PROTOCOL COVER PAGE

TITLE: Phase II study of Telomelysin (OBP-301) in combination with pembrolizumab in esophagogastric adenocarcinoma

NCT#: 03921021

Version 8.0, Date: March 2, 2022

TITLE: Phase II study of Telomelysin (OBP-301) in combination with pembrolizumab in esophagogastric adenocarcinoma

IRB Protocol #: 1807019403
IND/IDE # (if applicable): 18482
Version 8.0, Date: March 2, 2022

Principal Investigator:
Manish Shah, MD
1305 York Avenue, 12th Floor
New York, NY 10021
Phone 646-962-6200
Fax 646-962-1607
mas9313@med.cornell.edu

Statistician:
Paul J Christos, PhD
Healthcare Policy and Research
402 E. 67th St, Room 34
New York, NY 10065
pac2001@med.cornell.edu

Participating Sites:

Sites	Investigator(s)	Academic Center(s)
1.	Manish A. SHAH, MD mas9313@med.cornell.edu	Weill Cornell Medicine/ New-York Presbyterian
2.	Peter ENZINGER, MD Peter_Enzinger@dfci.harvard.edu	Dana Farber Cancer Institute
3.	Jennifer EADS, MD Jennifer.Eads@uphs.upenn.edu	University of Pennsylvania

Confidentiality Statement

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ad5	adenovirus serotype 5
AE	adverse event
AJCC	American Joint Commission on Cancer
ALT	alanine transaminase
APC	antigen presenting cell
AST	aspartate transaminase
BSLD	baseline sum longest diameters
CAR	Coxsackie adenovirus receptor
CBC	complete blood count
CBER	Center for Biologics Evaluation
cGMP	current Good Manufacturing Practice
CR	complete response
CRF	case report form
eCRF	electronic case report form
CRO	contract research organization
CSR	clinical study report
CT	computed tomography
CV	coefficient of variation
CTCAE	Common Toxicity Criteria for Adverse Events
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
ECG	electrocardiogram
EDC	electronic data capture
FDA	Food and Drug Administration
FSH	follicle stimulating hormone

GCP	Good Clinical Practice
GEJ	Gastroesophageal Junction
GLP	Good Laboratory Practice
GM-CSF	granulocyte-macrophage colony stimulating factor
HBV	hepatitis B virus
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HED	human equivalent dose
HEK	human embryonic kidney (cell)
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
hTERT	human telomerase reverse transcriptase
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IL	interleukin
IM	intramuscular
IND	Investigational New Drug
INR	international normalized ratio
IP	investigational product
irCR	immune-related complete response
irPD	immune related progressive disease
irPR	immune-related partial response
irRC	immune-related response criteria
irSD	immune-related stable disease
IRB	institutional review board

IRES	internal ribosomal entry site
ITT	intent-to-treat
IV	intravenous
KPS	Karnofsky Performance Status Scale
LD	longest diameter
LDH	lactate dehydrogenase
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MFD	maximum feasible dose
MRI	magnetic resonance imaging
mRNA	messenger RNA
MTD	maximum tolerated dose
NCI	National Cancer Institute
NOAEL	no adverse effect level
NOEL	no effect level
NTL	non-target lesion
ORR	objective response rate
OS	overall survival
PD	progressive disease
PD-L1	Programmed death-ligand 1
PFS	progression free survival
PI	Principal Investigator
PP	per-protocol
PR	partial response
PTT	prothrombin time
qPCR	quantitative polymerase chain reaction

RECIST	response evaluation criteria in solid tumors
iRECIST	Immune response evaluation criteria in solid tumors
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
SD	stable disease
SOP	standard operating procedure
SUSAR	suspected unexpected adverse reaction
TIL	tumor infiltrating lymphocytes
TL	target lesion
TNF	tumor necrosis factor
TNM	tumor; lymph nodes; metastasis: classification of malignant tumors
T_{reg}	regulatory T cell
TTR	time to treatment response
T-VEC	talimogene laherparepvec
US	United States
USP	United States Pharmacopoeia
ULN	upper limit of normal
VP	viral particles
WCM	Weill Cornell Medicine
WCMC	Weill Cornell Medical College
WHO-DD	World Health Organisation Drug Dictionary
XTT	cell viability assay

STUDY SYNOPSIS

Protocol Title:	Phase II study of Telomelysin (OBP-301) in combination with Pembrolizumab in Esophagogastric Adenocarcinoma
Protocol Number:	1807019403
Study Sites:	1. Weill Cornell Medical College 2. Dana Farber Cancer Institute 3. University of Pennsylvania
Study Phase:	Phase II
Study Objective(s):	**Primary Objective:** • To examine the efficacy of OBP-301 with pembrolizumab in PD-L1 positive advanced gastric and gastroesophageal junction adenocarcinoma in the 3rd or 4th line setting, as assessed by the RECIST response rate. • To examine the safety of multiple OBP-301 intratumoral injections in combination with pembrolizumab in advanced gastroesophageal adenocarcinoma. **Secondary Objectives:** • To examine other measures of efficacy of the combination of OBP-301 with pembrolizumab in advanced gastric and esophageal adenocarcinoma including the disease control rate, duration of response, overall survival and progression free survival. **Exploratory Objectives:** • To examine the tumor-immune microenvironment prior to and following OBP-301 and pembrolizumab therapy as assessed by bulk RNA sequencing and single-cell RNA sequencing. • To examine and characterize the immune infiltrate by multi-parameter flow cytometry.

Study Design:	This is a phase II study of OBP-301 with pembrolizumab in advanced gastric and gastroesophageal junction adenocarcinoma that has progressed on at least 2 lines of prior therapy for advanced disease. This study will examine the addition of the oncolytic virus, OBP-301, administered prior to pembrolizumab in this patient population. Patients will be enrolled in a two-stage design, with 18 patients in the first stage. All patients will receive OBP-301 at $2x10^{12}$ viral particles (VP)/ tumor injection administered every two weeks x 4 injections as well as standard dose pembrolizumab 200 mg IV every 3 weeks. The tumor will be injected with OBP-301 four times (d1, d15, d29, d43). The preference is to inject the primary tumor endoscopically. Metastatic lesions may be injected on a case-by-case basis after discussion with the PI (Shah). All patients treated with OBP-301 will be eligible for the safety cohort.
Investigational Product Administration:	OBP-301, the investigational product (IP) is formulated in 20 mM Tris pH 8.0, 25 mM NaCl with 2.5% glycerin, USP by volume. OBP-301 will be injected into the target tumor lesions.
Planned Patients:	41 patients in total for both stage 1 and 2, to achieve 37 evaluable patients.
Participant Selection Criteria:	**Inclusion Criteria:** Patients who meet all of the following criteria will be considered for inclusion in the study: 1. Be willing and able to provide written informed consent for the trial. 2. Be ≥18 years of age on the day of signing the informed consent. 3. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2. 4. Have histologically or cytologically confirmed advanced or metastatic gastroesophageal adenocarcinoma, at least 1 cm in size and amenable to intratumoral injection. 5. Patient must have received at least 2 line of systemic therapy for advanced disease. 6. Tumor must be PD-L1 positive, as defined by a combined positive score (CPS). 7. Have one or more measurable lesions based on iRECIST. 8. Be willing to provide tissue; newly obtained biopsy specimens or formalin-fixed, paraffin-embedded (FFPE) block specimens. 9. Female subjects of childbearing potential have a negative urine or serum pregnancy test within 7 days prior to enrollment. If the urine test is positive or cannot be confirmed as negative, a serum

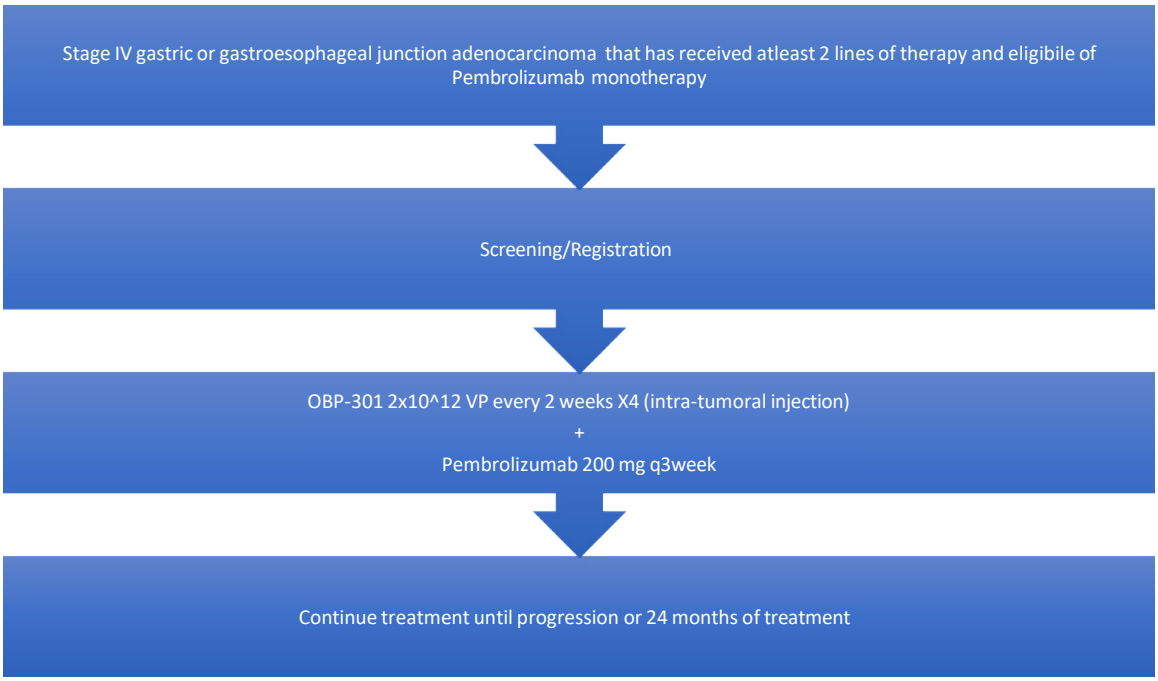
	pregnancy test will be required. If a serum pregnancy test is required it can be performed on the same day as the urine pregnancy test. The serum pregnancy test must also be completed 7 days prior to enrollment. And male / female subjects of childbearing potential must agree to use an adequate method of contraception starting with signing the informed consent through 120 days after the last dose of study medication. 10. Demonstrated adequate organ function as defined in following criteria. All screening labs should be performed within 14 days of enrollment. Note: Subject must not have taken transfusion, hematopoietic agent; granulocyte-colony stimulating factor (G-CSF) etc., and/or oxygen supplementation within 7 days before the screening labs. a. Absolute neutrophil count (ANC)≥1,000 /mm3 b. Platelets≥100,000 /mm3 c. Hemoglobin≥8.0 g/dL d. Serum total bilirubin≤2.0 mg/dL e. Aspartate aminotransferase (AST) (SGOT) and alanine aminotransferase (ALT) (SGPT)≤ 3x Upper limit of normal (ULN). For subjects with liver metastases≤ 5x ULN. f. Serum creatinine≤ 2.0 mg/dL; or if serum creatinine > 2.0 mg/dL, measured or calculated creatinine/clearance ≥45 mL/min (Cockcroft-Gault formula). 11. Life expectancy of ≥ 4 months from the first OBP-301 treatment. 12. Understand the study requirements and the treatment procedures, and is willing to comply with all specified follow-up evaluations, and provides written informed consent before any study-specific tests or procedures are performed. **Exclusion Criteria:** The presence of any of the following criteria will constitute cause for the exclusion of the participant: 1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy within 4 weeks of study Day 1. 2. Has an active autoimmune disease that has required systemic treatment in past 2 years. 3. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy (greater than equivalent of prednisone 20 mg/day) or any other form of immune-suppressive therapy within 7 days prior to study Day 1. 4. Has known active central nervous system metastases and/or carcinomatous meningitis. 5. Has had prior anti-cancer monoclonal antibody chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1, who has not recovered from adverse events due to a previously administered agent. 6. Has a known additional malignancy within 3 years of first injection of OBP-301 that is progressing or requires active

	treatment, with the exception of prostate cancer controlled with androgen deprivation therapy. 7. Has received a live vaccine within 30 days of planned start of study therapy. 8. Patients known to have acute or chronic active hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV). 9. Has any evidence of active, non-infectious pneumonitis or interstitial lung disease requiring steroids. 10. Has an active infection requiring systemic therapy within 2 weeks of Day 1. 11. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial. 12. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of trial treatment. 13. Previous severe hypersensitivity to another monoclonal antibody 14. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator. 15. Uncontrolled intercurrent illness including, but not limited to, uncontrolled diabetes, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/psychological incompetence, whereby in the opinion of the Investigator the patient is assessed as being unable to provide information, consent, or comply with the study requirements and procedures. 16. Patients who are pregnant or breastfeeding, or expecting to conceive or father children within the projected timeframe of the study, starting from the time of the Screening Visit through 4 months (120 days) after the last OBP-301 administration. Females of childbearing potential must have a negative serum or urine pregnancy test at Screening. A female not of childbearing potential is one who has undergone bilateral oophorectomy or who has had no menses for 12 consecutive months.
Study Procedures:	1. OBP-301 $2x10^{12}$ VP / tumor injection every 2 weeks x four injections. * Up to one additional injection may be considered in discussion with the PI (Shah). 2. Pembrolizumab 200 mg intravenous every 3 weeks
Study Endpoints:	**Primary Endpoint:** • To examine the efficacy of OBP-301 with pembrolizumab in PD-L1 positive advanced gastric and gastroesophageal junction adenocarcinoma in the 3rd or 4th line setting, as assessed by the RECIST response rate.

	• To examine the safety of multiple OBP-301 intratumoral injections in combination with pembrolizumab in advanced gastroesophageal adenocarcinoma. **Secondary Endpoints:** • To examine other measures of efficacy of the combination of OBP-301 with pembrolizumab in advanced gastric and esophageal adenocarcinoma including the disease control rate, duration of response, overall survival and progression free survival. **Exploratory Analysis (optional):** Correlative endpoints include the following: • To examine the tumor-immune microenvironment prior to and following OBP-301 and Pembrolizumab therapy as assessed by bulk RNA sequencing and single-cell RNA sequencing. • To examine and characterize the immune infiltrate by multi-parameter flow cytometry.
Statistical Analysis:	The primary endpoint will be the objective response rate (RR), as measured by iRECIST criteria. Sample size recommendations for the phase II design are determined according to Simon's two-stage Minimax design [1]. We project a RR of 15% (historical RR for pembrolizumab alone), below which the combination regimen will be unacceptable, and a RR of 30% (expected RR rate for OBP-301 + pembrolizumab), above which the combination regimen will be considered worthy of further exploration. The null hypothesis that the RR is less than or equal to 15% will be tested against the alternative hypothesis that the RR is greater than or equal to 30%. The sample size computations were performed assuming a 10% level of significance and 80% power. If 2 or fewer patients respond out of the first 18 evaluable patients, the study arm will be terminated and declared to have a negative result. If 3 or more patients respond out of the first 18 evaluable patients, enrollment will be extended to 37 patients. The treatment will be declared effective and worthy of further testing if 9 or more patients respond among the 37 patients entered. This two-stage design yields a ≥ 0.80 probability of a positive result if the true RR is $\geq 30\%$. It yields a ≥ 0.90 probability of a negative result if the true RR is $\leq 15\%$. A 95% confidence interval constructed around the expected RR of 30% can be estimated to be within $\pm$ 14.8% of the observed RR proportion. Assuming 10% are unevaluable/ineligible, we anticipate that a total of 41 patients will be enrolled in the study (assuming the study proceeds to the second stage). *Table 1*. Numbers of observed responders required to accept or reject H_0 at each stage. Under this design, H_0 will not be rejected at Stage 1.

	<table><tr><td></td><td>N</td><td>Accept H_0</td><td>Reject H_0</td></tr><tr><td>Stage 1</td><td>18</td><td>≤ 2</td><td>---</td></tr><tr><td>Stage 2</td><td>37</td><td>≤ 8</td><td>≥ 9</td></tr></table> **Analysis Plan for Endpoints:** *Primary Endpoint:* The primary endpoint is the objective response proportion; a 95% confidence interval will be estimated for the response proportion via binomial proportions. *Secondary endpoints:* Secondary endpoints include the disease control rate (the study will be adequately powered (≥80%) to detect an expected disease control rate of 60% in the combination regimen versus 33% historical control), duration of response, progression-free survival (PFS), and overall survival (OS). Median PFS and OS, including survival curves, will be estimated using Kaplan-Meier methodology. Greenwood's formula will be used to calculate 95% confidence intervals for the Kaplan-Meier estimates. The frequency of subjects experiencing toxicities will be tabulated. Toxicities will be assessed and graded according to CTCAE v. 4.0 terminology. Exact 95% confidence intervals around the toxicity proportions will be calculated to assess the precision of the obtained estimates. All analyses will be performed in SAS Version 9.4 (SAS Institute, Inc., Cary, North Carolina) and STATA Version 15.0 (Stata Corporation, College Station, Texas).
Study Duration:	Approximately 24 months Screening evaluations may be conducted up to 4 weeks before the first anticipated OBP-301 administration. Each patient will be in the study for 96 weeks (i.e 24 months) following the first intratumoral injection of OBP-301.

STUDY SCHEMA



1 BACKGROUND

1.1 Rationale

Despite advances in developing targeted therapies across solid tumor malignancies, progress in cancers of the upper gastrointestinal tract (esophagus and gastric cancers) has been limited. Recent advances in gastroesophageal cancers have included the use of trastuzumab in HER2 positive gastric and esophageal adenocarcinoma and ramicirumab (VEGFR2 Ab inhibitor) either with or without chemotherapy in the 2nd line setting for metastatic disease. Despite these advances, median survival for metastatic disease ~10 months and more than 80% of patients diagnosed with an esophageal or gastric cancer will ultimately die of their disease. However, the advent of immune checkpoint inhibitor therapy has transformed solid tumor oncology. The Programmed Death Ligand-1 (PD-L1) and its receptor (PD-1) are important regulators of T cell activity[2], and their inhibitors have now demonstrated antitumor activity in several malignancies[3, 4] including melanoma[5, 6], lung[7-10], renal cell[11], and bladder cancers[12]. There has also been demonstration of activity of PD-1/ anti-PD-L1 antibody immune checkpoint inhibitors in upper GI malignancies. Activity has been demonstrated with the PDL-1 antibody MEDI14736 in gastroesophageal cancers a modest 25% (eg.4/16) response rate in patients with gastric or esophageal adenocarcinoma[13]. Similarly, nivolumab and pembrolizumab, both PD-1 inhibitors, have also demonstrated modest activity in treating esophageal carcinoma[14, 15]. Although activity is modest, those that respond tend to have durable responses of 12 months or greater with no patients stopping therapy for an adverse event [14] [14]. Our own experience in Keynote-059 in advanced GEJ cancer supported the recent approval of pembrolizumab in the 3rd line setting for gastroesophageal cancers[16] (Figure 1).

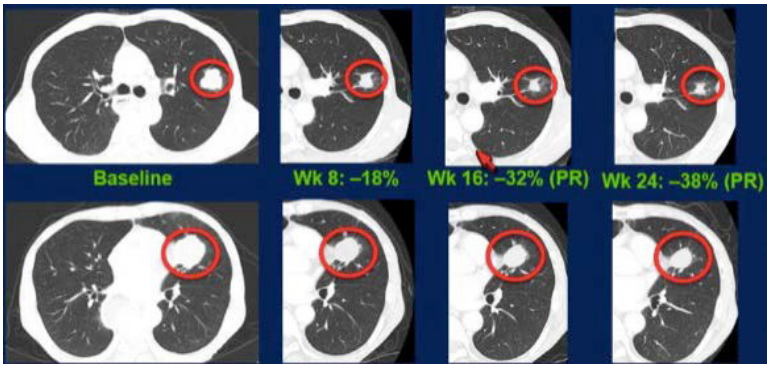


Figure 1. CT images of a patient with metastatic GEJ adenocarcinoma treated with pembrolizumab. Two lesions in the left lung are encircled in red, top and bottom panels. Patient achieved a RECIST partial response.

March 2022 Update

Although Pembrolizumab has demonstrated activity in the 3rd line treatment setting, more recent studies have demonstrated efficacy of immunotherapy in the first line treatment setting for metastatic disease (Keynote-590[16] and Checkmate-649[17]). As a result, immunotherapy is now approved in the first line treatment setting, and the FDA has withdrawn approval of pembrolizumab in the 3rd line treatment setting. It is still available for compassionate use. Oncolys Biopharma has agreed to reimburse pembrolizumab for patients enrolled on this study.

Immunotherapy for gastroesophageal adenocarcinoma has primarily been examined as monotherapy, with response rates ranging from 10-15%, and patient selection only marginally improved by PD-L1 status. A novel concept in immuno-oncology is the use of cancer specific oncolytic viral therapy to induce an immunogenic cell death in the tumor to augment the immune activation driven by PD-1 inhibition. This involves selective replication within neoplastic cells, resulting in a direct lytic effect on tumor cells, and induction of systemic antitumor immunity.[18]

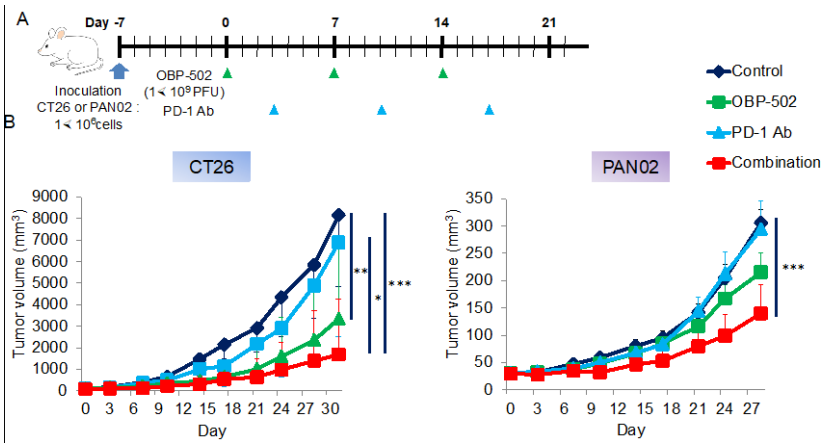


Figure 2. Antitumor activity of the combination of OBP-502 in combination with PD-1 Ab inhibitor. (A) study schema, CT26 and PAN02 are murine colon and pancreatic tumor models. OBP-and PD-1 Ab were administered 3 times as indicated. (B) Tumor growth curves with control, PD-1 Ab, OBP-502, or the combination.

OBP-301 is a telomerase-specific, replication-selective adenovirus in which the human telomerase reverse transcriptase (hTERT) promoter drives viral replication efficiently killing only cancer cells[19-22]. OBP-301 has been examined in a phase I study in solid tumor patients, with an established safe dose of $1x10^{12}$ viral particles/tumor injection. In this study, there was evidence of systemic immune response with the development of a neutralizing Ab response in all tested patients, and up regulation of serum IL-6 and

IL-10 levels in 8/9 and 7/9 patients respectively[23]. Notably, 7/16 patients had stable disease at 56 days, suggesting preliminary efficacy. The most common side effects were local irritation and erythema from the injection (3/16 grade 3), as well as fever, chills, and fatigue (grade 1-2) in 30-40% of patients[23]. There is preclinical evidence that OBP-301 is synergistic with anti-PD-1 therapy (**Fig 2**), providing the rationale for this proposal.

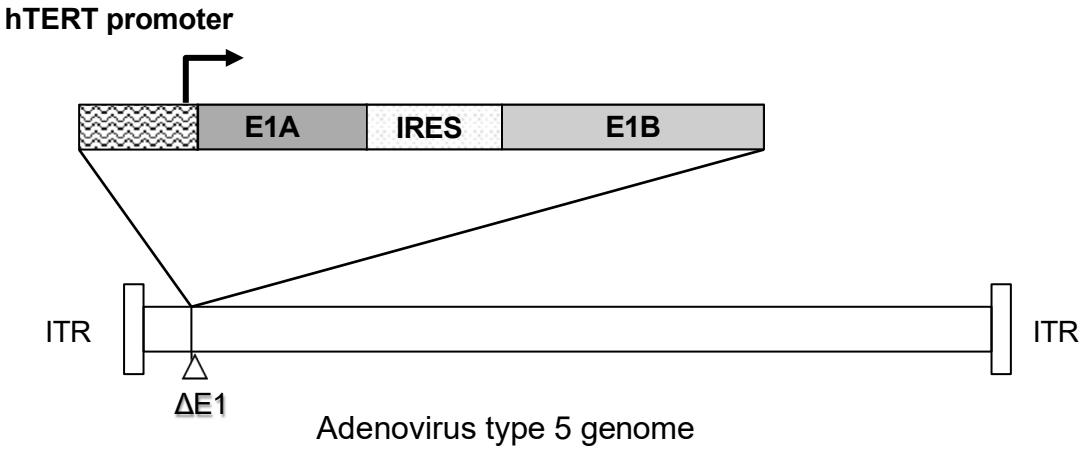
Specifically, in a murine model of pancreas and colon cancer, the combination of PD-1 inhibition with the murine equivalent of OBP-301 (OBP-502), the combination therapy was associated with immunogenic cell death[24]. OBP-502 induced a significant accumulation of tumor infiltrating lymphocytes within the tumors compared with control. The combination of OBP-502 and PD-1 Ab significantly suppressed CT26 colon cancer subcutaneous tumors and successfully eradicated 4/12 (33% of mice. When these 4 tumor-free mice were then re-challenged by inoculation with CT26 colon cancer cells, 2/4 (50%) remained tumor free, indicating continued immune surveillance[24].

The optimal treatment for human cancer requires a therapeutic ratio that increases cytopathic efficacy to target tumor cells, rather than surrounding normal cells. Thus, focus on the genetic and epigenetic targets present only in cancer cells is providing an expanding repertoire of clinically applicable targeted therapeutics, including the use of oncolytic viral vectors.

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 oncolytic virus-based product (Onyx-015 adenovirus), which entered a Phase III clinical trial in the United States (US), and a similar virus, H101, 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* (T-VEC), 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%, $p<0.0001$) [25]. Based on these results, in October 2015, IMLYGIC (*talimogene laherparepvec)* was granted approval as the first oncolytic viral therapy in the US indicated for the Local Treatment of Unresectable Cutaneous, Subcutaneous and Nodal Lesions in Patients With Melanoma Recurrent After Initial Surgery [26]. The European Medicines Agency also recommended authorizing Imlygic for the treatment of adults with unresectable melanoma that is regionally or distantly metastatic (Stage IIIB, IIIC and IVM1a) with no bone, brain, lung or other visceral disease [27].

Conditionally replicative oncolytic viruses are engineered for selective replication in cancer cells that express certain oncogenic phenotypes [28, 29]. To this end, multiple viral backbones have been employed, although the most commonly utilized is derived from the adenovirus serotype 5 (Ad5). OBP-301 is a novel, replication-competent Ad5-based adenoviral construct that incorporates a human telomerase reverse transcriptase gene (hTERT) promoter. The hTERT promoter encodes for 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. 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. 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 [28].



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

Figure 3 The construct of OBP-301

Specificity to cancer is derived by exploiting differential (normal versus tumor) cell surface or intracellular aberrations in gene expression that arise in malignancies. 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 [30, 31] with 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 [32] 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 improved recognition and destruction of tumors by cytotoxic T cells.

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.

Summary of Non-Clinical and Clinical Studies

1.1.1 Non-Clinical Studies

The pre-clinical experience with OBP-301 is described in further detail in the Investigator's Brochure [33]. Non-clinical studies have investigated OBP-301 mechanism of action and clinical proof-of-concept in a) cytotoxicity studies using normal and tumor cells, and b) tumor-bearing xenograft mouse models. The safety of OBP-301 has been characterized in animal models that exhibit tumors (xenograft models) as well as in non-tumor models (normal cotton rats, *Sigmodon hispidus*). The primary safety studies for OBP-301 were conducted in cotton rats, a species selected based on information available in the literature, which suggested that this was a relevant and semi- permissive model for adenoviral safety characterization and reflecting a pre-investigational new drug application (pre-IND) discussion with the Center for Biologics Evaluation and Research (CBER) at the US Food and Drug Administration (FDA).

Due to issues with the generation of homologous recombinants when using an human embryonic kidney cell (HEK)293 cell substrate for OBP-301 manufacture, transfer to a HeLa cell-based production system was implemented after pre-clinical studies had been initiated. The HEK293 cell-based material may have contained wild-type Ad5, which may have contributed to the observed oncolytic activity. Comparability studies undertaken in the rodent toxicology species, consisting of extended product characterization and a comparison of the toxicological effect of the HEK293 and HeLa OBP-301 material, have demonstrated equivalence between OBP-301 virus produced through either production system.

1.1.1.1 Non-clinical Pharmacology

In vitro studies have demonstrated cytopathic activity of OBP-301 on a variety of human tumor cell lines derived from different organs relevant to the clinical setting, as evaluated by a cell viability assay (XTT assay). OBP-301 oncolytic activity in tumor cells was found to be similar compared to another engineered oncolytic virus, and was similar to or slightly weaker than that of wild-type Ad-5 depending on the target tumor cell type. Cytopathic activity of OBP-301 in human normal cell lines was also evaluated, using a variety of methods (XTT assay, plaque assay, Coomassie Blue staining), including assays for E1A mRNA and protein expression, which served as surrogates for viral replication. A correlation between the expression of E1A, hTERT activity and viral replication at the early stage of infection appeared to be present. In normal cells, OBP-301 is less cytotoxic than wild-type Ad5, while in tumor cell lines, OBP-301 showed cytopathic effects equivalent to wild-type Ad5. This is indicative of selective replication in the tumor cells.

In mechanism-of-action studies, there appeared to be a correlation between E1A expression (assessed by immunoblot) and hTERT mRNA expression levels in tumor cells at the early stage of infection. In addition, a correlation between Coxsackie adenovirus receptor (CAR) expression and cytopathic effect of OBP-301 was noted for tumor cells. *In vivo*, the human tumor-bearing xenograft mouse model was utilized in order to define a potentially efficacious dose and regimen.

In the first study, multiple intratumoral injections of OBP-301 E3 (-) and E3 (+) both resulted in equivalent inhibition of tumor growth. A second study demonstrated OBP-301 replication in non-injected distant tumors, as evaluated by RT-PCR for E1A mRNA and immunohistochemistry using an anti-E1A antibody, indicating the potential for OBP-301 to reach and then replicate in distally located tumors. The dose of 3.6×10^9 viral particles (VP)/animal intratumoral injection in the xenograft model achieved the anticipated anti-tumor effect, and no drug-related death has been observed.

1.1.1.2 Toxicology

In total, 4 single dosing toxicological studies with OBP-301, including 2 single non-Good Laboratory Practice (GLP) study and 2 GLP studies, have been undertaken. Most of the data gathered from non-GLP and GLP studies assessed the route in animals most similar to human intratumoral dosing, being the intramuscular (IM) route. Toxicity evaluation following IM injection of OBP-301 in rats did not show a formal no effect level (NOEL). However, the low dose group, 10^9 VP/animal (approximately 10^{10} VP/kg) showed only sporadic, minimal-to-mild, transient microscopic changes in the liver on Day 28, and these changes were no longer apparent by day 85 after treatment. Therefore, this dose was designated to be the No Adverse Effect Level (NOAEL). A higher IM dose, 10^{11} VP/kg (dose group 3 in FXT00014) as well as the highest dose, showed minimal-to-mild sporadic hepatocellular damage on histopathological evaluation. No animals experienced morbid changes, weight loss, or deaths when 10^{11} VP/animal (approximately 10^{12} VP/kg) was administered via either the intravenous (IV) or IM route.

In the pilot repeated dose study, rats (15 males and 15 females) were assigned into 3 groups, Group 1: Control group, Group 2: 7 days repeated dosing and Group 3: 14 days repeated dosing. Both groups 2 and 3 had a 7-day Follow-Up Period following the dosing phase. The results of this study demonstrated that repeat dosing of IM administered OBP-301 to cotton rats at 1×10^{11} VP/animal for 7 or 14 day was well tolerated. No significant differences were observed in animals dosed for either 7 or 14 days.

In the single dose toxicity study of intra-hepatic injection, histopathologic findings were observed including adhesion, fibrinous inflammation, granulomatous inflammation, and necrosis in the liver on Day 5 and adhesion and fibrosis in the liver on Day 14 in both genders at 1×10^{11} VP/kg. However, no mortality, or observations of clinical signs, body weight and food consumption changes were reported. Systemic distribution of virus away from the injection site was noted in these studies with quantitative PCR (qPCR) data at 10^{11} VP/animal indicating the presence of viral DNA in the liver (and to a lesser extent in the lung) out to and including Day 85. These data showed continued presence of viral DNA in normal liver, which may signify that the toxicity on histopathologic evaluation in the liver was test article related (highest dose only, other dose groups were not evaluated for persistence). However, the continued presence of viral DNA in the highest dose group did not result in accrual of toxicity with lesions identified on Day 85, particularly the liver lesions noted above, no more severe than those observed at earlier time points. In addition, the pattern of persistence was consistent with results from other replication-competent oncolytic adenoviruses (see December 2005 RAC Meeting Documents Tab 2397, Protocol Number 0510-732: A Phase I/II Dose Escalation Trial of Intratumoral Injection with Oncolytic Adenoviral Vector INGN 007 (VRX-007) in Patients with Advanced Solid Tumours). IM compared to IV administration was shown to limit toxicity, and all toxicities were reflected in the biomarker surrogates of clinical pathology.

Dosing in human patients will utilize a conservative dose extrapolation paradigm and offers an adequate safety margin for systemic toxicity. The human equivalent dose (HED) is calculated on a body weight-adjusted basis (to 10^{10} VP/kg × 70 kg, or 7×10^{11} VP; the HED). The highest proposed clinical dose of 1×10^{12} VP (equivalent to ~1.4×10^{10} VP/kg) is equivalent to the NOAEL dose in the animal studies.

1.1.2 Clinical Studies

Clinical trials of OBP-301 administered by intratumoral injection have been conducted in patients with advanced cancer under the following protocols:

Completed studies

- OBP-301-001: an open-label, dose-escalation study. The first human study evaluating sequential doses of a single (1×10^{10} to 1×10^{12} VP/tumor) and repeated (1×10^{12} VP/tumor) intratumoral injection of OBP-301 in patients with advanced solid carcinoma. The first patient was enrolled on 30 October 2006 and the last patient completed follow-up on 24 October 2011. Twenty-two patients (n = 13 female, n = 9 male) were treated and analysed. Patients with the following tumor types participated in the Phase 1 trial: 5 patients with melanoma, 4 with squamous cell carcinoma of the head and neck, 3 with breast cancer, 2 with soft-tissue sarcoma, and 1 each with squamous cell carcinoma primary unknown, carcinoma of the salivary gland, non-small cell lung cancer, neuroendocrine tumor, basal cell carcinoma, carcinoma of the gall bladder, pancreatic carcinoma, and cancer of the vulva. Of these, 3 patients (1 patient with carcinoma of the breast and the 2 patients with carcinoma of the gall bladder and pancreatic carcinoma, respectively) had visceral administration of OBP-301, all into the liver. The study was conducted under the current IND for OBP-301.

  OBP-301 was found to be well tolerated in this Phase I study. No dose-limiting toxicities (DLTs) were experienced by any of the 22 patients treated with OBP-301 in this study; therefore, the maximum tolerated dose (MTD) could not be determined. Analysis of all safety data from this study demonstrated an acceptable safety profile with intratumoral injection of OBP-301 with types of events reported that were similar to those reported in patients receiving other adenovirus vectors. Signals of efficacy were seen even in patients treated with a single intratumoral injection. Most patients had stable disease after treatment (15/17); 2 patients reported progressive disease on Day 28. One additional patient who had stable disease on Day 28 reported progressive disease on Day 56. In patients followed for up to 5 years after study participation, there was no signal of a safety concern related to OBP-301 treatment.

  Retrospective analyses:
  - Biopsy samples were obtained from Case C-S, a 60-year-old black male patient enrolled in the Phase I study, who had a diagnosis of squamous cell carcinoma in mouth (Stage IVa). OBP-301 was injected in the tumor in submandibular area. Tumor shrinkage was observed within 7 weeks after injection. Biopsy samples were obtained from the injected site on Day 28 after injection and evaluated retrospectively for histopathological and immunological responses. Results demonstrated tumor cell degradation accompanied by increased numbers of antigen presenting cells (APC), CD8+ T cells, and a decreased regulatory T cell (T_{reg})

population, suggesting treatment-induced oncolysis and immunological activation. In this patient, the anti-tumor effect of OBP-301 appeared to be mediated by direct cytopathic activity following immunological activation.

- Five patients with melanoma were extracted from the total population of patients enrolled in the Phase I study for a retrospective analysis. Changes in tumor size in OBP-301 injected and non-injected lesions, and survival duration were calculated. As for the main study, the effect of treatment on tumor reduction was observed in distant non-injected lesions as well as injected lesions in patients with advanced melanoma. Furthermore, the median survival time was 31.3 months in 5 patients with melanoma [34]. In other reports, the median overall survival of patients diagnosed with unresectable melanoma in Stage IV M1a, M1b, and M1c, are 22.3 months, 11.2 months and 5.1 months, respectively [35]. Survival in patients with other types of tumor who had been treated in the Phase I study was 8.0 months. The results of this retrospective analysis in a subpopulation of patients were suggestive of a positive anti-tumor effect and extended survival in patients with melanoma treated with OBP-301 compared to other types of tumor and other therapies [34].

Ongoing studies

- CT-OT-21 (OBP-301-002-B-H): a Phase I/II Study to Evaluate the Safety and Efficacy of OBP-301 at doses of 1×10^{10} VP/tumor and 1×10^{12} VP/tumor in patients with hepatocellular carcinoma. The study aims to recruit a maximum of 36 patients from 2 clinical trial sites in two countries, Taiwan and Korea. A maximum of 36 patients with hepatocellular carcinoma meeting all inclusion criteria and none of the exclusion criteria will be recruited in the trial. For dosing cohorts of at least 3 patients each are planned to be sequentially accrued until study agent related DLT is met in equal or more than 2 patients at whichever dose level. Recruitment to this study commenced in November 2014.
- In this study, the safety of more than 2 mL has been investigated. In single dose cohorts, 3mL of 1x1012 VP/mL (3x1012 VP) was administered in 3 subjects and no DLT was observed.
- OBP-301-003-C-V: a Phase I/II dose-escalation study to determine the safety and efficacy of OBP-301 at doses of 1×10^{10} VP/tumor and 1×10^{12} VP/tumor in patients with head and neck, esophagus and lung cancers with radiation therapy is being conducted in Japan under Ministry of Health, Labour and Welfare (MHLW) approval (granted on 13 July 2012). The study was initiated on 29 November 2013 and aims to recruit a maximum of 24 patients from Okayama University Hospital in Japan. The primary objectives are assess the clinical safety of OBP-301 in combination with radiotherapy in patients with head and neck, esophagus, and lung cancers; to determine the MTD/maximum feasible dose (MFD) of OBP-301 and to assess the DLT of OBP-301. Secondary objectives for this study include determination of the tumor response rate of OBP-301 and the duration of response and overall survival.
    - In OBP-301-003-CV (OBP-301 + Radiation phase I study, NCT0321305), the safety was monitored when OBP-301 were administered in the dosing levels of; 1 mL of $1x10^{10}$ VP/mL(Level 1), I mL of $1x10^{11}$ VP/mL(Level 2), and 1mL of $1x10^{12}$ VP/mL (Level 3), 3 times of the administration, with the dose escalation study design. The dose difference between the level was 10 times and 100 times of OBP-301 compared to Level 1 was tested in the Level 3, resulted in no DLT and no any intolerable adverse events.

  - Thus far, no dose limiting toxicities have been observed. Specifically in esophageal cancer, 13 patients have been enrolled and have received OBP-301 with radiaiton, with no dose limiting adverse events.
- TL03001: a Phase IIa Study to Evaluate the Efficacy, Safety, and Immunological Response of OBP-301, Telomerase Specific Replication-competent Oncolytic Adenovirus in Patients with Unresectable Metastatic Melanoma. This study aims to recruit a maximum of 50 patients in the US. Recruitment to this study commenced in July 2017.

- TL04001: a phase 1 study to evaluate the safety of OBP-301, OBP-301 in combination with radiation therapy in patients with esophageal cancer not applicable for standard therapy. The study aims to recruit a maximum of 12 patients in two sites in Japan. Recruitment to this study commenced in July 2017.
  - In addition, in TL04001, the cohort 2 (1mL of 1x1012 VP/mL, 3 times biweekly) is ongoing and no DLT was observed so far.

- EPOC1505: a phase I study to evaluate the safety and potential efficacy of OBP-301 in combination with Pembrolizumab in patients with advanced or metastatic solid tumors. This study aims to recruit a maximum of 28 patients in two sites in Japan. Recruitment to this study commenced in December 2017.

1.2 Summary of Potential Risks and Benefits

The side effects listed below are expected based on those seen with the same type of adenovirus-based oncolytic agents in human trials and are anticipated from non-clinical toxicological studies of OBP-301 treatment:

Transient fever	Chills
Injection site pain	Injection site inflammation
Flu-like symptoms	Fatigue
Nausea	Vomiting
Headache	Lymphopenia
Myalgia	Neutropenia
Pneumonia	Leukopenia
Transaminitis	

In the Phase I study conducted in patients with advanced cancers (OBP-301-001), intratumoral injection with OBP-301 at dose of 1×10^{10} VP/tumor and 1×10^{12} VP/tumor was well tolerated with no DLTs reported [29]. Five patients reported 9 serious adverse events (SAEs) of dysphagia and difficulty breathing, syncope, intractable pain, ECG T-wave abnormality, abdominal pain, ascites, hypotension, chills and pyrexia. Although only patients in the 2 highest dose cohorts reported SAEs, none of the events occurred in more than one patient. SAEs of chills and pyrexia were considered related to drug treatment whereas the causality of all other SAEs was assigned to tumor progression, prior medical conditions, and/or dehydration. Decreases in lymphocytes were noted at the time of treatment, but these changes were transient and returned to baseline levels by no later than Day 7. The changes were not considered to reflect drug toxicity. No consistent changes were observed in the hematological or biochemical laboratory parameters and there was

no indication of hepatic, renal or bone marrow toxicity. In summary, analysis of available safety data from this Phase I study demonstrated an acceptable safety profile for intratumoral injection of OBP-301, which exhibited similar events to those reported in patients receiving comparable adenovirus vectors.

There are no known direct benefits to the participants in this study.

1.3 Dosage and Treatment Periods

The OBP-301 NOAEL in a GLP rodent species toxicology study was 10^9 VP/animal (approximately 10^{10} VP/kg). Higher doses (>10^{11} VP/kg), administered IM, showed minimal-to-mild sporadic hepatocellular damage on histopathological evaluation but no animals experienced morbid changes, weight loss, or deaths when 10^{11} VP/animal (approximately 10^{12}VP/kg) was administered either via the intravenous (IV) or IM route. Furthermore, administration of 3.6×10^9 VP via intratumoral injection in a pre-clinical xenograft model achieved demonstrated oncolytic activity. The HED is calculated on a body weight-adjusted basis (to 10^{10} VP/kg × 70 kg, or 7×10^{11} VP; the HED) with the highest proposed clinical dose in this study, 1×10^{12} VP (equivalent to ~1.4×10^{10} VP/kg), equivalent to the NOAEL dose in the animal studies. In the Phase I study in patients with advanced solid carcinoma, the starting dose was a single intratumoral injection of 1×10^{10} VP (1.4×10^8 VP/kg) per tumor. This was administered to an initial 3 cohorts (n = 3 each) and escalated to a maximum of 5×10^{12} VP/tumor. As DLT was not observed with single injections of the highest dose, a repeat dose ($5 \times 1 \times 10^{12}$ VP), was administered. No DLTs were reported in this patient population with the dosing regimen described.

1.4 Patient Population

Patients with advanced, metastatic gastric and gastroesophageal junction adenocarcinoma that has received at least 2 lines of therapy for advanced/metastatic disease. Patients must be PD-L1 positive, as defined as CPS ≥ 1, and have measurable disease for the primary study endpoint of overall response rate by iRECIST.

2 OBJECTIVES

2.1 Primary Objective

- To examine the efficacy of OBP-301 with pembrolizumab in PD-L1 positive advanced gastric and gastroesophageal junction adenocarcinoma in the 3rd or 4th line setting, as assessed by the iRECIST response rate.

- To examine the safety of multiple OBP-301 intratumoral injections in combination with pembrolizumab in advanced gastric and gastroesophageal junction adenocarcinoma.

Secondary Objectives

The secondary objectives of the study are:

- To examine other measures of efficacy of the combination of OBP-301 with pembrolizumab in advanced gastric and gastroesophageal junction adenocarcinoma including the disease control rate, duration of response, overall survival and progression free survival.

2.2 Exploratory Objective

- To examine the tumor-immune microenvironment prior to and following OBP-301 and pembrolizumab therapy as assessed by bulk RNA sequencing and single-cell RNA sequencing.
- To examine and characterize the immune infiltrate by multi-parameter flow cytometry.

3 STUDY DESIGN

3.1 Study Overview

This is a phase II study of OBP-301 with pembrolizumab in advanced gastric and GEJ adenocarcinoma that has progressed on at least 2 lines of prior therapy for advanced disease. This study will examine the addition of OBP-301 with pembrolizumab in this exact patient population.

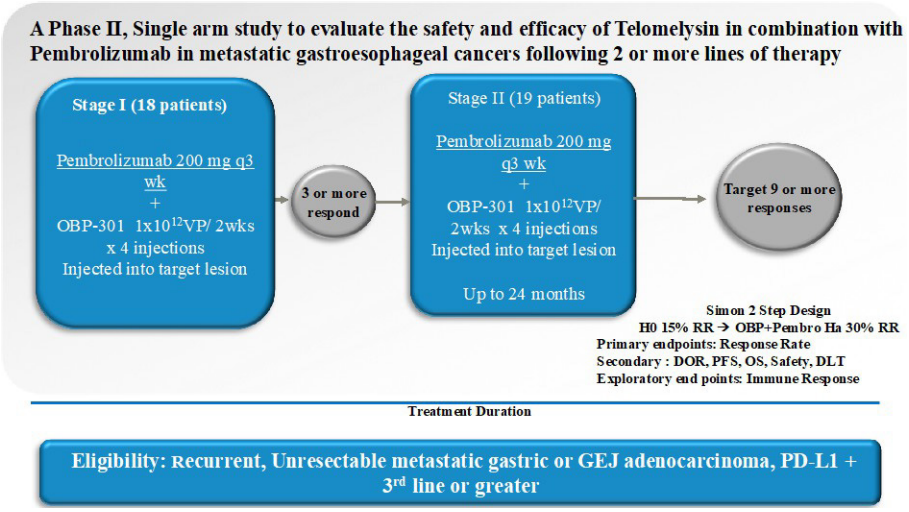


Figure 4. Study Schema. Patients will be enrolled in the study in two stages. The first stage will be 18 patients. If 3 or more patients respond to the combination therapy, the study will move forward to stage 2, with 19 more patients enrolled. In the overall study population of 37 evaluable patients, 9 or more patients with an objective response would indicate a positive study.

The study schema is provided in **Fig 4.** Patients will be enrolled in a two stage design, with 18 patients in the first stage. If 3 or more patients respond to the combination therapy, the study will move forward to stage 2, with 19 more patients enrolled. Patients will receive $1x10^{12}$ VP/ tumor injection with Pembrolizumab (200 mg IV every 3 weeks). The tumor will be injected with OBP-301 four times (d1, d15, d29, d43). The preference is to inject the primary tumor endoscopically. Metastatic lesions may be injected on a case-by-case basis after discussion with the PI (Shah). All patients treated with OBP-301 will be eligible for the safety cohort.

4 STUDY POPULATION

4.1 Selection of Study Population

Only those patients will be included in the study that meet inclusion and exclusion criteria as outlined below.

4.2 Inclusion Criteria

Patients who meet all of the following criteria will be considered for inclusion in the study:

1. Be willing and able to provide written informed consent for the trial.
2. Be ≥18 years of age on the day of signing the informed consent.
3. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2.
4. Have histologically or cytologically confirmed advanced or metastatic gastroesophageal adenocarcinoma amenable to intratumoral injection (i.e. at least 1 cm in size).
5. Tumor must be PD-L1 positive, as defined by a combined positive score (CPS).
6. Patient must have received at least 2 lines of systemic therapy for advanced disease
7. Have one or more measurable lesions based on RECIST v1.1.
8. Be willing to provide tissue; newly obtained biopsy specimens or formalin-fixed, paraffin-embedded (FFPE) block specimens.
9. Female subjects of childbearing potential have a negative urine or serum pregnancy test within 7 days prior to enrollment. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. It is allowed that the test at the same day at 7 days prior to enrollment. And male / female subjects of childbearing potential must agree to use an adequate method of contraception starting with signing the informed consent through 120 days after the last dose of study medication.
10. Demonstrated adequate organ function as defined in following criteria. All screening labs should be performed within 14 days of enrollment. Note: Subject must not have taken transfusion, hematopoietic agent; granulocyte-colony stimulating factor (G-CSF) etc., and/or oxygen supplementation within 7 days before the screening labs.

a. Absolute neutrophil count (ANC) ≥1,000 /mm3

b. Platelets ≥100,000 /mm3

c. Hemoglobin ≥8.0 g/dL

d. Serum total bilirubin ≤2.0 mg/dL

e. Aspartate aminotransferase (AST) (SGOT) and alanine aminotransferase (ALT) (SGPT)≤3x Upper limit of normal (ULN). For subjects with liver metastases ≤5x ULN.

f. Serum creatinine≤ 2.0 mg/dL; or if serum creatinine > 2.0 mg/dL, measured or calculated creatinine/clearance ≥45 mL/min (Cockcroft-Gault formula).

11. Life expectancy of ≥ 4 months from the first OBP-301 treatment.

12. Understand the study requirements and the treatment procedures, and is willing to comply with all specified follow-up evaluations, and provides written informed consent before any study-specific tests or procedures are performed.

4.3 Exclusion Criteria

The presence of any of the following criteria will constitute cause for the exclusion of the participant:

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy within 4 weeks of study Day 1.
2. Has an active autoimmune disease that has required systemic treatment in past 2 years.
3. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy (greater than equivalent of 20 mg/day) or any other form of immunosuppressive therapy within 7 days prior to study Day 1.
4. Has known active central nervous system metastases and/or carcinomatous meningitis.
5. Has had prior anti-cancer monoclonal antibody chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1, who has not recovered from adverse events due to a previously administered agent.
6. Has a known additional malignancy within 3 years before the first OBP-301 administration that is progressing or requires active treatment, with the exception of prostate cancer controlled with androgen deprivation therapy.
7. Has received a live vaccine within 30 days of planned start of study therapy.
8. Patients known to have acute or chronic active hepatitis B virus (HBV), hepatitis C virus (HCV) or human immunodeficiency virus (HIV).
9. Has any evidence of active, non-infectious pneumonitis or interstitial lung disease requiring steroids.
10. Has an active infection requiring systemic therapy within 2 weeks of Day 1.
11. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
12. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit through 120 days after the last dose of trial treatment.
13. Previous severe hypersensitivity to another monoclonal antibody
14. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.
15. Uncontrolled intercurrent illness including, but not limited to, uncontrolled diabetes, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/psychological incompetence, whereby in the opinion of the Investigator the patient is assessed as being unable to provide information, consent, or comply with the study requirements and procedures.
16. Patients who are pregnant or breastfeeding, or expecting to conceive or father children within the projected timeframe of the study, starting from the time of the Screening Visit through 4 months (120 days) after the last OBP-301 administration. Females of childbearing potential must have a negative serum or urine pregnancy test at screening. A female not of childbearing

potential is one who has undergone bilateral oophorectomy or who has had no menses for 12 consecutive months.

4.4 Registration Procedures

4.4.1 Patient Registration – Part 1 (WCMC Only)

Patients will be centrally registered with the Office of Billing Compliance. To register a patient, submit the following documents via the JIRA Registration Process:

- Legible copy of the HRBAF
- First and last page of signed informed consent form

Registration must be completed within 24 hours of the signing of informed consent.

4.4.2 Patient Registration – Part 2 (All Sites)

Study participants will be centrally registered Monday through Friday from 9:00am to 4:00pm with the Weill Cornell Medicine Joint Clinical Trials Office (JCTO). To register a new study subject, email the following documents to JCTOIIT@med.cornell.edu:

- Completed WCM subject registration form
- First and last page of the fully executed informed consent form, plus additional pages if checkboxes for correlative studies are required
- Fully executed HIPAA research authorization form
- Eligibility checklist signed and dated by investigator and research nurse
- Documentation of any eligibility waivers granted
- Redacted source documentation to verify eligibility

Note that attachments larger than 4.5 MB are not accepted, so larger attachments should be split into more than one email. Central registration information is reviewed and entered into the REDCap database.

4.5 Stopping Criteria

4.5.1 Patient Withdrawal Criteria

In accordance with applicable regulations, a participant has the right to withdraw from the study, at any time and for any reason, without prejudice to his future medical care.

Withdrawal from the study and therapeutic measures taken shall be at the discretion of the Investigator. A full explanation for the discontinuation from the study should be made in the participant's medical records and in the eCRF.

Premature discontinuation from active study participation may occur at any time due to any of the following reasons:

- Any situation where, in the opinion of the Investigator or the Medical Monitor, continued participation in the study would not be in the best interest of the patient.
- Positive pregnancy test (see Section for procedures to be followed in case of a pregnancy).
- Patient initiates other anti-cancer therapy. The anti-cancer therapy will be documented in the medical record and eCRF.
- Patient is unable or unwilling to comply with study procedures.
- Patient is lost to follow-up.
- Patient withdraws consent.
- Request by a Health Authority.
- Termination of the study by the study investigator(s).
- Progressive neoplastic disease, unless the Investigator feels this may be pseudo-progression, in which case the patient may remain on study until the next evaluation for extent of disease.

The reason(s) for premature study discontinuation/withdrawal should be reported in the eCRF and within the source documents along with the date of withdrawal. AEs should be documented according to Section 7.0 Adverse Events.

For all patients who discontinue, a Final Visit will be performed within 28 ± 5 days after the last IP administration. If a participant is withdrawn because of an AE, the Investigator must arrange for the participant to have appropriate follow-up care until the AE is resolved or has stabilized. Unresolved AEs will be followed until the last scheduled follow-up visit or until the Investigator determines that further follow-up is no longer indicated. Investigators should attempt to collect the survival information as dictated in section 6.1.3.

Patients who discontinue the active study participation will not be replaced.

Any enrolled patients who are discontinued before study dosing will be documented as screen failures in the eCRF and replaced. No follow-up is required for these patients.

4.5.2 Termination or Suspension of the Study

Investigator and the IEC/IRB reserve the right to terminate or suspend the study at any time for medical, safety, regulatory, administrative, or other reasons consistent with applicable laws, regulations, and Good Clinical Practice (GCP); however, this should be discussed between the relevant parties beforehand and the reason for such decision recorded. Should this occur, all data available will also be recorded in the eCRFs. The Investigator should notify the IEC/IRB in writing of the study's completion or early discontinuation.

We have included adverse events stopping rule (see Section 8.7.9).

In the event that the study is terminated early, the Investigator should make every effort to follow AEs and SAEs for all patients until the end of the defined follow-up period resolution, until the condition stabilizes, until the event is in the opinion of the Investigator stabilized or determined to be chronic, or until the participant dies or is lost to follow-up. SAEs that occur after the Final Visit, that are thought to be related to the administration of OBP-301, should be reported to Oncolys BioPharma Inc. or designee.

5 STUDY MEDICATION

5.1 Investigational Product (IP) Identification

The OBP-301 clinical study material and product is generated according to current Good Manufacturing Practice (cGMP). Table 1 shows an overview of OBP-301. OBP-301 will be provided by Oncolys BioPharma Inc.

Table 1 Investigational Product Information

Investigational product code:	OBP-301
Content:	2 mL/vial (1×10^{12} VP/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

5.2 Dosage and Treatment

1. OBP-301 $2x10^{12}$ viral particles/ tumor injection every 2 weeks x 4 injection encounters.

   * Up to 1 additional tumor injection encounter may be considered in discussion with the PI (Dr. Shah) which may be given 2 weeks after the 4th injection of OBP-301.

   * OBP-301 will be administered as a fixed dose to the tumor. The target dose of OBP-301 injection will be $2x10^{12}$ VP (i.e. 1 vial), and a minimum of $1x10^{12}$ VP (i.e. 1 ml). Specifically, inject as much OBP-301 as possible, but within 1 - 2 mL. The maximal injection volume of 2.0 mL is preferred ($2 x10^{12}$ VP per lesion) and should be injected as long as this is technically possible. At least 1 mL OBP-301 should be injected to the same location as the 1st and 2nd injection even though the primary tumor mass may have decreased and may be hard to locate.

   * OBP-301 will be administered to one lesion. If the gastroesophageal tumor is multifocal, only the largest lesion will be targeted.

   * For patients with tumors not assessable by endoscopy, injection via interventional radiology procedure is permitted, applying the same dosing principles above.

   * Note that it is expected that some amount of material will be used to prime the injection device (i.e. 0.8 mL for a Carre-Locke injection needle). This will be discarded as per standard procedure.

2. Pembrolizumab 200mg intravenous every 3 weeks until disease progression.

Please note that the Pharmacy manual, which is used for all OBP-301 studies globally, also indicates another OBP-301 dose administration option (plan B). This protocol will only use Plan A (i.e. fixed dose administration).

5.2.1 Study Supplies

Pembrolizumab will be supplied by commercial venders.

Oncolys BioPharma Inc. will be responsible for the preparation and labelling of the Investigational Product 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. will be 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. Specific ordering information will be provided to each participating site upon study activation.

5.2.1.1 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 ≤ -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. 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.

5.2.1.2 Accountability and Compliance

The Investigator, pharmacist, or designee, may only dispense clinical supplies in accordance with this protocol.

The Investigator, pharmacist, or designee, is responsible for maintaining an accurate and current record of all clinical supplies received from Oncolys BioPharma Inc., 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 Investigator is also responsible for the education of study staff in the correct administration of the IP.

Additional measures to ensure treatment compliance are not required because the IP will be prepared and administered by study personnel (see Section 5.2.1.3).

5.2.1.3 Study Material Preparation

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 the OBP-301 Pharmacy Brochure Appendix V: Handling Guidelines/Universal Precautions for Biosafety Level 2 Agents. Aseptic technique must be followed at all times during the preparation and transport of the IP to the treatment area. 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.

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

The Investigator is responsible for the education of study staff in the correct administration of the IP and a designated staff member experienced in the administration of investigational agents directly into tumor lesions will administer all treatments.

5.2.1.4 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 Drug Accountability Form.

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.

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 and the monitor will retrieve the original.

Further information of the storage, preparation, and administration of IP will be provided in the OBP-301 Pharmacy Brochure.

5.2.2 Selection and Timing and IP Administration for each Patient

OBP-301 (2×10^{12} VP/vial) is administered as intratumoral injection into a primary gastric or GEJ tumor mass that is suitable for injection, eg greater than 1 cm in size and in a location amenable for endoscopic injection. For patients with tumors not assessible by endoscopy, injection via interventional radiology procedure is permitted, applying the same dosing principles described herein. The largest diameter of the lesion will determine the volume of OBP-301 injected. The rationale for this is to ensure adequate penetration of the viral particals throughout the tumor.

Safety Guidelines for Clinical Study Patients and Caregivers

- Avoid direct contact with body fluids of patients who were injected with OBP-301.
- 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.

5.2.3 Injection Procedures

The optimum technique for injection of a primary tumor is to inject the viral particles circumferentailly around the lesion in 4 quadrants, similar to the technique used in endoscopic submucosal disection (Gotoda T, et al. J Gastroenterol 2006;41:929-942.). This will ensure that the viral partical will be maximally penetrated into the lesion.

Endoscopists who perform single polypectomies will be eligible to perform the procedure. A trianing video (prepared by Oncolys Biopharma) is available as well.

The procedure will be performed in the Endoscopy Unit or interventional suite with standard sterilization procedures. A standard gastroscope and needle (eg. GIF-HQ190 Olympus gastroscope with 25 gauge Carr-Locke injection needel made by US Endoscopy) is used for the procedure. Guidelines for each injection procedure are provided below.

Pre-Injection

- Confirmation of eligiiblity
- 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. OBP-301 may be associated with injection site pain if administered to dermal lesions. However, pain has not been observed for intralumenal injections previously.

5.2.4 Dose Delay and Adjustment

Pembrolizumab

Pembrolizumab is given at a flat dose of 200 mg IV every 3 weeks, per institutional guidelines. There are no dose modifications to this treatment.

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 2 below. See Section 5.2.2.6 and Events of Clinical Interest Guidance Document for supportive care guidelines, including use of corticosteroids.

Table 2. Dose Modification Guidelines for Pembrolizumab -Related Adverse Events (as per package insert).

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject from Study Treatment
Diarrhea/Colitis	2-3	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject from Study Treatment
	4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[1]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold pembrolizumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume pembrolizumab when patients are clinically and metabolically stable.
Hypophysitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted	Therapy with pembrolizumab can be continued while treatment for the thyroid disorder is instituted.
Infusion	3-4	Permanently discontinue	Permanently discontinue

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment	Discontinue Subject from Study Treatment
Reaction			
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	3-4	Permanently discontinue	Permanently discontinue
All Other Drug-Related Toxicity[2]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 12 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[1] For patients with liver metastasis who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[2] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

OBP-301

OBP-301 injection will be delayed for any ongoing Grade 3 toxicity attributable to OBP-301 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 1 toxicity will be discontinued from the study. In this circumstance, the patient will be evaluable for toxicity, but will be replaced (up to the maximum enrollment) for efficacy assessment.

5.2.5 General Concomitant Medication and Supportive Care Guidelines

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Principal Investigator. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician.

Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded.

Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than OBP-301
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describe other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.2.6 Rescue Medications & Supportive Care

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below and in greater detail in the ECI guidance document. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab.

Note: if after the evaluation, the event is determined not to be related, the investigator is instructed to follow the ECI reporting guidance but does not need to follow the treatment guidance (as outlined in the ECI guidance document). Refer to Section 5.2.1 for dose modification.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures, as appropriate, can be found in the ECI guidance document.

- **Pneumonitis**:
  - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
  - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
  - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.
- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).
  - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
  - For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
  - For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)**

- For **T1DM** or **Grade 3-4** Hyperglycemia
  - Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.
  - Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

- **Hypophysitis**:
  - For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
  - For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hyperthyroidism or Hypothyroidism**:

  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
    - **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 3 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab.

Table 3 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	from further trial treatment administration.	
<u>Grades 3 or 4</u> Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

6 STUDY PROCEDURES

Study procedures should be completed as outlined in the Schedule of Events. Section 6.1 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below.

6.1 Schedule of Events

	Screening Phase		Treatment Phase												
Treatment Encounter	Part 1	Part 2	Baseline	1	2	3	4	5	6	7	8	9	10	11	12
Scheduling Window	(-28 to -1)	(-14 to -1)	Day 0[e]	Day 1	Day 4	Day 11-14	Day 15	Day 25	Day 29	Day 39-42	Day 43	Day 46	Week 9	Week 10 onwards[g]	End of Study[h]
Informed Consent	X														
Inclusion/Exclusion Criteria	X														
Demographics and Medical History	X														
Concomitant Medication Review	X		X		X	X		X		X				X[g]	X
Physical Examination		X	X			X		X		X				X[g]	X
Vital Signs and Weight		X	X			X		X		X				X[g]	X
ECOG Performance Status		X	X			X		X		X				X[g]	X
Pregnancy Test – Urine or Serum β-HCG		X[m]													
PT/INR and aPTT		X						X							
CBC & Serum Chemistry[a]		X	X[k]			X		X		X				X[g]	X
Urinalysis, T3, FT4 and TSH		X								X				X[f]	X
ECG		X[i]	As clinically indicated												
Tumor Imaging - CT preferred[b]	X												X[b]		X
Tissue Biopsy[j]	X			X			X		X		X				
Correlative Blood Collection[c]		X						X				X[c]			X
OBP-301[l,n]				X			X		X		X				
Pembrolizumab Administration[d, o]					X			X				X		X[d]	
Review Adverse Events					X	X		X		X		X		X[g]	X

a. Serum chemistry includes sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, glucose, AST, ALT, Alk Phos, lactate dehydrogenase, bilirubin (total and direct), total protein, albumin, calcium, magnesium and phosphorous, may be drawn up to -2 days from schedule. b. Tumor imaging studies preferably CT scans of chest/abdomen/pelvis should be performed every three cycles of Pembrolizumab after week 10 until end of study.

c. Correlative blood collection will be done at screening, day 25, day 46, at week 19 and at the end of study. Correlative blood draws will be done pre-dose if treatment is given on the same day.

d. Pembrolizumab will be administered every 3 weeks ± 3 days.

e. Baseline assessment should be performed within 5 days of administrating first dose of OBP-301.

f. Urinalysis, T3, FT4 and TSH should be performed every 3rd cycle of Pembrolizumab thereafter until end of study.

g. From week 10 and onwards, concomitant medication review, physical exam, vitals/weight, ECOG performance status, CBC, comprehensive serum chemistry and review of adverse events should be performed every 3 weeks until end of study.

h. End of study will occur 28 days (+/- 5 days) following study removal, disease progression, or following 2 years on treatment. Patients may still be followed for survival for up to 96 weeks from study start.

i 12-lead ECG will be recorded after the patient has rested for 5 minutes

j. Tumor biopsy may be needed during screening phase for PD-L1 testing if there is no archival tissue available.

k. Only needed, if screening CBC and chemistry done more than 7 days prior to first cycle of pembrolizumab.

l. OBP-301 will be administered every 2 weeks (-2 business day/ +5 business day window). If the window is used, the following doses should be administered following the same window, ensuring at least 12 days between each OBP-301 administration.

m. Pregnancy test for screening should be done within 7 days of Day 1.

n. A CBC and CMP should be obtained within 10 days of OBP injection, if not already performed.

o. OBP-301 and Pembrolizumab should not be administered on the same day.

6.1.1 Screening Visit

- Informed consent
- Demographics
- Medical history
- Concomitant Medications
- Physical exam
- Vital signs and weight
- ECOG performance status (Appendix A: ECOG Performance Status Criteria)
- Urine or Serum Pregnancy Test
- PT/INR and aPTT
- Complete Blood Count (CBC) with differential
- Serum chemistry
- Urinalysis
- T3, FT4 and TSH
- ECG
- Tumor Imaging CT preferred
- Tumor Biopsy/Archival biopsy (for PD-L1 status)
- Correlative Blood Collection

6.1.2 Treatment Phase

Pembrolizumab infusions will be administered every 3 weeks ± 3 days until disease progression or patient is withdrawn for another reason. OBP-301 is to be administered prior to pembrolizumab in this patient population. All patients will receive OBP-301 at 2 $x10^{12}$ viral particles/ tumor injection administered every two weeks x 4 injection encounters. The tumor will be injected with OBP-301 four times (day 1, day 15, day 29, day 43). However, there may be some exceptions for OBP-301 injections which are outlined below.

A. Up to 1 additional tumor injection encounter may be considered in discussion with the PI (Shah), which may be given 2 weeks after the 4th injection of OBP-301.

B. Patients who initially respond to combination therapy may be rechallenged with OBP-301 at the time of disease progression, after discussion with the principal investigator.

Criteria for the additional single dose of OBP-301 is that the patient has not had disease progression and has had no grade 3 or 4 events related to OBP-301.

6.1.3 Follow up Phase

Patients with disease progression will be followed every 3 months for survival for up to 96 weeks from study start. This assessment can occur by telephone or some other remote monitoring.

6.1.4 Patient Withdrawal Criteria

In accordance with applicable regulations, a participant has the right to withdraw from the study, at any time and for any reason, without prejudice to his future medical care.

Withdrawal from the study and therapeutic measures taken shall be at the discretion of the Investigator. A full explanation for the discontinuation from the study should be made in the participant's medical records and in the eCRF.

Premature discontinuation from active study participation may occur at any time due to any of the following reasons:

- Any situation where, in the opinion of the Investigator or the Medical Monitor, continued participation in the study would not be in the best interest of the patient.
- Positive pregnancy test
- Patient initiates other anti-cancer therapy. The anti-cancer therapy will be documented in the medical record and eCRF.
- Progressive neoplastic disease, unless the Investigator feels this may be pseudo-progression, in which case the patient may remain on study until the next evaluation for extent of disease.
- An intercurrent illness or AE that requires concomitant medication that precludes further study participation
- Patient is unable or unwilling to comply with study procedures.
- Patient is lost to follow-up.
- Patient withdraws consent.
- Termination of the study by the Sponsor.
- Request by a Health Authority.

In addition, the Sponsor will discontinue a patient from active study treatment for any of the following criteria (subjects will continue to be followed as appriopriate for outcomes and toxicities:

- Patients who experience a Grade 4 hematologic toxicity or a Grade 3 or greater non-hematologic toxicity attributable to OBP-301 or the endoscopic procedure that has not resolved to Grade 1 (or basline) within 2 weeks of onset.
- OBP-301 injection will be delayed for any ongoing Grade 2 toxicity at the time of the next scheduled injection after consultation with the Principal Investigator. 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 1 toxicity will be discontinued from the study after consultation with Medical Monitor and Principal Investigator.

After apparent disease progression (PD) is observed, administration of OBP-301 may be continued as per iRECIST. Immune-related disease progression (irPD) will be confirmed if the increase in tumor burden is ≥ 25% relative to nadir (minimum recorded tumor burden). The confirmation is completed by a repeat, consecutive assessment no less than 4 weeks from the date the disease progression was first documented. If in the opinion of the investigator, the disease progression is not clinically significant, then the patient may continue to receive OBP-301 treatment.

The reason(s) for premature study discontinuation/withdrawal will be documented along with the date of withdrawal. AEs should be documented according to the study calendar.

For all patients who discontinue, a Final Visit will be performed within 28 ± 5 days after the last IP administration (see study calendar). If a participant is withdrawn because of an AE, the Investigator must arrange for the participant to have appropriate follow-up care until the AE is

resolved or has stabilized. Unresolved AEs will be followed until the last scheduled follow-up visit or until the Investigator and Medical Monitor determine that further follow-up is no longer indicated. Investigators should attempt to collect the survival information as dictated by the Schedule of Events in the Study calendar.

Patients who discontinue the active study participation will not be replaced.

Any enrolled patients who are discontinued before study dosing will be documented as screen failures in the eCRF and replaced. No follow-up is required for these patients.

6.2 Informed Consent Form

Each patient is required to provide informed consent before participating in the study.

Before performance of any study procedures, potential patients will attend a screening session at which time they will be provided with full information concerning details of the study assessments and procedures. They will also be provided with the ICF. Before being asked to sign the ICF, patients will be given time to review the study information and ask any questions. A copy of the signed ICF must be provided to the patient.

6.3 Demographic Data and Baseline Characteristics

Gender, date of birth, height, and weight, as well as race and ethnic origin will be recorded for each patient and age and body mass index calculated from these data.

6.4 Medical History, Concomitant Disease, Previous and Ongoing Medications

A detailed medical history, including surgical and cancer history, will be recorded and will include any conditions reported by the patient or noted by the Investigator in each body system. Medical history is defined as any condition that started and ended before the Screening Visit. A concomitant disease is defined as any condition detected at the Screening Visit that started after ICF signature or was started before the Screening Visit and is still ongoing at the Screening Visit.

Prior medication taken before the Screening Visit and any concomitant medication will be documented. Prior medication is defined as any medication taken within 4 weeks of screening visit. Concomitant medications are those medications which the patient is currently taking at any particular visit.

Records should include the drug name (preferably both generic and trade name), route of administration (e.g., oral, intravenous), injection and frequency (expressed in metric units, for example, mg, mL, or IU), indication, and the start and, if applicable, the stop date (day, month, and year). Therapy changes (including changes of regimen) during the study have to be documented. All concomitant medications will be recorded in the eCRF.

6.5 Safety Assessments

6.5.1 Adverse Events

Definitions, management of, and special considerations for AEs are provided in Section 7. Adverse events will be collected at each contact with the patient. Questions regarding the presence of adverse events should be open-ended without leading the patient.

6.5.2 Physical Examination

The physical examination is to be conducted by the Investigator or designated fully trained representative. Performance status will also be determined at screening as well as at each subsequent visit as oulined in the section study schedule. Patients will be graded according to the ECOG Performance Status Scale.

Performance Status	Definition
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

6.5.3 Vital Signs

Vital signs will include measurements of heart rate, systolic and diastolic blood pressures while the patient is in a seated position, and temperature.

6.5.4 Electrocardiogram

During screening of patients, a standard baseline 12-lead ECG will be recorded after the patient has rested for 5 minutes. The Investigator or designee will review all ECGs. The following parameters will be documented: rhythm, heart rate, PR interval, QRS interval, QT interval, and QTc. Every ECG must be assessed as normal, abnormal – clinically not significant, or abnormal – clinically significant. A diagnosis must be provided in the eCRF for all ECGs assessed as abnormal – clinically significant. If clinically indicated, additional ECGs may be obtained during the study.

6.5.5 Laboratory Assessments

6.5.5.1 Clinical Chemistry, Hematology, Coagulation Parameters, and Urine Analysis for Safety Panel

Following table illustrates laboratory assessments, which will be performed at prescribed intervals as per schedule of events.

Table 4 Clinical Laboratory Parameters for Safety Panel

Hematology	Coagulation	Serum Chemistry	Urine Analysis
• Hemoglobin • Hematocrit • Red blood cell count • White blood cell (WBC) count • Platelet count • WBC differentials	• International normalized ratio • Prothrombin time	• Sodium • Potassium • Chloride • Bicarbonate • Blood urea nitrogen • Creatinine • Glucose • Alanine transaminase • Aspartate transaminase • Alkaline phosphatase • Lactate dehydrogenase • Bilirubin (total and direct) • Total protein • Albumin • Calcium • Magnesium • Phosphorous	• pH • Specific gravity • Glucose • Ketones • Bilirubin • Hemoglobin • Protein • Microsopic if any dipstick value 2+ or greater

6.5.5.2 Pregnancy Testing and Post-Menopausal Status

A serum or urine pregnancy test will be performed on all women of childbearing potential at Screening.

The post-menopause is defined as the time after which a woman has experienced 12 consecutive months of amenorrhea (lack of menstruation) without a period. If a woman claims she is post-menopausal, but has had her menses within 12 months, a follicle stimulating hormone (FSH) test must be performed and must be within acceptable limits of the post-menopausal status. If the post-menopausal status of a female is confirmed, the pregnancy tests will not be performed.

All pregnancies detected during the study must be reported and handled as described in Section 7.2.

6.6 Efficacy Assessments

6.6.1 Imaging Assessment for the Study

All the patients who enrolled into study will undergo efficacy assessments for overall response rate, disease control rate, progression free survival and overall survival, as defined in Section 8.5. A baseline imaging study preferably CT scan of chest/abdomen/pelvis with IV contrast (permitting no contraindication to contrast) will be performed followed by every nine weeks interval while the patient is on study. Efficacy end points will be assessed according to RECIST 1.1 criteria.

6.6.2 Definitions of Measurable and Non-Measurable Disease

6.6.2.1 Measurable Disease

Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of 10 mm by CT scan (CT scan slice thickness no greater than 5 mm).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

6.6.2.2 Non-Measurable Disease

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with 10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

6.6.3 Response Criteria

All patients will undergo re-evaluation imaging scans every nine weeks from the start of treatment as per the study schedule to determine response to treatment. Following designations will be assigned according to iRECIST. (see appendix below for differences in RECIST 1.1 and iRECIST),

6.6.3.1 Complete Response (iCR)*

Defined as disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

6.6.3.2 Partial Response (iPR)*

At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

6.6.3.3 Progressive Disease (PD)*

At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

Unconfirmed PD (iUPD) requires confirmation, which is done on the basis of observing either a further increase in size (or in the number of new lesions) in the lesion category in which progression was first identified in (ie, target or non-target disease), or progression (defined by RECIST 1.1) in lesion categories that had not previously met RECIST 1.1 progression criteria. However, if progression is not confirmed, but instead tumor shrinkage occurs (compared with baseline), which meets the criteria of iCR, iPR, or iSD, then the bar is reset so that iUPD needs to occur again (compared with nadir values) and then be confirmed (by further growth) at the next assessment for confirmed progressive disease (iCPD) to be assigned. If no change in tumor size or extent from iUPD occurs, then the timepoint response would again be iUPD.

6.6.3.4 Stable Disease (iSD)*

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

* May have had unconfirmed progressive disease (iUPD) (1 or more instances), but not confirmed progressive disease (iCPD), prior to complete response (iCR), partial response (iPR) or stable disease (iSD). “i” indicates immune responses assigned using iRECIST.

6.6.4 Tumor Determination of Stage by Tumor, Lymph Nodes, Metastasis (TNM) Classification

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of CR depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

6.7 Biopsies and Immune Monitoring

All patients undergoing treatment with OBP-301 will have tissue biopsies along with correlative blood samples. Tissue biopsies will be performed with every injection of OBP-301. Correlative blood samples will be taken before the start of study treatment, during treatment and post-treatment as described in the section of study schedule. Blood and biopsy samples will be retained for future research if informed consent has been obtained from the patient.

7 ADVERSE EVENTS

7.1 Definitions

7.1.1 Definition of an Adverse Event

An AE is any event, side effect, or other untoward medical occurrence that occurs in conjunction with the use of a medicinal product in humans, whether or not considered to have

a causal relationship to this treatment. An AE can, therefore, be any unfavorable and unintended sign (that could include a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Events meeting the definition of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after IP administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either IP or a concomitant medication (overdose per se will not be reported as an AE/SAE).

"Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE.

Events that do not meet the definition of an AE include:

- Medical or surgical procedure (e.g., appendectomy). The condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (e.g., social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

If there is evidence of an AE through report or observation, the Investigator or designee will evaluate further and record the following information:

- Date of onset and resolution
- Classification of 'serious' or 'non-serious'
- Severity
- Causality/relation to study treatment
- Action taken regarding IP (no action taken, study treatment interrupted, study treatment discontinued)
- Medication or additional treatment (no medication additional treatment, concomitant medication, non-drug therapy, other)
- Outcome /Status (recovered, recovered with sequelae, recovering, ongoing, fatal unknown)

7.1.2 Severity of an Adverse Event

The intensity of AEs/SAEs will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03 (dated 14 June 2010) [36].

Should an event be missing in the NCI-CTCAE, the following 5-point scale is to be used:

- Mild: Discomfort noticed, but no disruption of normal daily activity
- Moderate: Discomfort sufficient to affect normal daily activity

- Severe: Inability to work or perform normal daily activity
- Life-threatening: Risk of death at the time of the event
- Fatal: The patient died

The correspondence between the 2 scales is as follows:

NCI-CTCAE	**5 point scale**
1	Mild
2	Moderate
3	Severe
4	Life-threatening
5	Fatal/Death

NCI-CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events

7.1.3 Causal Relationship of an Adverse Event

Causal relationship of an adverse event to the IP will be assessed by the Investigator as follows:

Not related: The event is clearly related to other factors such as the participant's clinical state, therapeutic interventions or concomitant drugs administered to the participant. This is especially so when an event occurs before the commencement of treatment with the IPs.

Unlikely: The experience was most likely produced by other factors such as the participant's clinical state, therapeutic interventions or a concomitant drug administered to the participant and does not follow a known response to the trial IP.

Possible: The experience follows a reasonable temporal sequence from the time of IP administration and/or follows a known response to the trial IP, but could have been produced by other factors such as the participant's clinical state or other therapeutic interventions or concomitant drugs administered to the participant.

Probable: The experience follows a reasonable temporal sequence from the time of IP administration and follows a known response to the trial IP, and cannot be reasonably explained by other factors such as the participant's clinical state or other therapeutic interventions, or concomitant drugs administered to the participant.

Definite: The experience follows a reasonable temporal sequence from the time of IP administration and follows a known response to the trial IP, and cannot be reasonably explained by other factors such as the participant's clinical state or other therapeutic interventions or concomitant drugs administered to the patient and in addition one or more of the following:

- Occurs immediately following trial IP administration
- Improves on stopping the trial IP
- Reappears on repeat exposure

7.1.4 Definition of a Serious Adverse Event

An SAE is any untoward medical occurrence regardless of the grade that:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or,
- is a congenital anomaly/birth defect.

Important medical events that may not be one of the above may be considered an SAE by the Investigator when, based upon appropriate medical judgement, are considered clinically significant and may jeopardize the participant, or may require medical or surgical intervention to prevent one of the outcomes listed above.

"Life-threatening" means that the participant was at immediate risk of death from the experience that, had it occurred in a more serious form might have caused death.

A congenital anomaly in an infant born to a mother who was exposed to the IP during pregnancy is considered an SAE. However, a newly diagnosed pregnancy in a patient that has received the IP is not considered an SAE unless it is suspected that the IP interacted with a contraceptive method and led to the pregnancy. Procedures for notification of pregnancies and follow-up are described in Section.

7.1.5 Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events and Serious Adverse Events

Abnormal laboratory findings (e.g., clinical chemistry, hematology, and urinalysis) or other abnormal assessments (e.g., ECG, X-rays, and vital signs) per se are not reported as AEs. However, those abnormal findings that are deemed **clinically significant** or are associated with signs and/or symptoms must be recorded as AEs if they meet the definition of an AE (and recorded as an SAE if they meet the criteria of being serious) as described previously. Clinically significant abnormal laboratory or other abnormal findings that are detected after consent or that are present at the time of consent and worsen after consent are included as AEs (and SAEs if serious).

The Investigator should exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Usually, the abnormality should be associated with a clinically evident sign or symptom, or be likely to result in an evident sign or symptom in the near term, to be considered clinically significant. A clinically significant laboratory abnormality in the absence of clinical symptoms may jeopardize the participant and may require intervention to prevent immediate consequences. For example, a markedly low serum glucose concentration may not be accompanied by coma or convulsions, yet be of a magnitude to require glucose administration to prevent such sequelae.

7.2 Documenting Adverse Events

Any AE occurrence (spontaneously volunteered and enquired for, as well as observed AEs) during the study must be documented in the patient's medical records in accordance with the Investigator's normal clinical practice and on the AE page of the eCRF. SAEs that occur during

the study must be documented in the patient's medical record, on the AE eCRF, and on the SAE form.

The Investigator should attempt to establish a diagnosis of the event based on the signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE (and SAE if serious) and not the individual signs/symptoms.

If a clinically significant abnormal laboratory finding or other abnormal assessment meets the definition of an AE, then the AE eCRF page in the REDCap database must be completed as appropriate. In addition, if the abnormal assessment meets the criteria for being serious, the SAE form must also be completed. A diagnosis, if known, or clinical signs or symptoms if the diagnosis is unknown, rather than the clinically significant laboratory finding or abnormal assessment, should be used to complete the AE/SAE page. If no diagnosis is known and clinical signs or symptoms are not present, then the abnormal finding should be recorded. If an SAE report is completed, pertinent laboratory data should be recorded on the SAE form, with baseline values and copies of laboratory reports.

The SAE page should be completed as thoroughly as possible and signed by the Investigator before transmittal to Oncolys BioPharma Inc at safety@oncolys.com.

It is very important that the Investigator provide an assessment of the causal relationship between the event and the IP at the time of the initial report, as this will be useful for submissions to regulatory authorities.

7.2.1 Recording of Adverse Events

All adverse events will be recorded on a patient specific AE log after W1D1 and entered onto REDCap. The AE log will be maintained by the research staff and kept in the patient's research chart.

7.2.2 Reporting of AE to WCMC IRB

All adverse events will be recorded on a patient specific AE log after Day 1. The AE log will be maintained by the research staff and kept in the patient's research chart.

7.2.3 Follow-up of Adverse Events and Serious Adverse Events

In this study, non-serious AEs will be reported in all participants from Treatmet 1. Non-serious AEs reported from the time of consent to injecting on Day 1 will be recorded as medical history. Serious adverse events (SAEs) will be reported in all patients (enrolled and not enrolled) from the time of consent. Study procedure-related AEs will be evaluated specifically from the time of consent until the administration of OBP-301. Treatment-emergent AEs will be evaluated from the time of injecting with IP until the Final Visit. The Investigator is responsible to ensure that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical the nature and/or causality of the AE/SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

All non-serious AEs that are ongoing at the Final Visit will be marked as ongoing on the AE eCRF page in the REDCap database.

All SAEs that are ongoing at the Final Visit must be followed until resolution, until the condition stabilizes, until the event is, in the opinion of the Investigator, stabilized or determined to be chronic, or until the participant dies or is lost to follow-up. SAEs that occur after the Final Visit, that are thought to be related to the administration of OBP-301, should be reported to Oncolys BioPharma Inc. or designee.

If a participant is withdrawn because of an AE, the Investigator must arrange for the participant to have appropriate follow-up care until the AE is resolved or has stabilized. Unresolved AEs will be followed until the last scheduled follow-up visit or until the Investigator and Medical Monitor determine that further follow-up is no longer indicated. Oncolys BioPharma Inc. may request that the Investigator perform or arrange for the conduct of supplemental measurements and/or evaluations. If a participant dies during participation in the study or during a recognized Follow-up Period, Oncolys BioPharma Inc. should be provided with a copy of any post-mortem findings, including histopathology.

7.2.4 Reporting of SAE to IRB

All SAEs occurring on this study will be reported to the IRB according to the IRB policy,. For WCM investigator-initiated studies, the following reporting guidelines apply:

a. A Mandatory MedWatch Form 3500A must be completed and sent to the FDA. Specific instructions on how to complete the MedWatch Form 3500A can be found on the FDA website.
b. If the protocol requires SAEs to be reported to an outside agency (i.e., to the supplier of the study drug), specific instructions will be stated in the protocol.
c. If the study utilizes the WCM Data and Safety Monitoring Board (DSMB), a narrative of the event will be submitted to the Regulatory Coordinator on a modified SAE cover sheet. The completed form serves as acknowledgement of the SAE's occurrence and will be used to complete the DSMB periodic report. A copy should be filed in the regulatory binder.

d. For multicenter studies, the multicenter core within the Quality Assurance Unit (QAU) will distribute the IND Safety Report

7.2.5 Notification of Serious Adverse Event to Oncolys

An SAE must be reported by the Investigator within 24 hours of discovery if it occurs during the clinical study or within 28 days of receiving the IP, whether or not the SAE is considered to be related to the IP. An SAE report consists of the SAE form, the AE form, and the concomitant medication form. A copy of these forms must be e-mailed to the attention within 24 hours of discovery:

E-mail: safety@oncolys.com

Please note: If the SAE can be categorized into one or more of the "Outcomes Attributed to the Adverse Event" outlined on the Mandatory Medwatch form 3500, the form and SAE Cover Sheet will be submitted to WCM Quality Assurance Unit (QAU) within 24 hours of PI notification.

The primary site will submit the Mandatory Medwatch form 3500 to Oncolys, Inc within 72 hours of receiving the form and to the FDA as specified by FDA guidelines.

The Investigator should not wait to receive additional information to document fully the event before notification of a SAE, though additional information may be requested. Where applicable, information from relevant laboratory results, hospital case records, and autopsy reports should be obtained.

Instances of death, congenital abnormality, or an event that is of such clinical concern as to influence the overall assessment of safety, if brought to the attention of the Investigator at any time after cessation of IP administration and linked by the Investigator to this study, should be reported to.

All SAEs must be reported to the investigational site's IEC/IRB by the Investigator in accordance with their regulations.

Reporting of any SAEs to applicable regulatory authorities will be the responsibility of Oncolys BioPharma Inc. in compliance with local regulations.

Details of the procedures to be followed if a pregnancy occurs are provided in Section7.2.7.

7.2.6 Notification of Suspected Unexpected Adverse Reactions

An unexpected adverse reaction is any untoward and unintended response that is related to the administration of the IP at any concentration that is not consistent with the applicable version of the Investigator's Brochure [33].

All suspected unexpected serious adverse reactions (SUSARs) will be the subject of expedited reporting in accordance with local requirements. All Investigators should follow-up SUSARs until the event is resolved or until, in the opinion of the Investigator, the event is stabilized or determined to be chronic. Post study SAEs that are deemed to be related to IP that occur after the patient has completed the clinical study must be reported by the Investigator to Oncolys BioPharma Inc.

7.2.7 Notification of Pregnancies

All pregnancies in female patients and female partners of male patients receiving IP will be recorded from treatment until 90 days after the patient has completed therapy.

Should a patient or male patient's partner become pregnant or suspect she is pregnant while participating in this study, or in the 90 days following when the patient received the last injection of IP, the treating Investigator should be informed immediately. All pregnancies will be reported on a pregnancy report form and submitted to safety along the same timelines as an SAE.

The IEC/IRB and Oncolys BioPharma Inc. will be informed. The patient will be asked to provide information on the outcome of the pregnancy, including premature termination should the case arise. For pregnancies in a female partner of a male patient consent to follow-up on the pregnancy will be obtained in agreement with the US Health Insurance Portability and Accountability Act (HIPAA) and other local laws and regulations as outlined in Section. Pregnancy outcomes will be reported on a pregnancy follow-up report form.

Spontaneous miscarriage and congenital abnormalities will be reported as SAEs The Follow-up Period for any and all pregnancies will be deemed to have ended when the health status of the child has been determined on its birth and followed up for 8 weeks after the birth for any potential abnormalities.

Full details will be recorded on the withdrawal page of the eCRF.

7.3 Multicenter Study Monitoring

The study will be monitored by the Weill Cornell Multicenter Study group, according to the Clinical Trial Monitoring Plan, which addresses site qualification, regulatory documentation, and site monitoring and auditing.

8 STATISTICAL ANALYSES

8.1 General Considerations

Full details of the statistical analysis will be provided in a statistical analysis plan (SAP). Any changes to the planned statistical methods from the SAP will be documented in the clinical study report (CSR). The statistical evaluation will be performed using SAS®, version 9.4 or later.

All data will be presented in listings, ordered by patient, unless otherwise specified.

Descriptive statistics for continuous variables (number of patients [n], number of patients with missing data, mean, standard deviation, minimum and maximum) and categorical data (n, percent, number of patients with missing data, by category) will be presented.

8.2 Definitions for Statistical Data Analysis

The following definitions were used in the assessments of safety and efficacy.

All patients who receive at least one injection of OBP-301 will be included in the Safety Population and are analysed according to the actual treatment received. Any patient that has at least one radiographic assessment for response will be included in the Efficacy Population.

8.3 Handling of Missing Values and of Values outside the Detection Limits

Missing values will not be imputed. All missing values will be reported in the listings as either "missing" or as the justification for the value being missing (e.g., "ND" [test not done] or "BQL" [below quantitation limit], where appropriate.

While efforts will be made to avoid incomplete or missing data, in rare cases the AE date could be incomplete or missing. If this happens, incomplete or missing dates will be imputed. For the calculation of AE duration and AE onset from dosing, missing AE dates will be imputed using a conservative approach. This approach will assume that the AE is treatment emergent where insufficient date information exists to determine if the AE occurred before or after dosing, except where year is missing. If the year is also missing then it will be assumed that the start is before the start of the study.

Imputed onset dates of AEs will be checked to ensure they occur after the first study day and before the resolution date. Imputed resolution dates will be checked to ensure they occur after the onset date and before date of last study visit (where AE is not flagged as ongoing).

Descriptive statistics for demographics, body weight, vital signs, and ECG data will be calculated from actual data only, with the number of actual data points used for the calculation of the summary statistics reported.

For censored safety lab data (i.e., hematology, clinical chemistry and urinalysis results that are outside the limit of quantification) the actual values reported by the laboratory (e.g., "< 2" for bilirubin) will be presented in the data listings. For the calculation of summary statistics, safety laboratory data values below the lower limit of quantification (LLOQ) will be set to ½ the LLOQ.

8.3.1 Data Collection

The data collection plan for this study is to utilize REDCap to capture treatment, toxicity, efficacy, and adverse event data for all enrolled patients.

8.3.2 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

8.4 Analysis Populations

8.4.1 Safety Population

All patients who receive at least one injection of OBP-301 will be included in the Safety Population and are analysed according to the actual treatment received.

8.4.2 Evaluable Population

Any patient that has at least one radiographic assessment for response will be included in the Efficacy Population. Imaging studies preferably CT scan of the chest/abdomen/pelvis will be performed every 9 weeks as per the study schedule. Patients will be evaluated according to RECIST v1.1 criteria. Definitions of response evaluation are detailed in section 6.6 for further explanation. Only those patients whose response can be measured according to RECIST v1.1 will be included in this population.

8.4.3 Intent-to-Treat (ITT) Population

All patients who are enrolled and allocated to treatment are included in the intent-to-treat population analysis.

8.4.4 Per-Protocol (PP) Population

Per-Protocol population will only include individuals who completed all four doses of OBP-301 and followed the study protocol

8.5 Efficacy Analysis

8.5.1 Primary Efficacy Endpoint

The primary efficacy endpoint will be the objective response rate (RR), as measured by RECIST 1.1 criteria.

Objective response rate is defined as the sum of partial responses plus complete responses as defined by RECIST v1.1 criteria.

8.5.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints include the disease control rate, duration of response, progression-free survival (PFS), and overall survival (OS).

Duration of response is defined as the duration that subjects who have responded to combination therapy remain without disease progression.

Disease control rate is defined as the percentage of patients who have achieved complete response, partial response and stable disease.

Progression-free survival (PFS) is defined as time from registration to progression or death due to any cause. Progression is defined as radiologic progression of disease by RECIST v1.1 criteria.

Overall survival (OS) is defined as the time from registration until death from any cause,

8.5.3 Exploratory Analysis

Correlative endpoints include the following:

• To examine the tumor-immune microenvironment prior to and following OBP-301 and pembrolizumab therapy as assessed by bulk RNA sequencing and single-cell RNA sequencing.

• To examine and characterize the immune infiltrate by multi-parameter flow cytometry.

8.5.4 Methods for Analysis

Endpoints involving response rates will be analysed by tabulating the response rates along with 95% confidence interval and number of patients with data for each time point that the endpoint is collected. Waterfall plots for tumor response (CR, PR, and ORR) will be provided.

Endpoints involving time to event or time to response will be analysed using survival analysis, including Kaplan-Meier plots. Median survival time with 95% confidence interval, 25th-75th percentiles will be provided.

Exploratory subgroup analyses for tumor response will be defined in the statistical analysis plan (SAP).

8.6 Correlative Study

All patients undergoing treatment with OBP-301 will have tissue biopsies along with correlative blood samples. Tissue biopsies will be performed with every injection of OBP-301. Correlative blood samples will be taken before the start of study treatment, during treatment and post-treatment as described in the section of study schedule. Correlative studies are focused on a detailed and comprehensive evaluation of the tumor immune microenvironment as it

relates to induction of the anti-tumor immune response. Specific procurement and shipping instructions will be provided to participating sites upon study activation. Our specific correlative studies include the following:

A) To examine the tumor-immune microenvironment prior to and following OBP-301 and pembrolizumab therapy as assessed by bulk RNA sequencing and single-cell RNA sequencing.

The focus in this application is to perform detailed cellular characterization of gastric and GEJ tumors and their microbial environment towards better understanding of the factors that shape the response to immunotherapies and mechanism of resistance. To address this, we propose to perform single cell expression analysis and microbiome profiling of tissue samples collected from patients with esophageal/gastric tumors.

We hypothesize that surveying the immune infiltration in the context of immunotherapy and OBP-301 will provide important biomarker for response to treatment. To address this, we propose the following studies**:** Sub-aim (1) Identify and quantify the cellular composition of tumor and adjacent normal tissues from esophageal and gastric cancer patients. Single cell profiling will be performed on each sample collected from patient recruited to the study at Weill Cornell and microbiome detection by whole genome sequencing at baseline (prior to treatment). Sub-aim (2) Generate a patient-specific profile of tumor microenvironment. In this aim the data collected in Aim1 will be analyzed to identify the cell types that are present in each patient at onset of treatment and how cellular composition changes in response to treatment. By computational approaches we will cluster the cells from each sample by their expression profile to distinct groups representing specific cell types (e.g. mucosa layer, lymphocytes, CD45+, squamous epithelium). Similarly, we will identify various immune cells such as CD8+ and CD4+, effector T cells, T_{reg} and others immune subtypes and correlate with microbiome composition. In addition, we will survey levels of PD-1, PD-L1/2, CD28 and other known marker of checkpoint inhibitor response. Sub-aim (3) Longitudinal profiling of patients before and after immunotherapy + OBP-301 to identify possible biomarkers for response and mechanism of resistance. In this aim we will summarize the results from all patient data to assess what are the consistent changes observed in cellular composition in response to immunotherapy and we will investigate how these changes impact response to treatment.

B) To examine the T-cell response in responding and resistant individuals by TCR-sequencing on pre- and post- treatment tumor biopsies.

The primary aim of this proposal is to overcome inherent resistance to immunotherapy in foregut tumors with the addition of a replication-selective oncolytic virus. This project will be focused on analyzing the immune landscape of individual tumors from tumor biopsies taken at baseline and on therapy. We will analyze changes associated with relapse and differences between refractory and sensitive patients. Our proposed analysis will take as input somatic mutations, raw WES reads, and raw RNA-Seq reads. In addition we will perform direct TCR-seq. We will run our existing pipeline for neoepitope prediction, expression, and TCRseq analysis. The output will be an immune landscape per tumor, including (1) mutation burden and high-affinity predicted neoepitopes, (2) checkpoint expression and immune composition, and (3) TCR-seq estimated profiles together with infiltration and diversity values. Comprehensive analysis of entire cohorts will reveal tumor type specific patterns such as correlation specific mutations and immune infiltration and repertoires as well as over-representation of specific T cell clones and signature of neoepitope burden.

For sub-aim (1) we will perform whole exome sequencing via the Englander Institute pipeline and apply state of the art neoantigen discovery tools. We will validate high affinity neoantigens using in vitro assay involving synthetic peptides and a patient's own T cell where we measure T cell clonal expansion using TCR-seq from Archer DX (test already available in Weill Cornell's genomics core). We will explore evolution of neoantigen in relapse biopsies. For sub-aim (2), we will perform RNAseq and TCR-seq using ArcherDX, then deconvolute using Cibersort and another in-house approach that takes into account lineage relationship between reference cell types. As needed we will perform single cell RNAseq to obtain orthogonal validation data. We will correlate immune cell type abundance and TCR repertoires with clinical outcomes. In sub-aim (3) we will develop an immunoscore that uses features defined by immune landscape analysis to build a predictive model of who will respond to immunotherapy. We will pre-train the model using genome profiling results from two recent studies of melanoma datasets treated with anti CTLA4 antibodies, then fine tune via transfer learning using the data from the trials. A variety of machine learning models such as logistic regression, random forest and deep learning will be tested and evaluated using cross-validation. We note that we have extensive experience building such models and that machine learning models we built have shown clinical value. We will determine how the immunoscore correlates with clinical outcome such as progression free survival (PFS) in prospective future trials. We will evaluate the immunoediting patterns of these cohorts, that is, identify variants whose presence is inversely correlated with T cell infiltration. The results will be presented as heatmaps and tables.

C) To examine and characterize the immune infiltrate by multi-parameter flow-cytometry.

In this application, innovative multi-parameter flow cytometry will be utilized that will provide single-cell high resolution of immune cell suppression or activation. Our collaborator, Dr. Gregory Sonnenberg has developed technologies to cryopreserve viable immune cells from primary human tissues and tumor biopsies or resections for side-by-side analyses of immune cells from multiple patients, thus reducing sample variation and allowing direct single-cell comparisons of cytokine production. This powerful approach will be utilized to characterize tumor immune cell infiltrates at baseline, following the induction of combination therapy and at the time of resection. This will be one of the first longitudinal analyses of tumor immune cell infiltrates by multi-parameter flow cytometry following immunotherapy.

8.7 Safety Analyses

8.7.1 Safety Endpoints

The primary safety endpoint is to examine the safety of multiple OBP-301 intratumoral injections in combination with pembrolizumab in advanced esophagogastric cancer.

8.7.2 Summary of Adverse Events

All adverse events, as defined in Section 7, will be recorded for each patient. Adverse events will be tabulated for all patients by: first occurrence of each adverse event, maximum severity, and relationship to treatment. Treatment-related AEs and serious AEs will be tabulated separately, by first occurrence, maximum severity, and strongest relationship to treatment. A listing of all AEs will also be included, per patient, including onset/resolution date, severity, relationship to study treatment, and action regarding IP. A listing of all discontinuations, injection delays, and deaths due to adverse events in the study will be included.

8.7.3 Summary of Laboratory Assessments

Summary statistics will be presented for all blood chemistry, hematology, coagulation, and urinalysis results by study visit, where data are collected.

In addition, all laboratory values will be flagged as low (L1, L2, L3, or L4), high (H1, H2, H3, or H4) or normal (N) based on the investigational site laboratory normal range and/or the NCI toxicity criteria. Shifts in assessments from baseline to all post-baseline visits will be presented (shift tables).

8.7.4 Summary of Special Laboratory Assessments

Summary statistics and shift tables will be presented for all special laboratories results including cytokines, and immune cell phenotypes by study visit, where data are collected.

8.7.5 Summary of Vital Signs

Summary statistics will be presented for all vital signs including temperature, blood pressure (systolic and diastolic), pulse, respiration rate, and body weight by study visit, where data are collected. Graphs of vital signs at each visit may also be provided.

8.7.6 Summary of Physical Examinations

The physical examination results will be evaluated by shift tables that compare baseline examination results to other study visit results in each exam. Frequency counts and percentages will be displayed. All abnormal results will be provided in a listing with a description of the abnormality.

8.7.7 Summary of ECG Measurements

The results of the ECG will be evaluated by transition tables that compare Screening exam results to Final Visit results. Frequency counts and percentages will be displayed. All abnormal results will be provided in a listing with a description of the abnormality.

8.7.8 Concomitant Medications

Concomitant medications will be tabulated for all patients by: the first occurrence of medication classes, the first occurrence of concomitant medications, and all concomitant medications. A listing of all concomitant medications will also be provided.

8.7.9 Adverse Event Stopping Rule

The stopping rule specified below is based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

> Accrual will be temporarily suspended to the study to allow for a full review of data according to the following AE stopping rule:

- If at any time more than 3 of the first 12 patients enrolled (or ≥ 33% of all patients enrolled after 12 patients) have experience at least one grade 3 or worse immune related adverse event or adverse event related (possibly, probably, or definitely) to OBP-301 injection.
    - Immune related adverse events for the purposes of this adverse event stopping rule, will include diarrhea/colitis, pneumonitis, renal failure/nephritis, or increased LFTS (bilirubin, AST, ALT). Rash, joint aches, fatigue, thyroid abnormalities, or other immune related events that are not life threatening will not be included for the purposes of this Adverse Event Stopping Rule.
- If grade 5 (death) event at least possibly, probably or definitely related to OBP-301 injection, the study will be halted.
- If 3 or more grade 4 adverse events that are possibly, probably, or definitely attributed to OBP-301 occur, that do not resolve back to grade 1 within 30 days, the study will be halted.

8.8 Determination of Sample Size

Sample size is determined according to Simon's two-stage Minimax design. We project a RR of 15% (historical RR for pembrolizumab alone), below which the combination regimen will be unacceptable, and a RR of 30% (expected RR rate for OBP-301 + pembrolizumab), above which the combination regimen will be considered worthy of further exploration. The null hypothesis that the RR is less than or equal to 15% will be tested against the alternative hypothesis that the RR is greater than or equal to 30%.

The sample size computations were performed assuming a 10% level of significance and 80% power. If 2 or fewer patients respond out of the first 18 evaluable patients, the study arm will be terminated and declared to have a negative result. If 3 or more patients respond out of the first 18 evaluable patients, enrollment will be extended to 37 patients. The treatment will be declared effective and worthy of further testing if 9 or more patients respond among the 37 patients entered. This two-stage design yields a ≥ 0.80 probability of a positive result if the true RR is ≥ 30%. It yields a ≥ 0.90 probability of a negative result if the true RR is ≤ 15%. A 95% confidence interval constructed around the expected RR of 30% can be estimated to be within ± 14.8% of the observed RR proportion. Assuming 10% are unevaluable/ineligible, we anticipate that a total of 41 patients will be enrolled in the study (assuming the study proceeds to the second stage).

9 REGULATORY REQUIREMENTS

9.1 Regulatory Approvals

Once protocol amendments or consent form modifications are implemented at the lead site, Weill Cornell Medicine, updated documents will be provided to participating sites. Weill Cornell Medicine must approve all consent form changes prior to local IRB submission.

Relevant study documentation will be submitted to the regulatory authorities of the participating countries, according to local/national requirements, for review and approval before the beginning of the study. On completion of the study, the regulatory authorities will be notified that the study has ended.

As required by local regulations, the Investigator will ensure all legal aspects are covered, and approval of the appropriate regulatory bodies obtained, before study initiation.

9.2 Institutional Review Board/Ethics Committee Approval

Before initiation of the study at each study center, the protocol, the ICF, other written material given to the patients, and any other relevant study documentation will be submitted to the appropriate Ethics Committee. Written approval of the study and all relevant study information must be obtained before the study center can be initiated or the IP is released to the Investigator. Any necessary extensions or renewals of IEC/IRB approval must be obtained for changes to the study, such as amendments to the protocol, the ICF, or other study documentation. The written approval of the IEC/IRB together with the approved ICF must be filed in the study files.

The Investigator will report promptly to the IEC/IRB any new information that may adversely affect the safety of the patients or the conduct of the study. The Investigator will submit written summaries of the study status to the IEC/IRB as required. On completion of the study, the IEC/IRB will be notified that the study has ended.

Neither the Investigator nor Oncolys BioPharma Inc. will modify or alter this protocol without the agreement of the other. All agreed protocol amendments will be clearly recorded on a protocol amendment form and will be signed and dated by the original protocol approving signatories. All protocol amendments will be submitted to the relevant institutional IEC/IRB for approval before implementation, as required by local regulations. The only exception will be when the amendment is necessary to eliminate an immediate hazard to the trial participants. In this case, the necessary action will be taken first, with the relevant protocol amendment following shortly thereafter.

9.3 Ethical Conduct of the Study

The Investigators and all parties involved should conduct this study in adherence to the ethical principles based on the Declaration of Helsinki, GCP, ICH guidelines and the applicable national and local laws and regulatory requirements.

This study will be conducted under a protocol reviewed and approved by the applicable ethics committees and investigations will be undertaken by scientifically and medically qualified persons, where the benefits of the study are in proportion to the risks.

9.4 Participant Informed Consent

The Investigator is responsible for ensuring that no subject undergoes any study-related examination or activity before that subject has given written informed consent to participate in the study.

It is the Investigator's responsibility to ensure that each participant gives informed consent to participate in the study. The Investigator will explain the nature of the study, its purpose, procedures, expected duration, and the potential benefits, risks and inconveniences in participation. In addition, a participant information sheet/consent form containing relevant information will be prepared by Oncolys BioPharma Inc. in conjunction with the investigational site and will be provided to all participants.

Participants must be informed that their participation is voluntary and that they have the right to withdraw from the study at any time without prejudice.

The participants will be informed of their rights to privacy but will be made aware that the study data will be submitted to Oncolys BioPharma Inc. and possibly to drug regulatory authorities for review and evaluation. They will be informed also that the study monitor may inspect their medical records to verify the accuracy and completeness of the study records and results.

The ICF will contain a separate section that addresses the use of remaining blood and tissue samples for future research. Participants will be informed that they are free to refuse to these additional tests being carried out and may request at any time for the samples to be destroyed.

The participants will be given an opportunity to ask questions and allowed sufficient time to decide whether they wish to participate. If the participant decides to participate in the study, they will voluntarily sign the written ICF.

The acquisition of informed consent should be documented in the participant's medical records, and should meet the requirements of GCP. The ICF will be signed and personally dated by the participant and by the person who conducted the informed consent discussion (not necessarily an Investigator). The original signed ICF will be retained in accordance with institutional policy, and a copy of the signed consent form will be provided to the participant or legal representative. The date that informed consent was signed will be recorded on the eCRF.

If new information becomes available that may be relevant to the patient's willingness to continue participation in the study, the ICF will be updated and approved by the IEC(s)/IRB(s) (and regulatory authorities, if required). The study participants will be informed about this new information and reconsent will be obtained.

Participants who are rescreened are required to sign a new ICF.

9.5 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor-Investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

10 DATA AND SAFETY MONITORING PLAN (DSMP)

The study will be reviewed by the Weill Cornell Medical College Data and Safety Monitoring Board (DSMB) as an independent means of data and safety monitoring. Enrollment information, adverse event and safety information, protocol changes, and other interim data will be evaluated by the DSMB. After each evaluation, the Board will provide the principal investigator with recommendations for protocol modification, continuation, or termination.

DSMB reports will be made to the DSMB every 6 months.

11 ADMINISTRATIVE PROCEDURES

11.1 Financing, Liability/Indemnity/Insurance

Investigators are required to follow financial disclosure policies at their respective institutions. Any investigator with a conflict of interest with this study must report the conflict to the Sponsor-investigator.

The study contract will govern all provisions relating to liability, indemnity and insurance.

11.2 Recording of Data and Retention of Records

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study (end of study defined as the date of the last follow-up contact at Week 48 with the last patient), all documents and data relating to the study will be kept in an orderly manner by the Investigator in a secure study file. Essential documents should be retained for 2 years after the final marketing approval in an ICH region or for at least 2 years since the discontinuation of clinical development of the IP. In addition, all patient medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

11.2.1 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill Cornell Medical Center CTSC. It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

11.3 Retention of Records

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study (end of study defined as the date of the last follow-up contact at Week 48 with the last patient), all documents and data relating to the study will be kept in an orderly manner by the Investigator in a secure study file. Essential documents should be retained for 2 years after the final marketing approval in an ICH region or for at least 2 years since the discontinuation of clinical development of the IP. In addition, all patient medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

11.4 Disclosure and Data Confidentiality

The Investigator must ensure that the participant's anonymity is maintained. Participants should only be identified by their initials and a participant identification number on the eCRFs and other source documents. Other study-related documents (e.g., signed ICFs) should be kept in strict confidence by the Investigator.

Data is captured using database REDCap. Participants will be informed that data will be held on file by Oncolys BioPharma Inc. and that these data may be viewed by staff including the study monitor and by external auditors on behalf of Oncolys BioPharma Inc. and appropriate regulatory authorities. Participants will also be informed that a study report will be prepared and may be submitted to regulatory authorities and for publication. However, participants will be identified in such reports only by study identification number, gender, and age. All participant data will be held in strict confidence.

Auditors, the IEC(s)/IRB(s) approving this research, and the US FDA, as well as that of any other applicable agency(ies), will be granted direct access to the study patients' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the patients to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the patients' identity will remain confidential.

All personal data collected and processed for the purposes of this study should be managed by the Investigator and his/her staff with adequate precautions to ensure confidentiality of those data, and in accordance with the HIPAA [37], applicable to national and/or local laws and regulations on personal data protection.

11.5 Publication of Results

Study results will be reported at appropriate international congresses and meetings as data are available, and the final study will be reported in a peer-reviewed oncology journal.

12 CLINICAL STUDY REPORT

We will provide the clinical study report within 1 year of study completion.

13 APPENDIX

13.1 ECOG performance status

Performance Status	Definition
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours.
3	Capable of limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair.

13.2 Comparison of RECIST 1.1 and iRECIST

	RECIST 1.1	iRECIST
Definitions of measurable and non-measurable disease; numbers and site of target disease	Measurable lesions are ≥10 mm in diameter (≥15 mm for nodal lesions); maximum of five lesions (two per organ); all other disease is considered non-target (must be ≥10 mm in short axis for nodal disease)	No change from RECIST 1.1; however, new lesions are assessed as per RECIST 1.1 but are recorded separately on the case report form (but not included in the sum of lesions for target lesions identified at baseline)
Complete response, partial response, or stable disease	Cannot have met criteria for progression before complete response, partial response, or stable disease	Can have had iUPD (one or more instances), but not iCPD, before iCR, iPR, or iSD
Confirmation of complete response or partial response	Only required for non-randomised trials	As per RECIST 1.1
Confirmation of stable disease	Not required	As per RECIST 1.1
New lesions	Result in progression; recorded but not measured	Results in iUPD but iCPD is only assigned on the basis of this category if at next assessment additional new lesions appear or an increase in size of new lesions is seen (≥5 mm for sum of new lesion target or any increase in new lesion non-target); the appearance of new lesions when none have previously been recorded, can also confirm iCPD
Independent blinded review and central collection of scans	Recommended in some circumstances—eg, in some trials with progression-based endpoints planned for marketing approval	Collection of scans (but not independent review) recommended for all trials
Confirmation of progression	Not required (unless equivocal)	Required

Consideration of clinical status	Not included in assessment	Clinical stability is considered when deciding whether treatment is continued after iUPD

"i" indicates immune responses assigned using iRECIST. RECIST=Response Evaluation Criteria in Solid Tumours. iUPD=unconfirmed progression. iCPD=confirmed progression. iCR=complete response. iPR=partial response. iSD=stable disease.

1. Simon R. Optimal two-stage designs for phase II clinical trials. Controlled Clinical Trials. 1989;10: 1-10.
2. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12: 252-264.
3. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366: 2455-2465.
4. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, al. e. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366: 2443-2454.
5. Robert C, Long GV, Brady B, Dutriaux C, Maio M, al. e. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372: 320-330.
6. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus ipilumumab in advanced melanoma. N Engl J Med. 2015;372: 2521-2532.

7. Brahmer J, Reckamp KL, Bass P, Crino L, Eberhardt WE, al. e. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373: 123-135.
8. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, al. e. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015;373: 1627-1639.
9. Garon EB, Rizvi NA, Hui R, Leighl N, Balmonoukian AS, al. e. Pembrolizumab for the treatmnt of non-small cell lung cancer. N Engl J Med. 2015;372: 2018-2028.
10. Reck M, Rodriquez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemothreapy for PD-L1 positive non-small cell lung cancer. N Engl J Med. 2016;375: 1823-1833.
11. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373: 1803-1813.
12. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, al. e. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515: 558-562.
13. Segal NH, Antonia SJ, Brahmer JR, al. e. Preliminary data from a multi-arm expansion study of MEDI4736, an anti-PD-L1 antibody. J Clin Oncol. 2014;32: abs3002.
14. Doi T, Piha-Paul SA, Jalal SI, Mai-Dang H, al. e. Updated results for the advanced esophageal carcinoma cohort of the phase Ib Keynote-028 study of pembrolizumab. J Clin Oncol. 2016;34: Abs 7.
15. Kojima T, Hara H, Yamaguchi K, et al. Phase II study of Nivolumab (ONO-4538/BMS-936558) in patients with esophageal cancer: Preliminary Report of Overall Survival. J Clin Oncol. 2016;34: absTPS175.
16. Sun J, Shen L, Shah MA, Enzinger P, Adenis A, al. e. Pembrolizumab plus chemotherapy versus chemotherapy alone for first-line treatment of advanced oesophageal cancer (KEYNOTE-590): a randomised, placebo-controlled, phase 3 study. Lancet. 2021;398: 759-771.
17. Janjigian YY, Shitara K, Moehler M, Garrido M, al. e. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. . Lancet. 2021;398: 27-40.
18. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov. 2015;14: 642-662.
19. Kawashima T, Kagawa S, Kobayashi N, al. e. Telomerase-specific replication-selective virotherapy in human cancer. Clin Cancer Res. 2004;10: 285-292.
20. Umeoka T, Kawashima T, Kagawa S, al. e. Visualization of intrathoracically disseminated solid tumors in mice withoptical imaging by telomerase-specific amplificationof a transferred gene fluorescent protein gene. Cancer Res. 2004;64.
21. Taki M, Kagawa S, Nishizaki M, al. e. Enhanced oncolysis by a tropism-modified telomerase-specific replication-selective adenoviral agent OBP-405 (Telomyelysin-RGD). Oncogene. 2005;24: 3130-3140.
22. Hashimoto Y, Watanabe Y, Shirakiya Y, al. e. Establishment of biological and pharmacokinetic assays of telomerase-specific replication-selective adenovirus. Cancer Sci. 2008;99.
23. Nemunaitis JJ, Tong AW, Nemunaitis M, Senzer N, Phadke AP, al. e. A phase I study of telomerase-specific replication competent oncolytic adenovirus (telomelysin) for various solid tumors. Mol Ther. 2010;18: 429-434.
24. Kanaya N, Kuroda S, Morihiro T, Kakiuchi Y, al. e. Telomelysin-induced immunogenic cell death synergizes with anti-PD-1 antibody in non-immunogenic gastrointestinal tumors. Proceedings: AACR Annual Meeting 2018; April 14-18, Chicago IL. 2018: Abs 2744.
25. Andtbacka RH, Kaufman HL, Collichio F, et al. Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. J Clin Oncol. 2015;33: 2780-2788.

26. Goodin T. FDA approves first-of-its-kind product for the treatment of melanoma. FDA News Release, 2015:1.
27. Benstetter M. First oncolytic immunotherapy medicine recommended for approval. European Medicines Agency Press Release, 2015:1.
28. Hawkins LK, Johnson L, Bauzon M, et al. Gene delivery from the E3 region of replicating human adenovirus: evaluation of the 6.7 K/gp19 K region. Gene Ther. 2001;8: 1123-1131.
29. Nemunaitis J, Tong AW, Nemunaitis M, et al. A phase I study of telomerase-specific replication competent oncolytic adenovirus (telomelysin) for various solid tumors. Mol Ther. 2010;18: 429-434.
30. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997;33: 787-791.
31. Shay JW, Zou Y, Hiyama E, Wright WE. Telomerase and cancer. Hum Mol Genet. 2001;10: 677-685.
32. Kamradt J, Drosse C, Kalkbrenner S, et al. Telomerase activity and telomerase subunit gene expression levels are not related in prostate cancer: a real-time quantification and in situ hybridization study. Lab Invest. 2003;83: 623-633.
33. OBP-301 Telomelysin. Investigator's Brochure, version 8.0. In: Inc OB, editor, 2017.
34. Sub-analysis Report of OBP-301 Phase I study in patients with Melanoma, Study Number: 0301-1-0002. Implementation period: 2016/1/12-2016/1/15: Oncolys BioPharma Inc, 2016.
35. Song X, Zhao Z, Barber B, Farr AM, Ivanov B, Novich M. Overall survival in patients with metastatic melanoma. Curr Med Res Opin. 2015;31: 987-991.
36. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 In: Institute UDoHaHSNIoHNC, editor, 2010.
37. Services UDoHau. The Health Insurance Portability and Accountability Act (HIPAA) of 1996 (P.L. 104-191) [HIPAA], 1996.