tr>
<tr><td>DICOM Dose</td></tr>
<tr><td>DICOM Plan</td></tr>
<tr><td colspan="2">All required structures must be labeled per the tables in Sections 5.3.4
5.3.5</td></tr>
<tr><td colspan="2">Imaging needed for RT review:
Any imaging used for target delineation in the RT Planning process must be submitted along with RT Digital Plan</td></tr>
<tr><td colspan="3">Upon submission of the digital data via TRIAD, complete an online Digital Data Submission Information Form (DDSI):
https://www.irocqa.org/Resources/TRIAD</td></tr>
<tr><td colspan="3"><u>NOTE</u>: ALL SIMULATION AND PORTAL FILMS AND/OR DIGITAL FILM IMAGES WILL BE KEPT BY THE INSTITUTION AND ONLY SUBMITTED IF REQUESTED.</td></tr>
</table>

13.3 **Data Quality Portal** (19-MAY-2023)

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status, and timeliness reports. Site staff should review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff who are rostered to a site and have access to the CTSU website. Staff who have Rave study access can access the Rave study data via direct links available in the DQP modules.

CTSU Delinquency Notification emails are sent to primary contacts at sites twice a month. These notifications serve as alerts that queries and/or delinquent forms require site review, providing a summary count of queries and delinquent forms

for each Rave study that a site is participating in. Additional site staff can subscribe and unsubscribe to these notifications using the CTSU Report and Information Subscription Portal on the CTSU members' website.

To learn more about DQP use and access, click on the Help Topics button displayed on the Rave Home, DQP Queries, DQP Delinquent Forms. DQP Form Status, and DQP Reports modules.

13.4 Global Reporting/Monitoring

Required submission of patient demographic data for this study will be submitted automatically via OPEN.

Note: Serious adverse events must be submitted via CTEP-AERS per protocol guidelines.

14. STATISTICAL CONSIDERATIONS

14.1 Study Design (11-JAN-2022)

This is a limited multicenter phase I study of OBP-301 and chemoradiation (CRT) with carboplatin/paclitaxel for patients with locally advanced esophageal/GEJ cancer, for whom surgery is not planned. Up to 2 OBP-301 dose regimens will be assessed. If either dose regimen is determined to be safe, additional patients will be accrued to that dose regimen.

14.2 Study Endpoints

14.2.1 Primary Endpoint: Dose limiting toxicity to assess the safety of OBP-301 given in combination with chemoradiation.

14.2.2 Secondary Endpoints:

- Adverse events as measured by CTCAE v5.0
- Clinical complete response (cCR) – *if one of the OBP-301 regimens is declared to be safe*.
- Number of patients alive without progression and number of patients alive at 1 and 2 years - *if one of the OBP-301 regimens is declared to be safe*.

14.3 Primary Objectives Study Design

14.3.1 Primary Hypothesis and Endpoints

The primary hypothesis is that OBP-301 given in combination with chemoradiation using carboplatin and paclitaxel is safe.

14.3.2 How Primary Endpoint Will Be Analyzed

To assess the safety endpoint, the initial cohort of patients to be assessed will receive 3 doses of OBP-301 at 1 $x10^{12}$ vp/mL in conjunction with chemoradiation.

The dose-limiting toxicity (DLT) assessment period is from start of protocol treatment

until 30 days after the completion of CRT (total of ~10 weeks).

DLT is defined as the following that are definitely or probably attributed to OBP-301:

- Any grade ≥3 toxicity **EXCEPT** for the following:
  - *Grade 3 nausea/vomiting*
  - *Grade 3 esophagitis or dehydration*
  - *The first occurrence of grade 3/4 neutropenia*
  - *Grade 3/4 lymphopenia (since this is a known toxicity of chemoradiation and OBP-301)*
- Any toxicity that leads to a >14-day cumulative delay in chemoradiation

Evaluable patients for the safety endpoint will be defined as (i) patients who started all study treatment (OBP-301 and CRT) and had a DLT and (ii) patients who started all study treatment (OBP-301 and CRT) and completed DLT evaluation period without a DLT. If ≤1 of 6 evaluable patients in the 3 doses of OBP-301 at 1 $x10^{12}$ vp/mL cohort have a dose limiting toxicity, then the regimen will be declared safe and an expansion cohort of 9 more patients will be treated on this regimen to further evaluate toxicity and to get preliminary data on cCR.

If ≥2 of these 6 evaluable patients have a dose limiting toxicity, then the 3 doses of OBP-301 at 1 $x10^{12}$ vp/mL will be deemed too toxic and a second cohort will assess 3 doses of OBP-301 at 1 $x10^{11}$ vp/mL in conjunction with chemoradiation. If ≤1 of 6 evaluable patients in the 3 doses of OBP-301 at 1 $x10^{11}$ vp/mL cohort have a dose limiting toxicity, then the regimen will be declared safe and an expansion cohort of 9 more patients will be treated on this regimen to further evaluate toxicity and to get preliminary data on cCR. If ≥2 of 6 evaluable patients in this cohort have a dose limiting toxicity, then the 3 doses of OBP-301 at 1 $x10^{11}$ vp/mLwill be deemed too toxic, no more patients will be accrued, and the results will be reported.

If a grade 5 AE and/or 2 or more grade 4 AEs occur within 30 days of OBP-301 administration, there will be a pause in accrual in order for NRG to discuss these events with the FDA via teleconference.

If a safe OBP-301 dose is determined, then cCR will be evaluated and reported for the cohort of 15 patients treated at that dose, including the 6 used for the DLT assessment.

14.3.3 Sample Size and Power Calculations:

The DLT portion of the trial will accrue 6 or 12 evaluable patients, depending on whether or not the OBP-301 doses needs to be reduced. If a regimen is determined to be safe, based on the DLT endpoint, then the total number of evaluable patients accrued to the trial will be 15 patients, if OBP-301 3 doses at 1 $x10^{12}$ vp/mL is the DLT safe dose, or 21 patients, if OBP-301 3 doses at 1 $x10^{11}$ vp/mL is the DLT safe dose.

With a cohort of 6 patients, the probability of the treatment being judged to be too toxic

when the true toxicity rate is ≥42% is at least 80%. If the true toxicity rate is ≤18%, the probability that the treatment will be deemed to be safe is at least 70%.

Study reports focusing on accrual and adverse event data will be prepared regularly and the PI/NRG study team will have regular conference calls (biweekly or at most monthly) to review the accrual/safety data. Information from these calls will be reviewed regularly by the NRG Oncology Early Phase Oversight Committee.

14.4 Study Monitoring of Primary Objectives

Interim Reporting

Interim reports will be prepared twice each year until the final analysis has been accepted for presentation or publication. In general, these reports will contain information about the accrual rate with projected completion date for the accrual phase, exclusion rates, pretreatment characteristics of patients accrued, and the frequency and severity of AEs.

Required submission of patient demographic data for this study will be submitted automatically via OPEN.

Note: Serious adverse events must be submitted via CTEP-AERS per protocol guidelines. After the start of the accrual or 8 weeks following protocol activation, whichever comes first, the study team, including the study chairs, study statisticians, data managers will hold at least monthly conference calls to review the overall conduct of the study lead by the study statistician. A representative from each participating site will be invited to these calls. Data on accrual, eligibility, patient demographics, treatment dose delivery, and reported adverse events will be reviewed on the calls. Brief minutes of each meeting will be written by the study PI to document the review of information and any decisions that are made. The meeting minutes will be provided to the NRG Early Phase Trial Oversight Committee for review.

14.5 Accrual/Study Duration Considerations

It is anticipated that 1-2 patients will be accrued per month, after a 3 month ramp-up period. There will be a staggered enrollment of a 3 week duration for the first 3 patients enrolled onto the study to monitor for acute and subacute AEs.

The projected duration to accrue and assess DLTs for a given regimen is estimated to be 5.5-8.5 months. If both regimens need to be assessed, the projected maximum time from study activation to reporting the primary endpoint is ~ 20 months. This trial will have finite follow-up for each patient of 2 years from start of treatment.

The required number of evaluable patients is as follows:

- 15, if the initial cohort is deemed to be safe (6 for DLT and 9 expansion)
- 21, if the initial cohort is not deemed to be safe, but the reduced cohort is deemed to be safe (12 for DLT and 9 expansion)
- 12, if neither cohort is deemed to be safe (12 for DLT and no expansion)

14.6 Dose Level Guidelines

- Initial Dose Level: 3 doses of OBP-301 with chemoradiation, as specified in Section 5.2.1
- De-escalated Dose Level (IF NEEDED): 3 doses of OBP-301 with chemoradiation, as specified in Section 5.2.1

14.7 Secondary or Exploratory Endpoints (including correlative science aims)

14.7.1 Secondary Hypotheses and Endpoints:

- Adverse events as measured by CTCAE v5.0
- Clinical complete response (cCR) – *if one of the OBP-301 regimens is declared to be safe*.
- Number of patients alive without progression (PFS) and number of patients alive (OS) at 1 and 2 years - *if one of the OBP-301 regimens is declared to be safe*.

14.7.2 Definitions of Secondary Endpoints and How These Will Be Analyzed

Adverse events

Adverse events (AE) will be evaluated using the CTCAE v 5.0. Counts of all AEs by grade will be provided by treatment arm. Counts and frequencies will be provided for the worst grade AE experienced by the patient by treatment arm and within the subset of AEs related to treatment. Severe AEs will be any grade 4 or greater non-hematologic toxicities and will be summarized by frequency tables by treatment arm. No formal statistical testing will be performed on these summary data.

Clinical Complete Response (cCR)

For the regimen that is determined to be safe, out of all patients accrued to that regimen, the number of cCRs will be reported. No formal statistical testing will be performed on these summary data.

Progression-free Survival (PFS)

For the regimen that is determined to be safe, out of all patients accrued to that regimen, the number of patients that are alive without progression at 1 and 2 years will be reported. No formal statistical testing will be performed on these summary data.

Overall Survival (OS)
For the regimen that is determined to be safe, out of all patients accrued to that regimen, the number of patients that are alive at 1 and 2 years will be reported. No formal statistical testing will be performed on these summary data.

14.7.5 Expected Sample Size or Patient Cohorts: 6 evaluable patients will be accrued for up to 2 dose levels. If a dose level is determined to be safe, an additional 9 patients will be accrued at that dose level.

14.8 Exploratory Hypothesis and Endpoints

Tumor and blood immune and virus-based correlative assays will be performed (see Section 10).
These analyses will allow for the following exploratory hypotheses to be evaluated:

- Correlate qualitative and semi-quantitative in the tumor micro-environment and persistence of adenovirus infection with outcomes to identify biomarkers of response
- Correlate changes in immune cell subsets via multi-parameter flow cytometry with outcomes to identify biomarkers of response
- Correlates changes in cytokine profile via multiplex ELISA with outcomes to identify biomarkers of response

14.9 Gender/Ethnicity/Race Distribution

Racial Categories	**DOMESTIC PLANNED ENROLLMENT REPORT**				
	Ethnic Categories				
	Not Hispanic or Latino		Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian/Alaska Native	0	1	0	0	1
Asian	0	1	0	0	1
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	1	2	0	0	3
White	2	12	0	2	16
More Than One Race	0	0	0	0	0
Total	3	16	0	2	21

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APPENDIX I: PHARMACY MANUAL (19-MAY-2023)

1. Telomelysin (OBP-301)

Summary of Investigational Product

OBP-301 (Telomelysin, hereafter referred to as OBP-301) is an adenoviral vector containing the adenoviral E1A and E1B gene linked with an internal ribosome entry site (IRES) under the control of the human telomerase reverse transcriptase (hTERT) promoter.

Packaging and Labeling

OBP-301 will be provided as a frozen viral suspension formulated in 20 mM Tris pH 8.0, 25 mM NaCl with 2.5% Glycerin, USP by volume. The product is packaged using a 5 mL glass vial, siliconized gray butyl-rubber stoppered vials and an aluminum seal with a plastic cap. Each vial contains a volume of 2.0 mL at a concentration of 1 x 10^{12} viral particles (vp) / mL. The product in this configuration is stored at ≤ -60° C prior to administration

Vials will be labeled with the following information:

Vial label:
Name of the Sponsor, Protocol No., Contents, Nominal Concentration, Content, Route of Administration, Batch No., Lot No., Storage Condition

Label for storage box:
Caution: For Clinical Trial Use Only,
Name of the Sponsor, Protocol No., Contents, Nominal Concentration, Content, Route of Administration, Batch No., Lot No., Storage Condition

STUDY AGENT RECEIPT PROCEDURES

Shipping Conditions

Each vial is boxed in an upright position within a small box. Each storage box is placed in a plastic self-sealing leak-proof bag with an adequate number of absorbent pads in the event of leakage during shipping. The storage boxes are shipped to the clinical sites in leak-proof foam-lined cardboard boxes.

Due to product specific requirements, the shipments are made on dry ice. Within each shipment there will be an official shipping form to be completed by the study investigator or designee (e.g. pharmacist).

Receipt Procedures

The study investigator or designee is responsible for receiving each OBP-301 shipment. Prior to each shipment, the drug distributor, Catalent, will contact the assigned personnel with the delivery information.

Upon receipt the following should be performed:

1. Always wear disposable gloves when handling OBP-301.

2. Examine the outside of the shipment package(s) for integrity. If the shipment package or contents are not intact:
   - The outer surfaces of all containers should be decontaminated with a virucidal disinfectant such as CaviCide.
   - If a vial is damaged in any way, it should be placed in a biohazardous waste container for sharps, and destroyed immediately according to institutional and local regulations for biohazardous waste management. Be sure to record the destruction of any clinical material on the Clinical Trial Material Destruction Certificate (located on the NRG website). See Appendix III Guidelines for use and completion of Clinical Trial Material Destruction Certificate for instructions to complete the form.
   - Contact the drug distributor, Catalent, to report the damage.
3. Open the shipment package(s).
4. Confirm the package still contains dry-ice.
5. Transfer the leak-proof package to a Biological Safety Cabinet (BSC).
6. Remove the storage box from the plastic bag(s).
7. OBP-301 will be provided in glass, chlorobutyl rubber stoppered vials, with an aluminum seal as a frozen viral suspension. It will be transported to the pharmacy, following the current International Airway Transport Association (IATA) regulations.
8. Promptly, inventory the vials of OBP-301 contained in the storage box and examine the integrity of the vials. If the shipment package or contents are not intact, follow the steps listed above in step 2. If the shipment does not match the detailed description, the site should contact the drug distributor, Catalent.
9. Transfer vials to the appropriate location in the assigned ≤ -60°C freezer. The vials must be stored in the original storage box to facilitate supply tracking.
10. Wearing gloves, dispose of the dry ice, according to institutional procedures.
11. Decontaminate the biological safety cabinet with a virucidal disinfectant.
12. Complete the lower portion of the official shipping form**,** email it to the originator of the form as instructed and retain the original document in the site file.
13. Enter the number of vials of clinical trial material received on the NCI's Investigational Agent Accountability Record (located on CTEP/PMB website).

Storage Conditions

Prior to dilution, the vials of OBP-301 must be stored in a ≤ -60°C freezer, in an upright position. The vials must be stored in the original vial storage boxes to facilitate supply tracking. It is recommended to leave the vial storage boxes in the original leak-proof bag or in a substitute leak-proof container, particularly if the supplies are not segregated (i.e. in a separate section of the freezer) from other products or biological samples.

The freezer should be in a locked or secured area, with limited access. Additionally, the freezer should have a temperature recorder and an alarm system. The temperature plot should be properly maintained and checked. Documentation for calibration of the freezer at installation and at each maintenance inspection is required, (at least annually or as per manufacturer's recommendations). A biohazard symbol should be affixed to the outside of the storage compartment.

PREPARATION NOTES

OBP-301 should be prepared according to Biosafety Level 2 Guidelines, unless local regulations require a more rigorous containment level. An outline of Biosafety Level 2 precautions is located in Appendix III: Handling Guidelines/Universal Precautions for Biosafety Level 2 Agents.

Disinfectant

A disinfectant that has been qualified as an effective virucide for adenoviruses should be used for any decontamination procedure. *The recommended exposure time should be at least 5 to 15 minutes*. However, if the handling precautions recommended by the manufacturer differ, the manufacturer's instructions should be followed.

Study Material Expiration After Thaw

The duration that OBP-301 remains at room temperature should be minimized. It is recommended that OBP-301 be administered as soon as possible (within 4 hours) after reconstitution. Immediately prior to injection, it may be warmed by rolling the syringe between the palms of the hands, which may facilitate comfort of administration.

Aseptic Procedures

Aseptic techniques must be followed at all times during the preparation and transport to the treatment area.

Biosafety Level 2

As there is the potential of creating infectious aerosol during the preparation procedure, all work involving OBP-301 must be performed inside a properly maintained class II Biological Safety Cabinet (BSC). Biosafety Level 2 guidelines should be adhered to, unless local regulations require a more rigorous containment level. An outline of Biosafety Level 2 guidelines is given in Appendix II.

Universal Precautions

Universal precautions should always be adhered to, as defined by federal, state and institutional and local regulations.

Biohazardous Waste Management

All OBP-301 wastes are considered biohazardous and consequently must be handled by the site according to federal, state and local regulations on management of biohazardous materials.

Syringe Label

According to local practice, a label will be affixed either to each injection syringe containing the final OBP-301 suspension or to the leak-proof plastic bags used in the transfer of the syringes. Care should be taken not to cover the graduation markings on the syringe with the label.

The label should include but is not limited to the following information:

- Patient name or initials
- Patient study number
- Study investigational product: OBP-301
- Dose (vp) and volume of injection
- Expiration date
- Lot number

MATERIALS REQUIRED

1. Vials of OBP-301
2. Labels for injection syringes
3. Disposable needles with bore 22-25 Gauge
4. Self-sealing leak-proof plastic bags
5. Puncture resistant leak-proof container for transport from pharmacy to treatment area
6. Virucidal Disinfectant
7. Biological Safety Cabinet (Class II) for OBP-301 preparation
8. Polypropylene syringe(s)

PREPARATION PROCEDURES

1. Prior to assembly and placement of the OBP-301 preparation material, wipe Biological Safety Cabinet work surface with an appropriate disinfectant, following manufacturer's instructions. Allow to dry.
2. Wipe all outside surfaces of materials with an appropriate disinfectant, following manufacturer's instructions, prior to placement of study material inside the biological safety cabinet. Arrange materials for preparation toward the back of the cabinet in order to protect from contamination.
3. Remove the vial(s) of OBP-301 from the ≤ -60°C freezer on the day of administration, following confirmation that the patient will be treated on that day. Transfer the vials in a puncture resistant leak-proof container to the preparation area.
4. Prepare labels.
5. Assemble the appropriate needles and syringes.
6. Thaw vial containing OBP-301 by rolling the vial between the palms of the hands and mixing 10 times by inverting the vial. The OBP-301 should be allowed to thaw completely prior to removing the aluminum seal. Before proceeding, visually inspect the solution in the vials to ensure there are no ice particles. Remove the seal on the OBP-301 vials.

7. As there is a "dead space" of up to 1.5 ml in the tubing and syringe, it will be necessary to utilize 2 vials of OBP-301, drawing up 2 mL from vial 1 and 1.5 mL from vial 2. At the end of the endoscopy procedure, any OBP-301 in the syringe, tubing and the 2nd vial that has not been administered should be disposed of per biosafety protocols.
8. Using polypropylene syringe(s), aseptically pierce the rubber cap(s) with the needle(s) and draw up the required volume. Caution should be used to avoid excessive shear forces in aspirating and discharging through needles.
9. According to local practice, label either the OBP-301 syringe and/or the plastic transfer bag with a patient label.
10. Place the syringe into the primary self-sealing leak-proof plastic bag and seal tightly. Place this bag into the second transfer bag. Seal outer transfer bag tightly, assuring that the labels can easily be read.
11. When the syringe is to be transported to the treatment area, the transfer bags should be placed in a puncture resistant leak-proof container, marked with a biohazard label. *Effort should be made to minimize the time that the OBP-301 is placed at room temperature.*
12. Immediately after use in the preparation procedure, the OBP-301 vials should be placed in a biohazardous waste container. Thereafter, the local procedures for handling of biohazardous wastes must be followed.
13. Immediately dispose of all materials used in the preparation of the syringe, according to practices established by local health authorities and/or hospital for disposal of biohazardous waste.
14. Clean and disinfect the Biological Safety Cabinet with a virucidal disinfectant.
15. Record dispensing of study material on the *NCI Investigational Agent Accountability Record.*
16. If you have any questions concerning the preparation procedure, please contact NRG Oncology.

INJECTION TECHNIQUE

Please read Appendix II: Handling Guidelines/Universal Precautions for Biosafety Level 2 Agents before injecting OBP-301.

Pre-injection planning is the key to success, therefore, a sketch of the tumor and the planned injection sites should be made. (The sketch will be helpful in determining the total quantity of study drug to inject.)

Irrespective of the size of the tumor, every effort should be made to inject 1.0-2.0 mL of OBP-301; the maximal volume of 2.0 mL is preferred and should be injected as long as this is technically possible

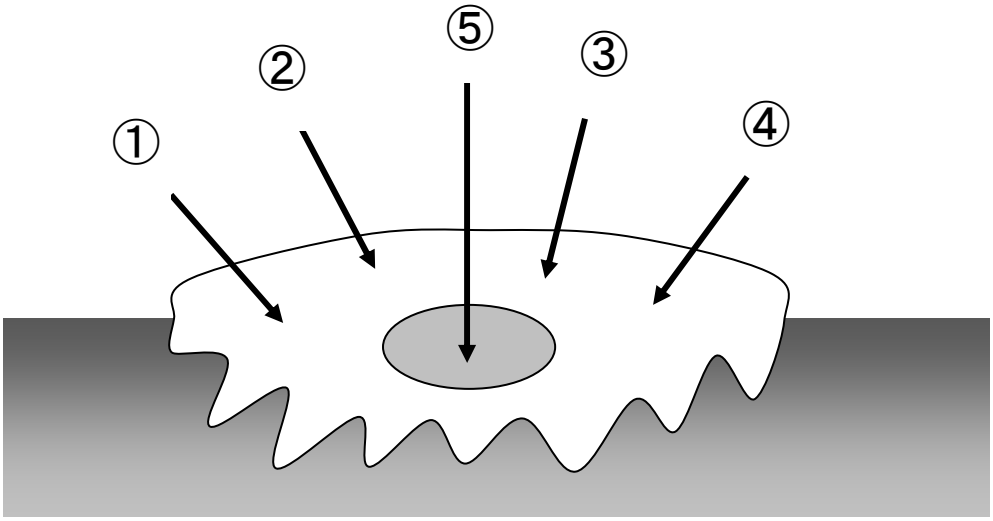


Figure 1. Example of Mapping into up to 5 Tracks

Specific endoscopic instructions are provided in a separate training document and video.

Pain Management for Intratumoral Injections with OBP-301

Injection site pain is the one of the more common adverse events in connection with intratumoral injections. Therefore, the following pain control measures are suggested to improve the comfort of the patient.

1. Review the analgesics already prescribed for the patient and determine the level of pain control.
   Additional pain medication will with all likelihood be needed during the day of the intratumoral injections and for a couple of days afterwards. Local anesthesia or nerve block may very well be required just before or during the intratumoral injection.

DISPENSING AND ACCOUNTABILITY

The study investigator, pharmacist, or designee may only dispense clinical supplies in accordance with the protocol.

The study investigator, pharmacist, or designee, is responsible for maintaining an accurate and current record of all clinical supplies received from the sponsor, dispensed to the investigator or destroyed. Accountability records must be maintained throughout the course of the study, showing receipt and disposition of the clinical supplies. At the end of the study, it must be possible to reconcile delivery records with that of used, unused and destroyed clinical supplies.

The NCI's Investigational Agent Accountability Record (located on CTEP/PMB website) must be used for accountability and destruction certificate to be completed upon destruction (located on the NRG website).

During site monitoring visits, the drug accountability forms will be verified and checked for accuracy and consistency.

DESTRUCTION OF EMPTY OR PARTIALLY USED VIALS

Immediately after use, the used vials should be placed in a biohazardous waste container. Empty or partially used vials should be destroyed by the study pharmacy, in accordance with institutional biosafety policy and procedures for biohazardous waste management. Proper accounting of the vials must be performed immediately using the Investigational Agent Accountability Record.

DESTRUCTION OF UNUSED VIALS

Written authorization must be obtained from Oncolys before any destruction of unused clinical material supplies.

This will occur at the completion of the clinical study or when the expiration date has been reached and could not be prolonged by the sponsor based on updated stability information. In addition, destruction of unused supplies may only be performed after product reconciliation by the pharmacist.

The unused vials must be destroyed by the study pharmacy, in accordance with institutional Biosafety policy and procedures for biohazardous waste management.

The investigator or designee must fill in, sign and date the appropriate section of *Clinical Trial Material Destruction Certificate* (located on the study-specific page on the NRG website). Discrepancies in vial accountability are to be explained in writing by the investigator or designee. The original will be retained for the site study file.

APPENDIX II: BIOSAFETY LEVEL II SUMMARY INFORMATION FOR INSTITUTIONAL BIOSAFETY COMMITTEE REVIEW

The site at which this trial is being conducted will ensure that an Institutional Biosafety Committee (IBC) is in place that is composed of at least 5 appropriately-qualified members. The IBC will ensure that the site conforms to the requirements set forth in the Section IV-B-2 of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, promulgated by the NIH/Office of Biotechnology Activities (NIH/OBA). The Principal Investigator at the site will be responsible for petitioning the IBC and obtaining approval prior to enrolling any subject in the study. The Principal Investigator will also be required to obtain and follow all biohazard safety guidelines promulgated by the IBC, and to report all findings as required to the IBC and to NRG Oncology for reporting to NIH/OBA.

This protocol and any accompanying material provided to the subject (such as subject information sheets, Informed Consent Form, or descriptions of the study used to obtain informed consent) will be submitted by the Principal Investigator to the legally constituted and chartered Institutional Biosafety Committee (IBC). Additional materials, such as the Investigator's Brochure, will be submitted to the IBC according to the specific Committee and federal (United States' National Institutes of Health or foreign equivalent) requirements. Each site will be approved by the IBC in accordance with local procedures and country specific regulatory requirements. Documentation of IBC approval must be in place with both the CTSU and locally prior to subject enrollment. At the discretion of the specific IBC and within federal requirements, IBC oversight of individual sites may be terminated provided (1) all subjects at that site have completed dosing by at least 90 days, and (2) all investigational materials have been fully accounted for and either returned to the distributor, destroyed on site, or shipped to a duly licensed destruction facility and a shipping and inventory reconciliation records have been filed.

Precautions appropriate to a Risk Group 2 virus are recommended for the OBP-301 (US NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules). There are 4 risk groups, with Risk Group 1 posing the least danger and Risk Group 4 being the most dangerous to work with. RG-2 viruses are defined as those agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. For comparison purposes, herpes simplex virus and hepatitis B virus are also classified as Risk Group 2 viruses. Dealing with Risk Group 2 viruses requires certain precautions referred to as Biosafety Level 2 (BSL-2) practices. These practices are summarized below. Further information can be obtained from the local site Institutional Biosafety Committee.

APPENDIX III: HANDLING GUIDELINES/UNIVERSAL PRECAUTIONS FOR BIOSAFETY LEVEL 2 AGENTS

Introduction

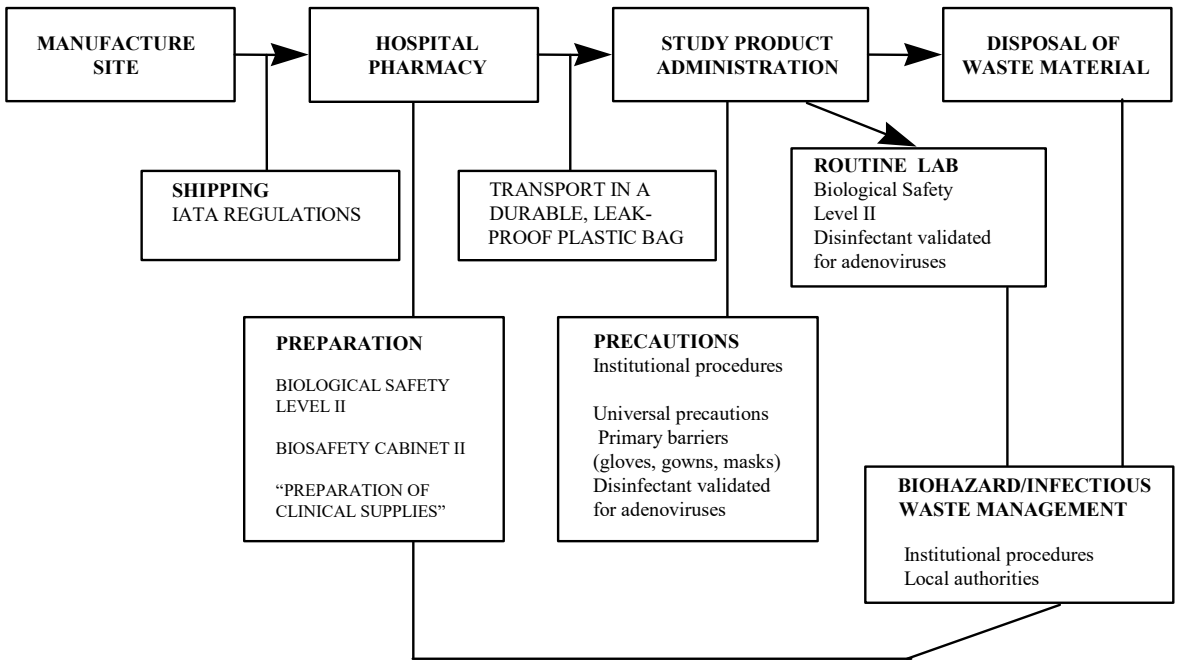
This document is designed to serve as a guide for pharmacists, laboratory staff, and healthcare workers involved with work involving agents of moderate potential hazard to personnel and the environment. It gives an overall view of the type of safety procedures and precautions that are recommended with work with these types of agents.

These instructions are based on two documents: Biosafety in Microbiological and Biomedical Laboratories and Guidelines for Isolation Precautions in Hospitals. Both documents are from the US Centers for Disease Control and Prevention (CDC) which should be consulted for further reference if needed.

Detailed specific study product preparation procedures are provided to the study pharmacist in the Pharmacy Brochure.

Overview

The following schema highlights key points for hospital team members participating directly or indirectly in patient care.



Biosafety Level 2 Guidelines

Standard Practices	Special Practices	Safety Equipment (Primary Barriers)	Laboratory Facilities (Secondary Barriers)
• Limited or restricted access • Hand washing • No eating, drinking, storing food, smoking, handling contact lenses, or applying cosmetics • Mouth pipetting prohibited • Minimize creation of aerosols, splashes • Procedures creating infectious aerosols or splashes to be conducted in BSC. • Work surfaces decontaminated • Wastes decontaminated before disposal • Insect and rodent control	• Policies and procedures • Hazard warning sign • Serum monitoring program • Biosafety manual • Appropriate training • Limit sharps • Use leak-proof containers • Decontaminate equipment and work surfaces • Report spills and accidents to appropriate hospital personnel	• Biosafety cabinets (BSC), preferably class II, and/or personal protective equipment • Face/eye protection for work outside BSC • Designated laboratory garments worn and removed before leaving lab • Gloves worn when handling materials, removed before leaving lab	• Sink available in room • Lab designed for easy cleaning • Impervious bench tops • Windows fitted with screens • Method for decontamination of waste available • Eyewash facility readily available

Universal Precautions

Patient Placement: Single room
Handwashing: After contact After glove removal
Gloves: Wear when handling patient/contaminated items Wear when entering room, change after contact, remove when leaving,
Mask/Eye Protection: When splashes or sprays are anticipated

Gown: When splashes or sprays are anticipated
Patient-care Equipment: Avoid contact with surfaces, ensure proper disposal/ decontamination
Environmental Control: Procedures for routine care, cleaning, and disinfection
Linen: Proper handling, transport, and processing
Occupational Health: Blood borne pathogens, sharps control, resuscitation devices

All Departments

All staff potentially working with agents of moderate potential hazard to personnel and the environment should receive appropriate training, regarding the potential hazards associated with the work involved and the necessary precautions to prevent exposures. The staff should receive additional training as necessary for procedural or policy changes.

Pharmacy Facilities

1. Properly maintained Level 2 Biological Safety Cabinets (BSC) and other appropriate physical containment devices and personal protective equipment are used, unless local regulations require a more rigorous containment level.
2. A method of decontamination of the infectious material is available (e.g., autoclave, chemical disinfection, incinerator, or other approved decontamination system).
3. The preparation room contains a sink for hand-washing.
4. The room is designed so that it can be easily cleaned.
5. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
6. An eyewash facility is readily available.
7. A hazard warning sign, incorporating the universal biohazard symbol is posted on the access door to the preparation room. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the responsible pharmacist or other responsible person(s), and indicates the requirement(s) for entering the room.
8. If the area has windows that open, they are fitted with fly screens.
9. Access to the preparation room is limited or restricted, at the discretion of the responsible pharmacist, when preparation is in progress.

General Recommendations for Product Preparation and Handling

1. Protective coats, gowns, smocks, or uniforms designated for pharmacy use should be worn while in the preparation room. This protective clothing is removed and left in the preparation room before leaving for another area (e.g. cafeteria, library, administrative offices). All protective clothing is either disposed into a biohazard waste container in the preparation room or laundered by the institution according to hospital policy and local regulations for garments potentially contaminated with infectious agents. Personnel must never take protective garments home.
2. Gloves must be worn and disposed of when contaminated, removed when work is completed, and are not worn outside the preparation room. Disposable gloves are not washed or reused.
3. Persons wash their hands after handling potentially infectious material, after removing gloves, and before leaving the preparation room.
4. Persons who wear contact lenses should also wear goggles or a face shield.
5. All procedures are performed carefully to minimize the creation of splashes or aerosols, Furthermore, face protection (goggles, mask, face shield, or other splatter guards) is used for anticipated splashes or sprays to the face
6. This is a sterile product for injection; therefore aseptic procedures should be followed at all times during the preparation of the required specific dose.
7. Mouth pipetting is prohibited. Mechanical pipette devices must be used.
8. A high degree of precaution should always be taken with any sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. Needles and syringes or other sharp instruments should be restricted in the pharmacy for use only when there is no alternative. Plastic ware should be substituted for glassware whenever possible.
    a) Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. If avoidable, used disposable needles should not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they should be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps should be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    b) Syringes that re-sheathe the needle, needle-less systems, and other safe devices should be used when appropriate.
    c) Broken glassware should not be handled directly by hand, but should be removed by mechanical means, such as a brush and dustpan, tongs, or forceps.

Accidental Inoculation

If an accidental inoculation (or blood spillage on broken skin) occurs:

1. The offending needle should be removed quickly.
2. The wound should be soaked as soon as possible in a good skin disinfectant for at least 15 minutes.
3. All actions that could lead to a deeper penetration (rubbing, scrubbing, etc.) should be avoided.
4. Apply a waterproof dressing.
5. Such incidents should be reported officially in accordance with the standard institutional procedures.
6. A blood sample should be taken and stored for possible future analysis.
7. The wound site should be monitored and a follow-up clinical evaluation should be performed 2 weeks after the incident.

Any accidental blood spillages onto unbroken skin should be washed off with water into a suitable receptacle. The skin should then be washed with soap and water. An accident form should be filled out in accordance with institutional policy.

Decontamination Procedures

1. Gloves are worn when handling contaminated surfaces or equipment. Gloves are disposed of when contaminated. Disposable gloves are not washed or reused.
2. Pharmacy equipment and work surfaces should be decontaminated with an appropriate disinfectant after work with these agents is finished and especially after overt spills, splashes, or other contamination. Contaminated equipment should be decontaminated according to hospital regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local or country regulations before removal from the facility.
3. All stocks and other regulated wastes will be double-bagged in biohazard bags, marked with a biohazard label and sealed before removal for destruction in accordance with institutional regulations on biohazardous waste management.
4. Materials to be decontaminated outside the pharmacy are to be placed in a durable, leak-proof container, marked with a biohazard label and closed for transport from the pharmacy.
5. All sharps (contaminated needles, sharp equipment, broken glass, etc.) should be placed in leak-proof, puncture-resistant sharps containers marked with a biohazard label. When procedures are finished, the container should be sealed and put into a biohazard waste bag. The bag is then sealed, marked with a biohazard label, and sent for destruction in accordance with institutional regulations on biohazardous waste management.
6. Routine daily cleaning procedures may be carried out as determined by hospital policy and local regulations.

Decontamination of Spills

<u>Small Spills on Hard Surfaces</u>

Blood or bodily fluids spilled on hard surfaces should be wiped up immediately with paper

towels (while wearing gloves, eye protection, mask, and a gown), and the area washed with a virucidal disinfectant. The material used in the clean-up procedure should be double-bagged in biohazard bags, sealed, marked with a biohazard label, and sent for destruction according to institutional regulations for biohazardous waste management.

Small Spills in Safety Cabinet

1. Close the cabinet sash and allow the ventilation system to operate for 20 to 30 minutes to remove any aerosolized material.
2. Wearing eye protection and gloves, cover the spill with absorbent material and saturate with disinfectant.
3. After 20 to 30 minutes of contact time, remove the absorbent and contained liquid.
4. Clean the area with disinfectant.
5. All material used in the clean-up should be double-bagged in biohazard bags, marked with a biohazard label, sealed, and sent for destruction according to institutional regulations for biohazardous waste management.

Large Spills

1. Evacuate the area. Wait for 20 to 30 minutes prior to entry to allow aerosols to settle.
2. Enter the area wearing full protective clothing, including eye protection and mask.
3. Place absorbent material on liquid and saturate with disinfectant.
4. Use mechanical means to pick up broken glassware or other sharps.
5. After 20 to 30 minutes of contact time, remove the absorbent and contained liquid.
6. Clean the area with disinfectant.

All material used in the clean-up should be double-bagged in biohazard bags, marked with a biohazard label, sealed, and sent for destruction according to institutional regulations for biohazardous waste management.

Transport, Reception and Handling of Biological Samples by Laboratory Staff

It is recommended that all biological samples be handled and disposed of according to Biosafety Level 2 guidelines, unless local regulations require a more rigorous containment level. Training and regular updating on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures should be performed. An outline of Biosafety Level 2 precautions is given below:

1. All specimens should be double-bagged and labeled with a biohazard label. Request cards also appropriately labeled.
2. Samples should be transported in a covered, leak-proof, autoclavable container, marked with a biohazard label.
3. In the laboratory, samples should be stored separately in autoclavable or disposable racks.

4. The reception area in the laboratory should have disposable gloves, disinfectant, and absorbent material ready to deal with contamination.
5. Gloves, eye protection, masks, and gowns should be worn when handling/transporting all biological samples, i.e., blood, urine, sputum and stools.
6. All materials containing or having come into contact with samples are discarded in sack holders containing doubled biohazard waste bags. These bags should be sealed, marked with a biohazard label, and sent for destruction according to institutional regulations for biohazardous waste management.
7. Contaminated needles, sharp equipment, and broken glass are placed in leak-proof, puncture-resistant sharps containers, which are closed securely, placed in biohazard bags, sealed, marked with a biohazard label, and sent for destruction according to institutional regulations for biohazardous waste management.

Clinical Waste

Universal Precautions should be observed when handling blood, urine, stool, and other bodily matter, unless local regulations require special procedures. An outline of Universal Precautions and Biosafety disposal guidelines is provided below:

1. The disposable gowns, gloves, and masks worn by the staff should be discarded in a biohazard waste bag kept on a regular holder with a lid just inside the door. When the holder is full, it should be sealed, marked with a biohazard label, and sent for destruction in accordance with institutional regulations on biohazardous waste management.
2. If re-usable gowns are worn they should be disinfected and laundered according to hospital policy and local regulations for garments potentially contaminated with infectious agents.
3. Sharps are a potential inoculation hazard; therefore extreme care should be taken in handling them. A leak-proof, puncture-resistant container for disposing sharps should be kept beside the patient's bed. When half full it should be sealed and put into a biohazard waste bag. The bag is then sealed, marked with a biohazard label and sent for destruction in accordance with institutional regulations on biohazardous waste management.
4. Used linen, whether it is soiled or not, should be handled, transported, and laundered as determined by hospital policy and local regulations for linen with an infectious risk.
5. Used dressings should be sealed securely in a plastic bag before disposal in a biohazard waste bag. The biohazard bag is then sealed, marked with a biohazard label, and sent for destruction in accordance with institutional regulations on biohazardous waste management.

Safety Guidelines for Clinical Study Patients and Caregivers

- Avoid direct contact with body fluids of patients.
- Immunocompromised individuals or pregnant women should not come into direct contact with body fluids of treated patients.

Safety Guidelines for Healthcare Staff when Handling OBP-301

- Immunocompromised individuals or pregnant women should not prepare or administer OBP-301 and should not come into direct contact with OBP-301 injection sites, dressings, or body fluids of treated patients.
- Staff must follow Biosafety Level 2 guidelines by avoiding accidental exposure to OBP-301 and follow the instructions for preparation, administration, and handling of OBP-301.
- Staff must wear personal protective equipment (protective gown or laboratory coat, safety glasses or face shield, and gloves) while preparing or administering OBP-301.
- Cover any exposed wounds before handling OBP-301.
- In the event of an accidental occupational exposure (e.g., through a splash to the eyes or mucous membranes), flush with clean water for at least 15 minutes.
- In the event of exposure to broken skin or needle stick, clean the affected area thoroughly with soap and water and/or a disinfectant.
- Clean all surfaces that may have come in contact with OBP-301 and treat all spills with a virucidal agent such as 1% sodium hypochlorite or 70% isopropyl alcohol and blot using absorbent materials.
- Dispose of all materials that may have come in contact with OBP-301 (e.g., vial, syringe, needle, cotton gauze, gloves, masks, or dressings) as biohazardous waste.

APPENDIX IV: Guidelines for Use and Completion of Clinical Trial Material (Drug) Destruction Certificate (11-JAN-2022)

GENERAL

After expenditure of each vial of OBP-301, the site must dispose of or destroy the vial in accordance with the existing disposal practices employed for biohazardous waste at your institution. If unused clinical trial material expires or will not be used in this study, please contact the drug distributor, Catalent, for instructions on accountability and subsequent destruction (see Catalent contact information on the *Clinical Trial Material Destruction Certificate* on the NRG website). This form is for documentation of the destruction of unused vials.

HEADER

Protocol number: Enter the number of the study

Clinical Trial Material: Enter the name of the study product OBP-301 (OBP-301)

Principal Investigator: Enter the name of the Principal Investigator (PI).

Site Number: Enter the number of the clinical site supplied by Sponsor.

Site Name: Enter the name of Institution/Hospital and country

TABLE

Date of destruction: Enter the date that the vials were destroyed, in DD/MMM/YYYY format as shown: 10/Jun/2020.

Lot Number: Enter the lot number indicated on the OBP-301 label.

Vial Status: Tick "Empty/Partially Used" and/or "Unopened"

Number of Vials Destroyed: Enter the number of the vials destroyed on the date given.

Reason for Destruction: Tick one box only. If destruction was requested or authorized by Sponsor, please attach the appropriate documentation. If for another reason, please specify in the space provided.

Initials of Person Destroying Material: Enter the name of the person responsible for destruction of the material. This may be the PI or other personnel as designated by the Investigator.

BOTTOM

Name of Investigator or Designee: Please print.

Signature of Investigator or Designee/date: Once a form is completed, please sign and date in

DD/MMM/YYYY format.

APPENDIX V: CARBOPLATIN DOSE CALCULATION INSTRUCTIONS (07-DEC-2020)

1) The Cockcroft-Gault formula will be used in NRG Oncology trials.

Dosing of Carboplatin:
1) The carboplatin dose will be calculated to reach a target area under the curve (AUC) according to the Calvert formula using creatinine clearance (mL/min) from the Cockcroft-Gault formula.

2) In patients with an abnormally low serum creatinine (less than 0.7 mg/dl), the creatinine clearance should be estimated using **a minimum value of 0.7 mg/dL**.

3) The initial dose of carboplatin must be calculated using GFR. In the absence of renal toxicity greater than or equal to CTCAE Grade 2 (serum creatinine >1.5 x ULN) or toxicity requiring dose modification, the dose of carboplatin will not need to be recalculated for subsequent cycles, but will be subject to dose modification for toxicity as noted in the protocol.

4) Carboplatin doses are required to be recalculated if the patient has a weight change of greater than or equal to 10%. Patients are permitted to have chemotherapy doses recalculated for < 10% weight changes.

5) At the time of dose modification, if the patient's age had changed (the patient has had a birthday), the site can use the current age.

CALVERT FORMULA:
Carboplatin dose (mg) = target AUC x (GFR [or estimated CrCl] + 25)

NOTE: the GFR used in the Calvert formula should not exceed 125 ml/min.
Maximum carboplatin dose (mg) = target AUC (mg/ml x min) x 150 ml/min.

The maximum allowed doses of carboplatin are:
AUC 2 = 300 mg
For the purposes of this protocol, the GFR is considered to be equivalent to the estimated creatinine clearance. The estimated creatinine clearance (mL/min) is calculated by the method of Cockcroft-Gault using the following formula:

$$\textbf{Creatinine Clearance (mL/min)} = \frac{\textbf{[140-Age (years)] x actual body Weight* (kg)}}{\textbf{72 x serum creatinine (mg/dl)}} \quad \textbf{\{x 0.85 } \textbf{\textit{if female}}\textbf{\}}$$

Notes:
1) Weight in kilograms (kg):
   a. Body Mass Index (BMI) should be calculated for each patient.

   b. Actual weight should be used for estimation of GFR for patients with BMI of less than 25.

c. **Adjusted** weight should be used for estimation of GFR for patients with **BMI of greater than or equal to 25**

d. Adjusted weight calculation:
Ideal weight (kg) = ((Height (cm)/2.54) – 60) x 2.3) (+ 45.5 females) or (+ 50 for men)
Adjusted weight (kg) = ((Actual weight – Ideal weight) x 0.40) + Ideal weight

At the time of a dose modification for toxicity:
If the creatinine at the time of a dose modification is lower than the creatinine used to calculate the previous dose, use the previous (higher) creatinine; if the creatinine at the time of a dose modification is higher than the creatinine used to calculate the previous dose, use the current (higher) creatinine. This will ensure that the patient is actually receiving a dose reduction.

APPENDIX VI: CORRELATIVE IMMUNE ASSSAYS

Tumor and blood will be collected for the following correlative immune analyses:

1. **Immunoflourescence:**
   Immunofluorescence (IF) is an emerging tissue-based methodology that allows for a single slide to be stained with multiple different antibodies conjugated to different fluorochromes. This permits for simultaneous semi-quantitative and qualitative characterization of multiple markers on one slide. Markers that will be analyzed include immune cell surface markers (CD3, CD4, CD8, FoxP3, CD68), pan-cytokeratin (for tumor cells) and Adhexon (for persistence of viral infection). Immunofluorescence will be performed in the laboratory of Dr. Prasad Adusumilli, MD at Memorial Sloan Kettering Cancer Center (MSKCC).

2. **Mulitparameter Flow Cytometry**
   Multiparameter flow cytometry will be used to characterize the peripheral blood mononuclear cell phenotype at various time-points. Based on the time-intensive nature of these analyses, they may be restricted to patients with extreme outlier responses, e.g. complete vs. minimal pathologic response. The panel will include CD3, CD4, CD8, FoxP3, Ki67, ICOS, PD-1, LAG-3, TIM-3 and CTLA-4. Analyses will be performed in the Immune Monitoring Facility (IMF) at MSKCC.

3. **Cytokine Profiles:**
   Cytokine profiles provide information about the status of immune activation and whether there is predominance of a Th1 or Th2 response. Th1 responses are associated with cytotoxic T cell responses and effective anti-tumor immunity, while Th2 responses are regarded as being anti-inflammatory and counteract Th1 responses. Cytokines can be measured using multiplexed ELISA kits, which can detect IL-1β, -2, -4, -6, -8, -10, -12p70, IL-13, tumor necrosis factor-α, interferon (IFN)-γ and others. Analyses will be performed in the IMF at MSKCC.