WINDOW-OF OPPORTUNITY TRIAL OF NEOADJUVANT ADXS11-001 VACCINATION PRIOR TO TRANSORAL RESECTION OF HPV-POSITIVE OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

CLINICAL TRIAL PROTOCOL

Version 11.2 (January 9, 2020)

IND# 15688

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1 OBJECTIVES

This is an investigator-initiated prospective clinical study of patients with stage I-IV squamous cell carcinoma of the oropharynx (OPSCC) who are to undergo ablative transoral surgery. We propose to test the hypothesis that the *Listeria*-based HPV vaccine ADX11-001 induces circulating and tumor-infiltrating antigen-specific T cells in HPV16+ oropharyngeal cancer patients undergoing transoral resection. The results of this trial will assess the ability of ADX11-001 vaccination to induce a robust HPV-specific cytotoxic lymphocyte (CTL) response in the blood and tumor.

1.1 Primary Aims

1.1.1 To determine the immunogenicity of ADX11-001 vaccination in patients with HPV+ squamous cell carcinoma of the oropharynx.

1.1.2 To evaluate the tolerability, safety, and nature and degree of toxicity of ADX11-001 by the numbers of patients with dose limiting toxicities (DLTs) and adverse events as assessed by the CTCAE v4.0

1.2 Primary Endpoints

1.2.1 The primary endpoint will be the change in HPV E6/E7-specific CD8+ cytotoxic lymphocyte (CTL) responses in the peripheral blood at time of surgery, with respect to baseline in vaccinated patients.

1.2.2 Toxicity (NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0)

1.3 Secondary Aim

The secondary objective will be to determine vaccine-induced HPV E6/E7-specific CD8+ CTL responses in the blood at various timepoints after surgery, with respect to baseline levels, in vaccinated patients.

1.4 Secondary Endpoint

The secondary endpoint will be the change in HPV E6/E7-specific CD8+ cytotoxic lymphocyte (CTL) responses in the peripheral blood at various times after surgery (3W, 6W, 3M, 6M, 12M), with respect to baseline in vaccinated patients.

1.5 Exploratory Aim

To analyze changes in the profile of tumor-infiltrating effector (NK cells, CD4+ and CD8+ T cells) and suppressor (Treg and MDSC) immunocytes by immunohistochemistry in vaccinated and unvaccinated (observational cohort) patients.

2 BACKGROUND/RATIONALE

2.1 DISEASE AND INCIDENCE

Squamous cell carcinoma is the most frequently occurring malignant tumor of the head and neck and is a major cause of morbidity and mortality worldwide. More than 90% of head and neck squamous cell carcinoma (HNSCC) originate from the mucosal linings of the oral cavity, pharynx, or larynx. Reports show that the estimated worldwide incidence of HNSCC in 2000 was more than 551,000 with greater than 217,000 deaths.[1] In the United States (US), in 2006 alone, more than 46,000 new cases of HNSCC and 11,000 deaths due to HNSCC were reported.[2] While HNSCC related to environmental carcinogens (tobacco, alcohol) has declined in the US, the incidence of certain subtypes of HNSCC, particularly HNSCC related to the human papilloma virus (HPV) is rising.[3]

2.2 HPV-MEDIATED HEAD AND NECK CANCER – A TARGET FOR THERAPEUTIC VACCINATION

Currently 60-80% of oropharyngeal cancer (OPC) is caused by the human papillomavirus (HPV), particularly the so-called "high-risk" oncogenic HPV16 and HPV18 genotypes. HPV 16 is strongly implicated in the etiology of OPC[4], and is found in up to 90% of virally-related OPC.[5] It has become increasingly clear that HPV-mediated OPC is a different disease from classical environmentally-related HNSCC, with distinct epidemiology and natural history. While patients with HPV+ tumors typically have an excellent response to surgical or radiation-based therapy[6,7], treatment can be associated with significant short- and long-term toxicity, and it is not yet known whether patients with HPV+ OPSCCA have better long-term control. Thus, approaches which target the unique biology of HPV-infected cancer cells, such as therapeutic vaccination, are attractive strategies for potentially de-escalating XRT and chemo/XRT regimens (and thus decreasing toxicity) and enhancing long-term disease control.

Prior to being internalized into epithelial cells in the basal layer of the oropharyngeal mucosa, HPV is susceptible to neutralization with specific antibodies, which is the mechanism of action of the preventative vaccine Gardasil.[8] However, once HPV is internalized, it can integrate into the epithelial cell genome, and is no longer susceptible to antibody-mediated clearance. HPV-infected cells can then only be cleared by mechanisms of cell-mediated immunity, including natural killer (NK) cells, and CD8+ cytotoxic T cells (CTL)[9]. In patients for whom these mechanisms are inefficient, progression of HPV-infected epithelial cells to malignancy is then driven by the E6 and E7 viral early genes, which inactivate p53 and Rb tumor suppressor genes respectively[10]. E6 and E7 are essential to maintenance of the transformed phenotype, and are also often recognized by T cells from HPV-infected patients[11-13]; thus these proteins are logical targets for monitoring HPV-specific immune responses, as well as therapeutic (cell-mediated, or T cell-based) vaccination.

Paradoxically, while patients with HPV-mediated tumors are unable to control and clear the virus, anti-HPV T cell responses can be detected[9,11-13]. Furthermore, a substantial amount of the HPV genome is devoted to various mechanisms of immune evasion[14], and HPV-induced cancers are more common in immunosuppressed patients[15,16]. This suggests that the immune system does, in fact, recognize viral antigens in HPV-infected cells and the efficacy of this response depends critically on the overall balance of immune activation and immunosuppressive/immunoregulatory mechanisms in the host.

In an effort to tip the immunologic balance towards beneficial immune activation, preventive and therapeutic vaccines targeting HPV have been developed. Therapeutic peptide vaccination using long peptides specific for HPV16 has recently demonstrated to have immunologic and clinical efficacy in the treatment of vulvar intraepithelial neoplasia (VIN)[49]. The etiologic similarities between cervical cancer/VIN and HPV+ OPSCC, where in both the most common HPV subtype is HPV16, provide a convincing rationale for therapeutic vaccination in HPV+ OPSCCA patients.

2.3 ADXS11-001 SUMMARY OF SAFETY

As of June 30, 2016, 722 doses of ADXS11-001 have been administered to 290 enrolled subjects at doses of 5 x 10^7, 3.3 x 10^8, 1 x 10^9, 3.3 x 10^9, and 1 x 10^{10} CFU. In the Phase 1 study, 100% of subjects (n=15) have experienced flu-like AEs or symptoms associated with cytokine release syndrome. The incorporation of NSAIDs and antiemetic medications pre and post infusions has effectively reduced the incidence of these symptoms from 100% to 37%. In addition, a course of antibiotics is given 3 days after each dose of ADXS11-001 as a precautionary measure to ensure clearance of the *Lm*. From the clinical experience in 290 subjects, a clear pattern of mild to moderate treatment-related AEs consistent with cytokine release symptoms (e.g., constitutional symptoms such as fever, chills, rigors, headache, nausea, vomiting, tachycardia, shortness of breath, hypotension, and rash) are commonly seen and typically appear 2-4 hours after infusion. Symptoms either self-resolve or respond quickly to symptomatic treatment.

A summary of AEs in ≥5% of subjects by MedDRA system organ class and preferred term is presented in **Table 1**. There have been no Grade 3-4 related AEs observed in over 5% of the subject population (see **Table 1**).

Table 1 Treatment-Related (Possibly Related, Probably Related, Related) Adverse Events Reported ≥5% with ADXS11-001 (Safety Population, N=290)

System Organ Class (SOC), Preferred Term, [N (%)] [1]	Grades 1-4 (N=290)	Grade 3-4 (N=290)
Blood And Lymphatic System Disorders		
Anaemia	178 (61.4)	3(1.0)
Cardiac Disorders		
Sinus Tachycardia	16(5.5)	2 (0.7)
Tachycardia	32 (11.0)	
Gastrointestinal Disorders		
Abdominal Pain	49 (16.5)	-
Constipation	47 (16.2)	-
Diarrhoea	30 (10.3)	1 (0.3)
Dysphagia	15 (5.2)	-
Nausea	107 (36.9)	-

System Organ Class (SOC), Preferred Term, [N (%)] [1]	Grades 1-4 (N=290)	Grade 3-4 (N=290)
Vomiting	88 (30.3)	4 (1.4)
General Disorders And Administration Site Conditions		
Chills	184 (63.5)	5 (1.7)
Disease Progression	23 (7.9)	-
Fatigue	74 (25.5)	1 (0.3)
Oedema Peripheral	24 (8.3)	-
Pain	34 (11.7)	-
Pyrexia	167 (57.6)	3 (1.0)
Immune System Disorder		
Cytokine Release Syndrome	24 (8.3)	13 (4.5)
Investigations		
Alanine Aminotransferase Increased	18 (6.2)	2 (0.7)
Aspartate Aminotransferase Increased	21 (7.2)	1 (0.3)
Blood Alkaline Phosphatase Increased	24 (8.3)	3 (1.0)
Blood Creatinine Increased	25 (8.6)	-
Gamma-Glutamyltransferase Increased	25 (8.6)	4 (1.4)
Haemoglobin Decreased	16 (5.5)	-
White Blood Cell Count Decreased	29 (10.0)	2 (0.7)
Metabolism And Nutrition Disorders		
Decreased Appetite	33 (11.4)	-
Hyperglycaemia	19 (6.5)	-
Hypertriglyceridemia	20 (6.9)	-
Hypoalbuminaemia	43 (14.8)	-
Hypokalaemia	18 (6.2)	-
Hyponatraemia	31 (10.7)	1 (0.3)
Musculoskeletal And Connective Tissue Disorders		
Back Pain	33 (11.4)	1 (0.3)
Nervous System Disorders		
Dizziness	19 (6.5)	-
Headache	69 (23.8)	-

System Organ Class (SOC), Preferred Term, [N (%)] [1]	Grades 1-4 (N=290)	Grade 3-4 (N=290)
Psychiatric Disorders		
Anxiety	17 (5.9)	-
Depression	17 (5.9)	-
Respiratory, Thoracic And Mediastinal Disorders		
Cough	16 (5.5)	-
Dyspnoea	24 (8.3)	2 (0.7)
Vascular Disorders		
Hypertension	17 (5.9)	3 (1.0)
Hypotension	58 (20.0)	18 (6.2)
Note: [1] Percentage is calculated using column header count as denominator for percentage calculation.		

3 ADXS11-001

3.1 Vaccine Design:

ADXS11-001 (ADXS-HPV) is a live attenuated *Listeria monocytogenes* -listeriolysin O (*Lm*-LLO) immunotherapy developed for the treatment of HPV-associated cancers. ADXS11-001 is bioengineered to secrete an antigen-adjuvant fusion protein (tLLO-HPV-E7) consisting of a truncated fragment of the listeriolysin O (truncated LLO, tLLO) fused to the full length E7 peptide of HPV-16.

3.2 Mechanism of Action:

ADXS11-001 is rapidly taken up by antigen presenting cells (APC) within the subject. This causes activation of the APC and results in a multi-factorial stimulation of innate immunity. To the subject, this activation can manifest as flu-like symptoms or symptoms associated with cytokine release that occur during or in the hours immediately following administration. Once inside the APC, ADXS11-001 can escape the phagolysosome into the cytoplasm where it secretes the HPV-E7-tLLO fusion protein. This peptide, along with other *Lm* peptides, is very rapidly ubiquitinated and transported to the proteasome where the peptides are broken down and cross-presented through major histocompatibility complex (MHC) Class 1 and Class 2 pathways. This cross-presentation, in immunologic context of responding to a “perceived” acute infection, stimulates the development of adaptive immunity culminating in HPV-specific effector T-cells that can infiltrate into the tumor microenvironment (TME) and destroy tumor cells immunologically.

Advaxis *Lm*-LLO immunotherapies have broad effects on the immune system and the ability to neutralize mechanisms of immune tolerance. These *Lm*-LLO immunotherapies take advantage of the ability of *Lm* to present target antigens in the cytoplasm of APCs that generate a target-specific T-cell immunity. High avidity T-cells are generated where possible,

but when they are not, *Lm* stimulates an up-regulation of T-cell responses to sub-dominant epitopes. Advaxis *Lm*-LLO immunotherapies secrete tumor peptides fused to LLO from multiple copies of plasmids. This increased LLO secretion triggers endocrine and exocrine signaling of the immune system that results in a relative reduction in the number and function of regulatory T-cells and myeloid-derived suppressor cells (MDSC) in the TME, which enables tumor cell killing, even when the T-cells are lower avidity. Tumor antigen specific T-cell immunity generated in the context of *Lm*-LLO immunotherapies can be effective even when targeting self-antigens or viral targets that are partially cross-reactive.

Studies have shown that ADXS11-001 has anti-tumor activity against multiple types of high-risk HPV, including cross-reactive activity where there are minor differences in HPV E7 T-cell epitopes. As an investigational drug product, ADXS11-001 has no direct effect on the tumor tissue, but is designed to stimulate the subject's own immune system to generate an effective immune response targeting the tumor-associated antigen like HPV-E7.

4.0 TRANSORAL SURGERY – A UNIQUE OPPORTUNITY TO PROFILE THE TUMOR IMMUNE MICROENVIRONMENT (TIME)

Increasing evidence suggests that the tumor immune microenvironment (TIME) of head and neck cancer is immunosuppressive, and that tumor-mediated immunosuppression may play a unique role in HPV-associated head and neck cancer. The TIME includes both anti-tumor effector cells (CD8+ cytotoxic T lymphocytes, CD4+ helper T cells, natural killer [NK] cells) and regulatory/suppressive immunocytes such as regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC). Other factors, such as local concentrations of cytokines and chemokines also play a role in determining the TIME. The balance of effector and regulatory cells determines whether an individual TIME is associated with productive anti-tumor immunity or localized immunosuppression and unrestrained tumor growth. While the majority of tumor vaccine studies examine circulating antigen-specific T cell responses, these responses do not always correlate with infiltration of antigen-specific T cells into the tumor and objective tumor responses.

Transoral resection of oropharyngeal tumors can in some cases be accomplished under direct vision with conventional instruments, but generally involves either robot- or laser-assisted resection. Both transoral robotic surgery (TORS) and transoral laser microsurgery (TOLM) are intended to minimize or avoid morbidity of radiation therapy or chemo-radiation by completely removing the tumor in three dimensions with negative histological margins, thus allowing de-escalation of adjuvant therapy in some patients and sparing adjuvant therapy altogether in others. When clinically indicated, a neck dissection is performed simultaneously. *Both TORS and TOLM are FDA approved for head and neck cancer, and considered standard of care therapy of OPSCC in appropriate patients.*

An important distinction between transoral surgery and definitive chemoradiation as treatment for oropharyngeal tumors is that only surgery yields a pathological specimen which can be used to provide postoperative risk-stratification and guide the use of adjuvant therapy. The pathological specimen also permits evaluation of the response of tumor biomarkers and the TIME following a course of preoperative (also described as "neoadjuvant") treatment. Transoral resection of oropharyngeal cancer provides a unique opportunity to evaluate the ability of vaccination to induce an immune response in the tumor and draining lymph nodes.

5.0 ELIGIBILITY AND SELECTION OF STUDY POPULATION

5.1 INCLUSION CRITERIA

To be enrolled in the study, the patients must meet the following inclusion criteria:

1. The patient has newly-diagnosed, biopsy proven squamous cell carcinoma of Stage I-IV (T1-3, N0-2b) of the oropharynx.
2. The patient's tumor is HPV positive by PCR or ISH assay of tumor biopsy.
3. The patient is able/eligible to undergo treatment with transoral surgery (including TOLM or TORS) with or without neck dissection and with or without adjuvant radiation therapy or chemoradiation.
4. The patient is able to understand and give informed consent.
5. The patient is at least 18 years old.
6. The patient's ECOG performance status is </= 2.

5.2 EXCLUSION CRITERIA

Patients who meet any of the following criteria will be excluded from the study:

1. The patient has had prior head and neck squamous cell carcinoma (HNSCC), with the exception of superficial cutaneous basal cell or squamous cell carcinomas.
2. The patient has active cancer in another part of the body, with the exception of superficial cutaneous basal cell or squamous cell carcinomas.
3. If a cancer survivor, the disease free interval is less than 3 years, with the exception of superficial cutaneous basal cell or squamous cell carcinomas.
4. If a cancer survivor, the patient received prior systemic chemotherapy or radiotherapy.
5. If prior standard-of-care pre-treatment biopsy is inadequate for analysis by immunohistochemistry, and the patient is unwilling to undergo an additional biopsy procedure.
6. The patient is a prisoner.
7. The patient has a psychiatric illness or developmental delay that would interfere with understanding of the study and provision of informed consent.
8. The patient has previously received definitive surgical, radiation, or chemoradiation treatment for HNSCC.
9. The patient has a history of HIV or other known cause of immunosuppression, or is actively taking immunosuppressive medications due to organ transplantation, rheumatoid disease, or other medical conditions.
10. The patient is allergic to naproxen or ibuprofen.
11. The patient has a history of liver disease (excluding Gilbert's disease and non-active Hepatitis C) and/or elevation of transaminases or bilirubin above the normal limit.
12. The patient has a contraindication (e.g. sensitivity/allergy) to both trimethoprim/sulfamethoxazole **and** ampicillin.

13. The patient has implanted medical device(s) that pose a high risk for colonization and/or cannot be easily removed (e.g., prosthetic joints, artificial heart valves, pacemakers, orthopedic screw(s), metal plate(s), bone graft(s), or other exogenous implant(s)). NOTE: More common devices and prosthetics which include arterial and venous stents, dental and breast implants and venous access devices (e.g. Port-a-Cath or Mediport) are permitted. The study chair must be contacted prior to consenting any subject who has any other device and/or implant.
14. Patients who are receiving or may receive future treatment with PI3K or TNFα inhibitors.
15. Patients who have undergone a major surgery, including surgery for a new artificial implant and/or device, within 6 weeks prior to the initiation of ADXS11-001 treatment. NOTE: All toxicities and/or complications must have recovered to baseline or Grade 1 prior to the initiation of ADXS11-001 study therapy. Sponsor must be consulted prior to enrolling subjects on the study who recently had a major surgery or have new artificial implant, and/or devices.
16. Patients who have a history of listeriosis or prior ADXS11-001 therapy.
17. Patients with a known allergy to any component of the study treatment formulations.
18. Pregnancy. The effects of this vaccine on the developing human fetus are unknown. For this reason women of child-bearing potential and men must use two forms of contraception (i.e., barrier contraception and one other method of contraception) at least 4 weeks prior to study entry, for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

    Pregnancy Testing. Women of childbearing potential are required to have a negative serum pregnancy test (with a sensitivity of at least 25 mIU/mL) within 10-14 days prior to the first vaccine, within 24 hours prior to the first and second doses of vaccine and prior to surgery.

    Women of childbearing potential are defined as follows:

    - Patients with regular menses
    - Patients with amenorrhea, irregular cycles, or using a contraceptive method that precludes withdrawal bleeding
    - Women who have had a tubal ligation

    Women are considered not to be of childbearing potential for the following reasons:

    - The patient has undergone hysterectomy and/or bilateral oophorectomy.
    - The patient is post-menopausal defined by amenorrhea for at least 1 year in a woman > 45 years old.

6.0 RESEARCH PLAN AND METHODS

This is a window of opportunity single-arm phase II clinical trial in which a group of surgically-resectable HPVOPC patients will receive the ADXS11-001 vaccine before standard-of-care transoral surgical resection. Vaccinated patients will be enrolled according to a Simon's two-stage design, with an initial cohort of 9 patients enrolled before preliminary analysis, and a

subsequent cohort of 13 patients enrolled if statistical criteria are met. To better understand the immunology of un-vaccinated HPVOPC patients, we will also enroll an optional observational group of up to 10 patients with the same characteristics who will undergo transoral resection without vaccination. We will collect, process and store tumor specimens, tumor-derived leukocytes, and serum and cellular components of peripheral blood, at different time points from study patients prior to analysis of batched samples at a later date once the specimens from 5 or more patients are available.

6.1 SCHEME:

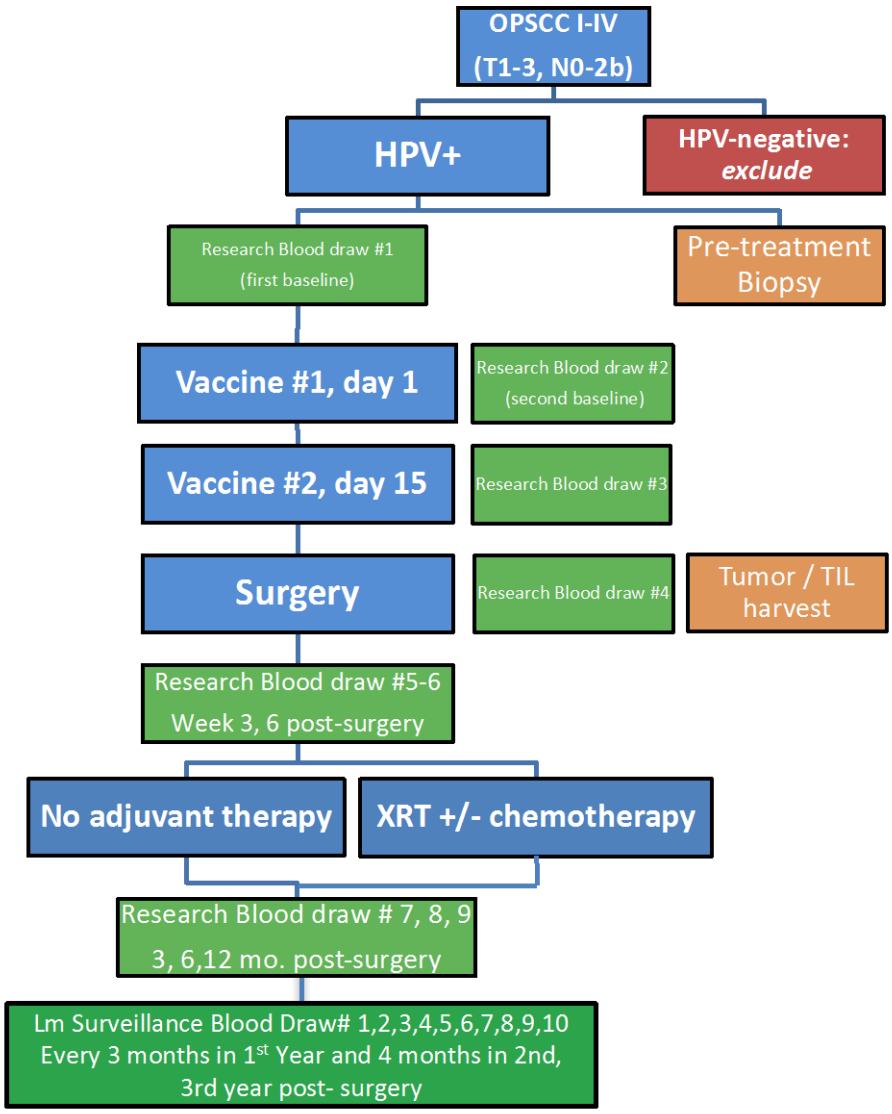


Figure 2. Flow diagram for clinical trial of ADXS11-001 therapeutic HPV vaccine in HPV+ OPC patients. OPSCC – oropharyngeal squamous cell carcinoma. XRT – radiation therapy.

7.0 STUDY CALENDAR

7.1Treatment Group

		Screening	Treatment Week						Pre-Op *5	Surgery *6	Post-Op									
		Day -14 to 0	Day 1	Day 2	Day 4**9	Day 15	Day 16	Day 19**9	**Day 25 - 35**	**Day 28 - 38**	Week 3 +/- 1 week	Week 6 +/-1 week	3 months +/-3 weeks	5 months +/-2 weeks	6 months +/-3 weeks	8 months +/- 1 month	9 months +/- 1 month	10 months +/- 1 month	12 months +/- 1 month	Year 2 and Year 3 +/- 1 month
1	Informed Consent	**1**																		
2	Inclusion/Exclusion Criteria	**1**																		
3	Complete Medical History	**1**																		
4	Concomitant Medications**	**1**	**2**		**3**	**4**		**5**	**6**		7	8	9	10	11	12		13	14	
5	Assessment of Symptoms	**1**	**2**	**3 *8**	**4**	**5**	**6 *8**	**7**	**8**		9	10	11	12	13	14		15	16	
6	ECOG Performance Status	**1**																		
7	Physical Examination	**1**	**2**		**3**	**4**		**5**	**6**		7	8	9	10	11	12		13	14	
8	Tumor Assessment ***1**	**1**	**2**		**3**	**4**		**5**	**6**											
9	CBC with differential	**1**				2			3											
10	CMP	**1**				2			3											
11	PT - PTT	**1**																		
12	Research Blood	**1**	**2**			**3**			**4**		**5**	**6**	**7**		**8**				**9**	
13	Pregnancy Test	**1*14**	**2**			**3**			**4*13**											
14	CT Scan or MRI of the Head & Neck With and Without Contrast ***2**	**1**							**2**											
15	Tumor Measurements ***3**	**1**							**2**											
16	PET CT Scan ***2**	**1**							2 ***7**											
17	Pathology for Tissue Analysis ***4**	**1**								**2**										
18	HPV Testing (PCR) and p16 ***4**	**1**																		
19	Vaccine Administration		1			2														

		Screening	**Treatment Week**						**Pre-Op *5**	**Surgery *6**	Post-Op									
		Day -14 to 0	Day 1	Day 2	Day 4***9**	Day 15	Day 16	Day 19***9**	**Day 25 – 35**	**Day 28 – 38**	Week 3 +/- 1 week	Week 6 +/-1 week	3 months +/-3 weeks	5 months +/-2 weeks	6 months +/-3 weeks	8 months +/- 1 month	9 months +/- 1 month	10 months +/- 1 month	12 months +/- 1 month	Year 2 and Year 3 +/- 1 month
20	Vital Signs		1 ***11**			2***11**														
21	*Lm* Surveillance Monitoring***10**												1		2		3		4	Every 4 months (5-10)
22	Dispense/ Prescribe Oral Prophylactic Antibiotics		1 ***12**			2***12**														

*1 Evaluate tumor or inflammation, size change or other indication of immune flare
*2 Within 45 Days of registration
*3 By RECIST 1.1
*4 Local site's Pathology Laboratory
*5 Maximum 72 hrs before surgery
*6 Pathology Report and Surgery Note will be collected as available
*7 Optional to patient (Desired but not required)
*8 by telephone call
*9 Day 4 and 19 visits may be replaced by a phone call if patient is asymptomatic
*10 Monitoring includes patient interview/exam and blood draws for labs and *Listeria*
*11 Monitor vital signs every 30 minutes (± 5 minutes) immediately prior to and for the first 6 hours following the completion of every ADXS11-001 infusion
*12 7-day course of oral antibiotic therapy starting approximately 72 hours after administration of ADXS11-001, if Patient discontinues the study anytime - 6 month course of oral trimethoprim/sulfamethoxazole or ampicillin
*13 Standard of care test
*14 To be done within 10 -14 days prior to vaccine 1

7.2 Observational Group

		Screening	Pre-Op *5	Surgery *6	Post-Op				
					Week 3 +/-1 week	Week 6 +/-1 week	3 months +/-3 weeks	6 months +/-3 weeks	12 Months +/- 1 month
1	Informed Consent	**1**							
2	Inclusion/Exclusion Criteria	**1**							
3	Complete Medical History	**1**							
4	List of Medications	**1**	**2**		3	4	5	6	7
5	Assessment of Symptoms	**1**	**2**		3	4	5	6	7
6	ECOG Performance Status	**1**							
7	Physical Examination	**1**	**2**		3	4	5	6	7
8	Tumor Assessment ***1**	**1**	**2**						
9	CBC with differential	**1**							
10	CMP	**1**							
11	PT - PTT	**1**							
12	Research Blood	**1**	**4*7**		**5**	**6**	**7**	**8**	**9**
13	CT Scan or MRI of the Head & Neck With and Without Contrast ***2**	**1**							
14	Tumor Measurements ***3**	**1**							
15	PET CT Scan ***2**	**1**							
16	Pathology for Tissue Analysis ***4**	**1**		**2**					
17	HPV Testing (PCR) and p16 ***4**	**1**							
18	Vaccine Administration								

***1** Evaluate tumor or inflammation, size change or other indication of immune flare

***2** Within 45 days of vaccination

***3** By RECIST 1.1

***4** Local Site's Pathology Laboratory

***5** Maximum 72hrs before surgery

***6** Pathology Report and Surgery Note will be collected as available

***7** Patient in this group will not undergo research blood Draw #2 and #3.

8.0 PATIENT IDENTIFICATION, CONSENT, SCREENING, HPV TESTING, ASSIGNMENT

8.1 Patients with biopsy proven squamous cell carcinoma of the oropharynx who are to undergo transoral surgical resection with or without neck dissection will be approached for participation in the study at the Head and Neck Clinic at each site.

8.2 Informed consent will be obtained by a co-investigator or authorized clinical research coordinator.

8.3 After the patient signs the informed consent, the patient will be registered for screening on the each site's EDC (Electronic Data Capture) system. If any question arises during screening process, please contact Study chair at 713-798-3909 and/or Yesenia Ramirez at 713-798-8541.

8.4 Once the investigator has verified that the patient meets all inclusion/exclusion criteria, the patient will be registered for and started on therapy. The screening process will consist of the following:

1. Signed informed consent form
2. Eligibility (Inclusion/exclusion) checklist
3. Complete medical history*
4. List of concomitant medications, taken in the 28 days prior to first vaccine dose
5. Assessment of baseline symptoms
6. ECOG Performance Status
7. Physical examination, including height, weight, and vital signs
8. Tumor Assessment by direct examination
9. Complete blood count with differential
10. Complete metabolic panel
11. Prothrombin time, partial thromboplastin time
12. PET/CT and CT/MRI with or without contrast of the head and neck (within 45 days before registration)
13. Tumor measurements by RECIST 1.1. [51]
14. Tissue analysis, HPV testing (PCR) and p16 tests by local lab – pathological material is required for both tests.

*Surgical history must be included within the complete medical history. Documentation of non-cancer surgeries, including, but not limited to artificial (prosthetic) joints, implants and/or devices, such as port/stent implant placed prior to study enrollment.

8.5 HPV Testing: HNSCC patients undergo routine pre-treatment biopsy of the primary tumor to ensure an accurate diagnosis and allow treatment planning. Following standard-of-care biopsy of the primary tumor, HPV testing will be confirmed prior to vaccination by HPV type-specific PCR for HPV subtype 16 and other oncogenic subtypes. HPV testing will be performed in the designated laboratory at each site. The results of PCR testing will be reported to the patients as a research, not a clinical procedure.

8.6 Following entrance into the study, appropriate patients will be assigned to the vaccine arm by the local site PI. This group will be compared with an observational group of similar patients that will be treated with transoral surgery without vaccination during the same period of time. Since the design of this study is to compare patients' pre- and post-treatment assay results rather than results from vaccinated versus unvaccinated patients, assignment to treatment or observational group will not be randomized. Rather we will offer enrollment in the observational group to patients who do not wish to receive the vaccine but are willing to enroll in the observational portion of this trial.

9.0 ADXS11-001 Safety Precautions, Handling and Preparation Instructions

9.1 Description: ADXS11-001 is a free flowing isotonic, aqueous, cream colored suspension at a pH of 6.8-7.8 supplied in a DIN 2R glass vial (4mL), stoppered with a grey rubber stopper and sealed with an aluminum seal and a blue flip off cap that must be stored frozen at -80 ± 10°C.

9.2 How Supplied: ADXS11-001 is provided on dry ice via bonded courier delivery with temperature monitors in 1.2 mL vials of which 1.0 ml is to be used in the preparation of a dose. ADXS11-001 must be received frozen on dry ice and immediately stored at -80 ± 10°C. ADXS11-001 is stable for 6 hours when stored at room temperature (temperatures at or below 25°C [77°F]). This 6 hour time allows for vial thaw, preparation of infusion and administration. **The 60 minute ADXS11-001 infusion at room temperature must be completed within 6 hours of product vial removal from freezer.**

9.3 Storage and Stability: ADXS11-001 must be received frozen on dry ice and immediately stored at -80 ± 10°C.

- Even though ADXS11-001 is non-pathogenic, all *L. monocytogenes* species are classified as Biosafety Level 2 (BSL-2) according to the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. Universal precautions and institutional guidelines should be used when handling investigational drugs and human specimens.
- Aseptic technique must be strictly observed throughout the preparation procedure including the use of a biologic safety cabinet or hood since ADXS11-001 is live, attenuated *L. monocytogenes.*
- Prior to preparation, the frozen vial of ADXS11-001 should be thawed at room temperature at or below 25°C (77°F) for approximately 5 to 10 minutes.
- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Discard the drug product vial if extraneous particulate matter other than slightly turbid white to off white suspension is observed.
- Do not use ADXS11-001 if discoloration is observed.
- Each dose of ADXS11-001 must be prepared in sterile 0.9% Sodium Chloride Injection, USP (normal saline) and patient must be dosed within 6 hours of drug product removal from the freezer.
- ADXS11-001 MUST **NOT** BE MIXED WITH OTHER DILUENTS.
- Once the vial is removed from the freezer, the ADXS11-001 product is stable at room temperature (temperatures at or below 25°C [77°F]) for 6 hours. The 6 hours

includes the drug product vial thaw, dose preparation, room temperature storage of the prepared dose in the IV bag AND the duration of infusion.

- DO NOT ADMINISTER THE PRODUCT AS AN (INTRAVENOUS [IV] PUSH OR BOLUS).
- DO NOT COMBINE, DILUTE OR ADMINISTER IT AS AN INFUSION WITH OTHER MEDICINAL PRODUCTS.
- ADXS11-001 **must be administered** through a separate and distinct infusion line. ADXS11-001 **must not be administered** via an existing or newly placed central venous catheter or infusion port which is planned to be used for another purpose. In addition, the central venous catheter or infusion port **must not be used** for 72 hours following the completion of the ADXS11-001 infusion **and** following the subject's first post-treatment dose of oral antibiotics.

9.4 Safety Precautions: ADXS11-001 is classified a BSL-2, minimally pathogenic. Precautions as stated in the BMBL 5th Edition[59] include:

Agent: ADXS11-001, live attenuated genetically engineered live vaccine

ADXS11-001 is a live attenuated strain of Listeria monocytogenes (Lm) that has been attenuated such that it is cleared by severe combined immunodeficiency (SCID) mice lacking cellular immunity and gamma interferon knock-out mice lacking adaptive immunity. It has also been altered such that it is impossible for it to recombine with wild-type *Lm*. In the Phase 1 study no bacterial shedding was detected from any subjects treated with ADXS11-001. It is considered as a non-infectious BSL-1 agent for transport by the CDC.

Wild type *Listeria* is Gram-positive, non-spore-forming, facultative bacilli that are hemolytic and catalase-positive. It is a naturally occurring bacterium that is present in the environment and is known to cause illness in some people when they eat foods contaminated with *Lm*. Although healthy adults and children can contract a wild-type *Listeria* infection, they do not usually become seriously ill. People at risk of severe illness from wild-type *Listeria* are pregnant women, newborns, and persons with impaired immune function.

Even though ADXS11-001 is non-pathogenic, all *Lm* species are classified as BSL-2 according to the BMBL 5th Edition. Universal precautions and institutional guidelines should be used when handling investigational drugs and human specimens. Except for the transmission of mother to fetus, human-to-human transmission of *Lm* is not known to occur[60] . Shedding studies completed in Phase 1 demonstrated that, in the absence of antibiotics, ADXS11-001 was rapidly cleared from the blood with no *Lm* detected in the blood of any subject beyond 48 hours post-dosing, and no *Lm* was detected in the urine and feces in any subject at the highest dose of ADXS11-001 tested (1 x 10^{10} CFU)[52] .

Based on the mechanism of action of ADXS11-001 and the inability of *Lm* to be transferred from human-to-human, there is no need for subjects who receive ADXS11-001 to avoid contact with people who are elderly, pregnant, newborns, or have weakened immune systems.

Precautions as stated in the BMBL 5th Edition include:

Wild-type L. monocytogenes poses a potential hazard to laboratory personnel. The Gram-positive, non-spore-forming, aerobic bacilli are hemolytic and catalase-positive. Bacteria have been isolated from soil, dust, human food, animals, and asymptomatic humans. Most cases of listeriosis have arisen from eating contaminated food products, most notably soft cheeses, raw meat, and unwashed raw vegetables. Although healthy adults and children can contract a Listeria infection, they do not usually become seriously ill. At risk of severe illness are pregnant women, newborns, and persons with impaired immune function.

Laboratory Hazards: Wild-type Listeria monocytogenes are ubiquitous in the environment and may be found in feces, cerebrospinal fluid (CSF), and blood, as well as food and environmental materials. Ingestion is the most common mode of exposure, but wild-type *Listeria* can also cause eye and skin infections following a direct exposure. Wild-type Lm infections in pregnant women occur most often in the third trimester and may precipitate labor. Transplacental transmission of *Lm* poses a grave risk to the fetus and may result in disseminated abscesses contributing to a mortality rate of nearly 100%.

Recommended Precautions: BSL-2 practices, containment equipment, and facilities are recommended for activities with clinical specimens and cultures known or suspected to contain the agent. Gloves and eye protection should be worn while handling the agent. Pregnant women who work with *Lm* in the clinical or research laboratory setting should be fully informed of the potential hazards associated with the organism, including potential risks to the fetus.

Disposing of Contaminated Materials: Gloves should be used while cleaning spills. Unless ingested orally or parenterally no pathologic hazard exists. Contaminated materials can be disposed of in sealed containers as medical waste (e.g. closed plastic bags). Spills should be washed and cleaned with the application of commercial chlorine bleach (e.g. Clorox).

9.5 Drug Preparation and Administration: Only sterile containers will be used in the preparation of these materials. They may be autoclaved glassware, disposable containers, or other sterile materials as provided for within each institution's SOP.

Please note that ADXS11-001, the Investigational Study Drug, is a live, highly attenuated microbe, L monocytogenes, which can multiply, thus increasing the dosage, or die off, thus decreasing the dosage. ADXS11-001 is stable for 6 hours when stored at room temperature (temperatures at or below 25°C [77°F]). This 6 hour time allows for vial thaw, preparation of infusion and administration. **The 60 minute ADXS11-001 infusion at room temperature must be completed within 6 hours from product vial removal from the freezer.**

A dose of study drug is prepared in the following manner:

Remove the appropriate number of vials from -80 ± 10°C and thaw the vials at room temperature for approximately 5 to 10 minutes. Gently agitate mildly by hand to ensure that the study drug is in suspension. Prepare the dose and infusion volume of ADXS11-001 as described below.

To make a dose of 1 x 10^9 cfu (Drug Product concentration of 1.9x10^9 cfu/mL): Thaw 1 vial of the ADXS11-001 study drug at room temperature (temperatures at or below 25°C [77°F]). Gently agitate the vial mildly by hand to ensure that the study drug is in suspension. Withdraw 0.53 mL of ADXS11-001 suspension and add it to the infusion bag for a final volume of 500 mL and mix thoroughly.

Each prepared dose of ADXS11-001 must be administered at room temperature (temperatures at or below 25°C [77°F]) within 6 hours from product vial removal from the freezer. This includes vial thaw, room temperature storage of the vial, dose preparation, storage of the infusion suspension in the IV bag and the duration of the infusion. Administer ADXS11-001 at room temperature intravenously over a 60-minute infusion time.

The time the vial is removed from the freezer is to be recorded (T0).

Doses will be administered to patients who have received the prophylactic medication specified in the protocol as a 60 minute intravenous (IV) infusion. The actual time of administration is to be recorded (T1). The actual time the infusion is completed is to be recorded (T2).

9.6 Drug Ordering and Distribution: Please see Appendix A.

10.0 VACCINATION OF PATIENTS WITH ADXS11-001

Two vaccinations with ADXS11-001 will be given at a dose of 1x10^9 cfu intravenously according to the schedule described in the **Study Calendar** above. The drug will be given by a 500ml infusion over 60 minutes through a separate and distinct infusion line with each infusion and this line is to be used for prophylactic medication, pre-medication and ADXS11-001 administration. The line is to be removed prior to patient leaving the clinic and a new line is to be placed with each administration. For safety monitoring purposes, enrollment of the first 3 subjects at any site will be staggered by at least 2 weeks. Once any site has enrolled 3 subjects, then all sites can enroll subjects concurrently.

ADXS11-001 **must not be administered** via an existing or newly placed central venous catheter or infusion port which is planned to be used for another purpose. In addition, the central venous catheter or infusion port **must not be used** for a minimum of 72 hours following the completion of the ADXS11-001 infusion AND following the subject's first dose of oral antibiotics.

10.1 Pre-treatment prophylactic medication regimen: Mild to moderate cytokine release symptoms (e.g., constitutional symptoms such as fever, chills, rigors, fatigue, headache, nausea, vomiting, tachycardia, shortness of breath, hypotension and rash) are commonly

seen and typically occur 2-4 hours after ADXS11-001 infusion and often resolve within 12-24 hours. Prophylactic medications are intended to reduce the inflammatory response. Subjects should receive the following pretreatment prophylaxis regimen.

IV Fluid Hydration:

- Normal saline (e.g., 500 mL over approximately 30-60 minutes)

Premedication Regimen:

- Antihistamine – PO or IV (e.g. diphenhydramine 25 mg or equivalent), once
- NSAIDs - PO (e.g., naproxen 220 mg or ibuprofen, 400 mg), once
- Antiemetic - PO or IV (e.g., promethazine or ondansetron), once
- Histamine H2-receptor antagonist – PO or IV (e.g., famotidine 20 mg or equivalent)

Pretreatment medication should be given on the day of dosing and completed at least 30 minutes prior to the start of the assigned study treatment. Additional NSAID doses and antiemetic administration should be given per label or package insert post initial administration on Day 1 and Day 2 of the ADXS11-001 infusion, as needed. The prescribed dosage of the selected NSAID and antiemetic will be at the discretion of the Investigator.
Do not substitute acetaminophen for the selected NSAID for prophylactic treatment since acetaminophen does not have similar anti-inflammatory properties that could ameliorate cytokine release symptoms.

10.2 After each vaccination, subjects will receive a 7-day course of oral antibiotic therapy starting approximately 72 hours after administration of ADXS11-001. Antibiotic therapy should consist of either 160 mg trimethoprim/800 mg sulfamethoxazole (DS) tablet administered three times a week for 7 days or 80 mg trimethoprim/400 sulfamethoxazole administered daily for 7 days. In subjects with a sulfa allergy, prophylactic antibiotic therapy should consist of ampicillin 500 mg four times daily for 7 consecutive days starting approximately 72 hours after administration of ADXS11-001.

10.3 Treatment is to be done on an outpatient basis. Patients will be observed in the outpatient area for 6 hours following the ADXS11-001 infusion. Vital signs will be taken pre-dose, and every 30 min for 6 hours after each dose.

10.4 Criteria for discharge from observation: At 6 hours after the dose, the patient must have a temperature less than 38.5 degrees C and other vital signs (heart rate, blood pressure, and respiration) must be within normal limits. The patient must not show signs or symptoms of moderate-severe nausea, vomiting, or headache. If the patient does not meet these discharge criteria, the patient should be admitted to the hospital for observation and treatment of side effects.

For those patients stable for discharge the study team will contact the patient by telephone at one and three days following administration of the vaccine to determine if any adverse events have occurred.

10.5 Safety follow-up will be conducted via a telephone call 30 days (±5 days) after the last ADXS11-001 infusion or in week 1 visit post surgery to confirm the resolution of any ongoing AEs and SAEs. Subjects will also continue to be monitored for adverse and serious adverse events during the 3 year Lm surveillance monitoring period of the study.

10.6 Subjects will receive a 6 month course of oral trimethoprim/sulfamethoxazole or ampicillin for subjects with sulfa allergies to be initiated approximately 72 hours of resuming oral intake after transoral surgery, or at the time of study discontinuation. For those patients for whom resumption of oral intake is delayed beyond 72 hours, an alternative delivery route (e.g. gastrostomy tube) may be employed. Trimethoprim/sulfamethoxazole therapy should consist of either 160 mg trimethoprim/800 mg sulfamethoxazole (DS) tablet administered three times a week or 80 mg trimethoprim/400 mg sulfamethoxazole administered daily for 6 months. The dose of ampicillin consists of 500 mg four times daily for 6 months. Review the approved product labeling for Bactrim and ampicillin, and monitor antibiotic tolerance as dosing adjustments may be necessary.

10.7 *Lm* Surveillance Monitoring: In addition, all subjects will participate in a three-year *Lm* surveillance period. Surveillance monitoring for the detection of *Lm* will be initiated at the completion of study treatment according to the protocol or at the time of study discontinuation if earlier. This surveillance monitoring period will consist of a 6-month course of oral antibiotics, obtaining a blood sample to monitor CBC, CMP, including CRP and ESR, and blood cultures at regular intervals. This testing will be performed on all subjects who have received at least 1 dose of ADXS11-001. It will occur at 3 months (+/- 2 weeks), 6 months(+/- 3 weeks), 9 months(+/- 1 Month), 12 months (+/- 1 Month), and every 4 months(+/- 1 Month) for year 2 and year 3 postoperatively. If a persistent increase in CRP and/or ESR is observed with negative blood cultures for *Listeria* during this time, the subject should be evaluated and treated, as appropriate, for another possible cause. In the event that a definite cause has not been identified, subjects must continue to be monitored closely, including additional testing and a blood culture, for possible signs/symptoms of listeriosis. This testing may be performed at the investigational site or at another acceptable location following consultation with the study chair.

10.8 **Treatment modification:** The dose of ADXS11-001 will not be modified (i.e., reduced or increased). However, treatment may be delayed or discontinued based on toxicities, as shown below in Table A.

Treatment modification and supportive care guidelines for cytokine release are included in Appendix A and for infusion related toxicities are included in Appendix B.

Table A: Treatment Delay/Discontinuation Guidelines for Drug-Related Adverse Events

Toxicity	Grade	Result	Action
Hematologic and non-hematologic, excluding cytokine release symptoms	1-2		Continue therapy
	3	Toxicity resolves to ≤ Grade 1 or baseline within 2 weeks of last infusion	Restart therapy
	3	Toxicity not resolved to ≤ Grade 1 or baseline within 2 weeks of last infusion	Discussion with study chair required
	4		Permanent discontinuation from Study

In case toxicity does not resolve to Grade 0-1 within 2 weeks after the last infusion, trial treatment should be discontinued after consultation with the Sponsor. With Investigator and Sponsor agreement, subjects with a laboratory AE still at Grade 2 after 2 weeks may continue treatment in the trial only if asymptomatic and controlled. For information on the management of AEs, see Section 4.6 (Supportive Care).

10.9 **MAJOR AND MINOR SURGERIES AND ADXS11-001 TREATMENT:** No formal studies of the effect of ADXS11-001 on wound healing have been conducted. However, based on its mechanism of action it is not expected that administration of ADXS11-001 would complicate wound healing. Therefore, a subject may initiate or resume study treatment 2 weeks after minor surgery (i.e., surgery involving little risk to the life of the subject; specifically an operation on the superficial structures of the body or a manipulative procedure that does not involve a serious risk) if the wound has completely healed and there are no wound healing complications. A subject who has wound healing complications following minor surgery, received major surgery or requires new implants and/or devices (permitted by the protocol) during the course of the study, must wait a minimum of 6 weeks and must have recovered from any toxicity (e.g., return to baseline or Grade 1) and/or complication before the next infusion of study treatment. Sponsor consultation is required prior to resuming study treatment for these subjects. If the treatment is delayed due to concomitant surgery beyond 12 weeks, the subject may be discontinued from the study.

10.10 **ADVERSE EVENTS AND SUPPORTIVE CARE:** The most likely AEs associated with ADXS11-001 are comprised primarily of individual flu-like symptoms (e.g., fever, chills, body ache, and fatigue) or cytokine release symptoms (e.g., headache, nausea, vomiting, tachycardia, shortness of breath, hypotension and rash). The symptoms usually present within 2-4 hours after the completion of infusions and are often mild to moderate and transient in nature or responds quickly to symptomatic treatment. In rare instances they may last up to 24 hours. No cumulative toxicity has been observed.

Less likely AE's include increase heart rate, low blood pressure, muscle aches, headaches, allergic reaction, changes in blood chemistry, changes in blood counts, and short term changes in liver function.

Rare but serious AEs include high fever, difficulty breathing and hypotension.

Like all Listeria, ADXS11-001 has a tropism for the liver. Transient asymptomatic elevations of ALT and alkaline phosphatase were observed after dosing in the Phase 1 trial without prophylactic medication administration. For that reason, patients with significant liver disease are excluded, and particular attention is to be paid to hepatic abnormalities.

10.10.1 Supportive Care Guidelines: The major safety findings with ADXS11-001 occurring in ≥5% of subjects, as of June 2016 (n=290) and being possibly, probably or definitely related include anemia (61.4%), chills (63.5%), fatigue (25.5%), fever (57.6 %), nausea (36.9%), vomiting (30.3%), headaches (23.8%). Most are Grade 1-2 in severity. Subjects should receive appropriate supportive care measures as deemed necessary by the treating Investigator with consideration given to the criteria the items outlined below.

Nausea and vomiting- should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. Subjects should be strongly encouraged to maintain liberal oral fluid intake.

Cytokine Release Symptoms- are a constellation of inflammatory symptoms resulting from cytokine elevations associated with T cell engagement and proliferation. Symptoms related to cytokine release may include constitutional symptoms such as fever, chills, rigors, fatigue, headache, nausea, vomiting, rash, tachycardia, hypotension and shortness of breath which usually presents several hours after the infusion and lasts for up to 24 hours. These symptoms are caused by an increase in cytokines such as TNFα, IFNγ and IL-6, all of which have been shown to occur after ADXS11-001 administration, resulting from the body's immune response to the therapy. Although, symptoms are often Grade 1-2 and transient, resolving with symptomatic management within 30 minutes to 1 hour, in some instances (~6%) Grade 3-4 hypotension has been seen. Therefore, close monitoring of blood pressure is strongly recommended at baseline, and during the post-infusion period. Increased levels of IL-6 have been strongly associated with capillary leak which manifests as hypotension due to the cytokines involved. We have observed elevated IL-6 levels after infusion of ADXS11-001, with peak levels occurring 2-4 hours after infusion. Emerging evidence indicates that IL-6 antagonists, such as tocilizumab, have demonstrated good results in treating cytokine-induced hypotension [61, 62, 63, 64] and are therefore recommended for cases of severe hypotension refractory to supportive care (e.g., fluids and/or pressors).

The management of cytokine release symptoms and guidelines for subsequent treatment for subjects who have experiences these AEs are shown in the table below.

Table B Recommended Management Guidelines for Adverse Events Associated with Cytokine Release

Toxicity	NCI CTCAE Grade or Severity	Treatment	Modification for Subsequent infusions
Hypotension	1 Mild	• Supportive care	• Increase pretreatment IV fluids (e.g., 500 ml -1L normal saline)
All other cytokine release symptoms (e.g. chills, rigors, fever, nausea, vomiting)	1	• Supportive care	• No modification
Hypotension	2 Moderate	• Fluids and 1 dose of pressor (e.g. 0.3 mg epinephrine IM) • Increase monitoring of vital signs • If hypotension persist for more than one hour consider low dose corticosteroids (e.g. hydrocortisone 100 mg IV over 30 seconds	• Extend infusion time to 2 hours. • Increase pretreatment IV fluids (e.g. 500 ml -1L normal saline) • Incorporate Glucocorticoid-Hydrocortisone or equivalent- 50 mg, IV, as premedication
All other cytokine release symptoms (e.g. chills, rigors, fever, nausea, vomiting)	2	• Appropriate supportive care measure	• Extend infusion time to 2 hours. • Consider increasing doses of prophylactic medications
Hypotension	3 Severe	• Fluids, high dose pressors (e.g. Dopamine 10 µg/kg/min) +1 dose tocilizumab*(4mg/kg over 1 hour) • If hypotension worsens or is unresponsive to above measures, administer corticosteroids	• Discuss with Sponsor

Toxicity	NCI CTCAE Grade or Severity	Treatment	Modification for Subsequent infusions
		• If the subject's condition does not improve or stabilize within 24 hours of the tocilizumab dose, administration of a second dose of tocilizumab +/- corticosteroids should be considered.	
All other cytokine release symptoms (e.g. chills, rigors, fever, nausea, vomiting)	3	• Appropriate supportive care measures	• Extend infusion time to 2 hours. • Consider increasing doses of prophylactic dose of NSAID, or antiemetic as appropriate
Hypotension/ Organ toxicity, mechanical ventilation	4 Life threatening	• Vigilant supportive care • Fluids • High dose pressors, • Tocilizumab (4mg/kg over 1 hour) +/- corticosteroids (hydrocortisone 100 mg IV infused over 30 seconds administered every 2 hours until symptoms resolve to <Grade 1)	• Permanently discontinue

***** Tocilizumab is a humanized, immunoglobulin G1k (IgG1k) anti-human IL-6R mAb approved for treatment of adult subjects with moderately to severely active rheumatoid arthritis (RA) who have had an inadequate response to Disease-Modifying Anti-Rheumatic Drugs (DMARDs), for the treatment of active polyarticular juvenile idiopathic arthritis (PJIA), and active systemic juvenile idiopathic arthritis (SJIA) in subjects 2 years of age and older. Tocilizumab works by preventing IL-6 binding to both cell-associated and soluble IL-6Rs. Although, it is not indicated for the treatment of cytokine release symptoms emerging clinical experience at several institutions has concluded that tocilizumab is an effective treatment for severe or life-threatening cytokine release symptoms. [61, 62, 63, 64]

Management of infusion reactions- While there is some overlap between the symptoms of cytokine release and infusion reactions, infusion reactions typically occur during the infusion, while symptoms associated with cytokine release typically occur after the infusion and are mediated by a different mechanism of action. Signs/symptoms of infusion reactions may include: allergic reaction/ hypersensitivity (including drug fever); arthralgia (joint pain); bronchospasm; cough; dizziness; dyspnea (shortness of breath); fatigue (asthenia, lethargy, malaise); headache; hypertension; hypotension; myalgia (muscle pain); nausea; pruritis/itching; rash/ desquamation; rigors/chills; sweating (diaphoresis); tachycardia; tumor pain (onset or exacerbation of tumor pain due to treatment); urticaria (hives, welts, wheals); vomiting.

Table C below shows the management guidelines for subjects who experience an infusion reaction associated with administration of ADXS11-001.

Table C: Management Guidelines for Infusion Reactions

NCI CTCAE Grade	Management
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.
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs.	1. Stop Infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics 2. 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. Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose.
Grades 3 or 4 **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	3. Stop Infusion. Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine 4. Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the Investigator. 5. Hospitalization may be indicated. 6. Subjects who experience a Grade 4 reaction should be permanently discontinued from study. Subjects who experience a grade 3 reaction may be discontinued. Discussion with the Sponsor is recommended

10.10.2 Listeriosis and Listeria Infection-Identification and Management

A person with wild type (*wt*) listeriosis usually presents with fever and muscle aches, sometimes preceded by diarrhea or other gastrointestinal symptoms. Almost everyone who is diagnosed with listeriosis has an "invasive" infection, in which the bacteria spread beyond the gastrointestinal tract. The symptoms vary with the infected person. Pregnant women typically experience fever and other non-specific symptoms, such as fatigue and aches. However, infections during pregnancy can lead to miscarriage, stillbirth, premature delivery, or life-threatening infection of the newborn. In people other than pregnant women, symptoms can include headache, stiff neck, confusion, loss of balance, and convulsions in addition to fever and muscle aches. Listeriosis can present in different ways. In older adults and people with immunocompromising conditions, septicemia and meningitis are the most common clinical presentations.[58] Subjects may need immediate evaluation with a brain CT scan or MRI and a lumbar puncture with the analysis of spinal fluid to rule out meningitis.

For symptomatic subjects, diagnosis is confirmed only after isolation of *Lm* from a normally sterile site, such as blood or spinal fluid (in the setting of nervous system involvement), or amniotic fluid/placenta (in the setting of pregnancy). Stool samples are of limited use and are not recommended. *Lm* can be isolated readily on routine media, but care must be taken to distinguish this organism from other Gram-positive rods, particularly diphtheroids. Selective enrichment media improve rates of isolation from contaminated specimens. You can expect that the cultures will take approximately 1-2 days for growth. Importantly, a negative culture does not rule out infection in the presence of strong clinical suspicion. Serological tests are unreliable, and not recommended at the present time [58].

Listeriosis is treated with a wide range of antibiotics. In preclinical studies, wt-*Lm* and ADXS11-001 are susceptible to the lowest tested concentration of the following antimicrobial agents: ampicillin, amoxicillin/K clavulanate, ciprofloxacin, erythromycin, gentamicin, penicillin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin (IV). ADXS11-001 is resistant to both streptomycin and chloramphenicol.

10.10.3 Management and Surveillance of Listeria during Study Participation

In the event a subject experiences a persistent fever lasting 72 hours after receiving study treatment then the oral antibiotic regimen will be replaced by broad spectrum IV antibiotic treatment. If symptoms consistent with sepsis occur close to ADXS11-001 administration or at any time after ADXS11-001 administration, immediate medical attention must be sought. A microbial culture will be taken to identify the agent of sepsis and antibiotic sensitivity testing should be performed to confirm susceptibility. An infectious disease consult should be obtained for further management of these subjects.

All subjects will receive a 6-month course of an oral antibiotic regimen as a prophylactic measure approximately 72 hours following the completion of the last dose of ADXS11-001 treatment or at the time of study discontinuation. This additional safety measure is intended to eradicate *Lm* from the body.

Lm surveillance monitoring will also be initiated following the completion of the last dose of ADXS11-001 treatment or at the time of study discontinuation. This monitoring will include obtaining a blood sample for CBC, comprehensive metabolic panel (CMP), including CRP and ESR, and blood cultures for the detection of *Listeria*. Testing will be performed on all subjects who have received at least 1 dose of ADXS11-001 and occur 3 months(+/- 2 weeks), 6 months(+/- 3 weeks), 9 months(+/- 1 Month), 12 months(+/- 1 Month), and every 4 months(+/- 1 Month) for year 2 and year 3 postoperatively.

Should a diagnosis of listeriosis be made at any point after treatment with ADXS11-001 is completed, immediate and intensive IV antibiotic treatment (ampicillin +/- gentamycin or other IV antibiotic regimen as indicated) is required. An infectious disease consult should be obtained. Based on each individual subject's case and at the discretion of the treating physician, the removal of any foreign medical object that has been present since treatment with ADXS11-001 was initiated may be warranted. It is extremely important that the Investigator, his/her research staff, other healthcare providers involved in the care of the subject as well as each subject participating in this study are educated and made aware of the signs and symptoms of listeriosis and the potential for delayed listeremia/listeriosis. Educational materials for the Investigator, research staff, health care providers and subjects will be prepared and educational training performed.

10.10.4 Potential for delayed/late Listeria infection:

ADXS11-001 has been attenuated by ≥4 logs in comparison to the wild-type (wt)-*Lm*. ADXS11-001 is cleared by SCID mice lacking a functioning cellular immune system, as well as by gamma interferon knockout mice lacking adaptive immunity. ADXS11-001 has been shown to be nonpathogenic in mouse models. In a Phase 1 clinical study, in the absence of antibiotics, *Lm* was rapidly cleared from the blood. No *Lm* was detected in the blood, urine or feces of any subject beyond 48 hours post-dosing and at the highest dose of ADXS11-001 tested (1×10^{10} CFU)[52].

wt-*Lm* is known to form and persist within biofilms especially on medical devices despite antibiotic treatment.[53] Although rare, medical device–related infections such as ventriculo-peritoneal shunt infection, peritoneovenous shunt infection, and prosthetic joint infection have been reported.[54-57] ADXS11-001 is highly sensitive to antibiotics such as ampicillin and trimethoprim/sulfamethoxazole which can be an effective treatment regimen for Listeria infection. However, to reduce the risk of ADXS11-001 persisting within a patient by attaching to the surface of a fixed medical device by establishing a biofilm, patients with indwelling medical devices require special consideration. Therefore, subjects with implanted medical device(s) that pose a high risk for colonization and/or cannot be easily removed are excluded from this study. In addition, all subjects will receive a course of oral antibiotics beginning on Day 4 (approximately 72 hours) after each dose of ADXS11-001 and for 6 months following the last dose of ADXS11-001 to aid in the eradication of the bacteria.

As of June 2016, approximately 290 subjects have received approximately 722 doses of ADXS11-001. During the course of treatment in this subject population, there has been one reported case of Grade 3 listeriosis. The subject was a 56 year old female who was enrolled in

November 2012 in Study GOG-0265. During the course of her participation in the study, she suffered a motor vehicle accident which required multiple orthopedic surgeries involving the placement of hardware and a bone graft. After completing her participation in the study, she went on to receive multiple other treatments for her metastatic cervical cancer including an investigational PI3K inhibitor. Two and a half years after completing her last administration of ADXS11-001, she presented to the hospital with mental confusion and a fever. Blood cultures were positive for *Lm*. Subsequent analyses revealed that the isolate was essentially the same as ADXS11-001 but without the plasmid. The subject was admitted to the hospital and treated with IV Ampicillin. She became afebrile and was discharged home three days after her admission. Prior to her discharge a spinal tap and a CT of the brain were recommended but the subject refused. Two weeks later the subject presented with acute respiratory distress caused by her metastatic disease and died. The investigator ruled that cause of death was due to disease progression (metastatic cervical cancer). It is hypothesized that dosing of attenuated *Lm* soon after her bone graft may have resulted in biofilm formation, which could have protected the *Lm* from both the immune system and antibiotics. At no time while the subject was on study or during the 2.5 years post-study did she show signs or symptoms of listeriosis. The *Lm* isolate also remained highly susceptible to multiple antibiotics.

Following recognition of this case of persistent Listeria colonization and delayed infection, new exclusion criteria and long-term monitoring provisions were added to this protocol to minimize the chance of this adverse event.

Complete information on the toxicity profile can be found in the ADXS11-001 Investigator's Brochure.

11 TRANSORAL SURGERY FOR OROPHARYNGEAL CANCER

1. Patients have previously been determined by an attending Head and Neck surgeon and the multidisciplinary tumor board at each site, to be eligible for transoral resection.
2. Standard-of-care transoral surgery with intent to perform complete resection of the primary tumor will be performed according to our previously described methods[50]. In brief: surgery will include transoral robotic resection or transoral laser resection of the tumor with negative intraoperative frozen section margins; in selected cases it may be appropriate to remove the tumor with hand-held electrocautery under direct vision. Appropriate reconstruction will be performed at the time of tumor resection as deemed necessary by the operating surgeon.
3. Selective neck dissection of levels II-IV will be performed routinely ipsilateral to the primary tonsillar tumors. Bilateral selective neck dissection of levels II-IV will be performed for all tongue base tumors. Additional levels (i.e. I, V) will be performed at the discretion of the operating surgeon if indicated on the size and location of the primary tumor. N+ patients will undergo a simultaneous appropriate therapeutic neck dissection.

11.1 POSTOPERATIVE ADJUVANT THERAPY AND CLINICAL FOLLOW-UP

1. Adjuvant radiation therapy or chemoradiation will be given to appropriate patients based on preoperative and pathological risk factors as per standard-of-care. Participation in this trial will in no way alter the decision to provide post-surgical radiation or chemoradiation. While there is some leeway based on clinical judgment, standard criteria for adjuvant therapy include: positive surgical margin; lymphovascular invasion (LVI); perineural

invasion (PNI); extracapsular spread (ECS) of lymph node metastases or matted lymph nodes; low (level IV) cervical lymph nodes; >2 positive lymph nodes. These criteria are fairly standard across many institutions. If adjuvant therapy is indicated, therapy usually begins 4 to 6 weeks after surgical resection.

2. Patients will undergo routine post-treatment clinical follow-up, which usually involves monthly visits for the first year and q2-3 month visits in the second year following treatment. Imaging is obtained at the discretion of the treatment team, but is generally obtained no less than q 3 months for the first 2 years after treatment.
3. Assessment of disease status (no evidence of disease / NED; recurrent disease; second primary upper aerodigestive tumor; other second primary cancer) is performed by the attending surgeon at each follow-up appointment, and recorded in the electronic medical record (EMR).

12.0 SCHEDULE OF BLOOD AND SAMPLE COLLECTION

12.1 TREATMENT GROUP

1. The day of the first dose of vaccination will be considered “day 1”.
2. Standard-of-care pre-treatment tumor biopsy will be performed in a timely fashion for those patients without a prior biopsy, whose prior biopsy is inadequate for clinical planning, or for whom prior biopsy material adequate for immunophenotyping by immunohistochemistry (IHC) is not available.
3. Initial blood draw (Draw 1) will be obtained prior to the first vaccination usually during screening visit.
4. Draw #2 will be performed on the day of the first vaccination (e.g. day 1) before vaccine infusion.
5. Draw #3 will be performed on the day of the second vaccination (day 15) before vaccine infusion.
6. Surgery, and harvest of tumor and TIL, will be performed on day 35 +/- 7 days following entry into the study and baseline blood draw.
7. Draw #4 will be performed preoperatively, on the day of surgery prior to induction of anesthesia, or up to 72 hours prior to the surgery.
8. Draw #5 will be performed 3 weeks (+/- 1 week) after surgery.
9. Draw #6 will be performed 6 weeks (+/- 1 week) after surgery, before radiation.
10. Draw #7 will be performed 3 months (+/- 3 weeks) post-surgery.
11. Draw #8 will be performed 6 months (+/- 3 weeks) post-surgery.
12. Draw #9 will be performed 12 months (+/- 4 weeks) post-surgery.

12.1.1 LISTERIA SURVEILLANCE MONITORING BLOOD DRAW: Up to 45 ml of additional blood will be collected for safety follow up testing as *Listeria* surveillance monitoring. It will include CBC, CMP, including CRP and ESR and blood cultures for the detection of Listeria.

Patients in the control group will NOT undergo additional blood draw or monitoring. These blood draws will be performed at 3 months(+/- 2 weeks), 6 months(+/- 3 weeks), 9 months(+/- 1 Month), 12 months(+/- 1 Month), and every 4 months(+/- 1 Month) for year 2 and year 3 as mentioned in schedule above.

12.2 OBSERVATIONAL GROUP

1. The day of screening will be considered “day 0”. Initial blood draw (Draw 1) will be obtained at screening.
2. Standard-of-care pre-treatment tumor biopsy will be performed in a timely fashion for those patients without a prior biopsy, whose prior biopsy is inadequate for clinical planning, or for whom prior biopsy material adequate for immunophenotyping by immunohistochemistry (IHC) is not available.
3. Draw # 2: Observational patients will NOT undergo this blood draw.
4. Draw # 3: Observational patients will NOT undergo this blood draw.
5. Draw #4 will be performed preoperatively, on the day of surgery prior to induction of anesthesia, or up to 72 hours prior to surgery.
6. Draw #5 will be performed 3 weeks (+/- 1 week) after surgery.
7. Draw #6 will be performed 6 weeks (+/- 1 week) after surgery, before radiation.
8. Draw #7 will be performed 3 months (+/- 3 weeks) after surgery.
9. Draw #8 will be performed 6 months (+/- 3 weeks) after surgery.
10. Draw #9 will be performed 12 months (+/- 4 weeks) after surgery.

12.3 COLLECTION AND PROCESSING OF PERIPHERAL BLOOD SAMPLES

1. Baseline blood samples will be collected either immediately after consent or prior to the first vaccination. Samples will be collected at baseline, on the day of surgery (following two rounds of vaccination), and according to the schedule described above.
2. At each collection, peripheral blood samples (eight 7mL tubes, 56 mL total) will be withdrawn by a clinical research coordinator, a medical assistant or a clinic nurse and promptly transported to the designated laboratory for processing. Immediately prior to drawing blood, the person in charge of the procedure will verify the subject‘s identity. Immediately after the blood has been collected, a label containing the appropriate subject identification number and the subject‘s initials is to be affixed to the vial.
3. Serum will be obtained by centrifugation, and stored in 250uL aliquots at -80°C. Whole blood will be processed over a Ficoll gradient to obtain PBMC, and PBMC will be washed, counted, aliquotted and frozen in DMSO-containing freezing medium prior to long-term storage in liquid nitrogen. All tubes will bear self-adhesive labels that clearly identify the trial code, the patient number, the patient initials, and the visit number. Sterile technique will be maintained throughout so that viable cells may be cultured and assayed after thawing.

12.4 COLLECTION AND PROCESSING OF TUMOR SPECIMENS

1. Following resection, tumor specimens will be expeditiously transported to the pathology suite, where a pathologist will evaluate and identify the portion of the specimen in excess of that required for pathological analysis.
2. A small piece will be flash-frozen and transported to each institution's designated banking resource facility as is routinely performed for all head and neck cancer specimens.
3. Where tumor size and quality permit, the remainder of the specimen will be transported on ice to the designated laboratory for harvesting tumor-infiltrating lymphocytes (TIL) according to a standard protocol. Briefly, after removal of fat, blood and necrotic areas, tumor tissue will be washed in RPMI-1640 containing 50 µg/ml gentamycin, and cut into 1 mm 3 pieces in a petri dish covered with RPMI-1640. The sample is washed again with RPMI-1640 and upon transfer to flasks, dissociated using 0.05% collagenase type IV and 0.02% DNase type I in RPMI-1640 supplemented with 5% (v/v) fetal calf serum and antibiotics. Tissues will then be dissociated for up to 4 h using a magnetic stirrer at 37°C. The digest will then be passed through 90 µm and 50 µm nylon mesh to remove clumps, and the filtrate washed in 2–3· mL medium followed by centrifugation at 350· g for 10 min and resuspension in 2 mL medium.
4. PBMC will be washed, counted, aliquotted and frozen in DMSO-containing freezing medium prior to long-term storage in liquid nitrogen. All tubes will bear self-adhesive labels that clearly identify the trial code, the patient number, the patient initials, and the visit number. Sterile technique will be maintained throughout so that viable cells may be cultured and assayed after thawing.

12.5 IMMUNOPHENOTYPING AND CHARACTERIZATION OF HPV-SPECIFIC T CELL RESPONSES

1. Following purification from whole blood or tumor, mononuclear cells will be cultured 48 hours in the presence or absence of pools of 15-20-mer overlapping peptides encompassing the full-length HPV16 E6 and E7 antigens, or control (CMV, EBV and Flu virus) peptide mixtures.
2. After 48 hrs of stimulation, an aliquot of supernatant from the culture will be harvested and frozen at -80°C for future studies of cytokine production.
3. E6/E7-specific T cell responses will be determined by ELISPOT, flow-cytometry for intracellular IFN-γ staining, or other comparable method. In brief, staining for T cell surface markers (CD3, CD4, CD8) with labeled antibodies is performed prior to fixation/permeablization of cells. Fix/permed cells are then stained with labeled antibody to IFN-γ, a marker of activated cytotoxic T cells. Multicolor flow cytometric analysis is then performed on an LSR II flow cytometer.
4. Additional flow cytometry staining panels to characterize NK cells (CD56), T cell activation and exhaustion markers (granzyme, perforin, CTLA-4, PD-1), regulatory T cell (CD127, CD25), and myeloid populations (CD11c, CD11b, CD33, HLA-DR) will be used to perform multi-dimensional immunophenotyping of effector and regulatory cell populations.

12.6 IMMUNOHISTOCHEMISTRY (IHC) AND MOLECULAR PROFILING OF TUMOR SPECIMENS

1. Tumor specimens obtained during standard-of-care resection will be harvested for preparation of tissue microarrays (TMA) from formalin-fixed, paraffin-embedded (FFPE) tissue specimens, according to each site's routine protocols. Histological slides which include tumor tissue and surrounding normal tissue from the tumor margin will be created with the help of the designated pathology facility at each site.

2. Histological slides will be subjected to immunofluorescent staining and analysis. These data are quantified using image analysis instrumentation and software. This approach has allowed successful characterization and quantification of up to 5-7 antigens in single sections[54].

3. We will utilize a comprehensive panel of IHC markers including markers for myeloid cells; Treg (CD4/FOXP3); NK cells (CD56); and CD3+, CD4+ and CD8+ T cells. Staining for these markers will be examined by multiplex immunofluorescence and their density in tumor quantitated by digital image analysis. Tumor infiltrating myeloid cells will be identified with the markers CD33 (myeloid-derived suppressor cells, MDSC), CD11b (MDSC, some macrophages, some DCs), and CD68 (macrophages), and neutrophils (CD66b).

4. A portion of the tumor specimen will be flash frozen according to each site's routine protocols, and cryopreserved for future molecular biology analyses of immune and cancer-related gene expression.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Subjects may withdraw consent at any time for any reason or be discontinued from the trial at the discretion of the investigator, should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the sponsor/Study Chair if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

Patients will be removed from the study, and notified of this fact, under the following circumstances:

1. Failure to obtain at least one baseline blood draw.
2. Failure to receive at least one vaccination (if in vaccine arm).
3. The patient experiences a grade IV toxicity.

If a patient is removed from the study or if the patient wishes to discontinue participation before all visits and monitoring are complete, the following will occur:

- **For patients in the treatment group, 3 years of *Lm* surveillance monitoring will be offered.**
- **Patients in the treatment group will be provided with sufficient antibiotics to complete the 6 months of post-vaccine treatment as described above.**

14.0 Concurrent Therapies and Procedures

All prescription and nonprescription medication (excluding vitamins, nutritional supplements and hormone replacement therapy) taken by the subject from 30 days prior to screening and up to and including completion of the 3-year *Lm* surveillance period will be recorded in the medical record and on the eCRF. Any addition, deletion, or change in the dose of these medications will also be recorded. Generic names should be used to eliminate confusion that may result from trade names. Protocol-mandated prophylactic medications, antibiotics and procedures administered/performed following the completion of study treatment, including during the 3-year *Lm* surveillance period, should also be captured in the eCRF.

Study subjects should be reminded that acetaminophen should not be used for pretreatment prophylaxis associated with the foreseeable AEs related to the study treatment since this medication can interfere with treatment.

Medications 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 specifically prohibited during the trial, discontinuation from trial therapy may be required. The Investigator should discuss any questions regarding this with the Sponsor. The final decision on any supportive therapy rests with the Investigator and/or the subject's primary physician. However, the decision to continue the subject's on-trial therapy requires the mutual agreement of the Investigator, the Sponsor, and the subject.

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

- Anti-cancer systemic chemotherapy or biological therapy
- Surgical treatment as per consultation with the sponsor
- PI3K and TNFα inhibitors
- Immunotherapy not specified in this protocol
- Investigational agents other than ADXS11-001
- Radiation therapy (except palliative radiation therapy for disease-related pain with a consult with the sponsor's medical monitor)
- 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, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines and are not allowed.
- Acetaminophen is not to be used for premedication but may be used for supportive care measures. NSAIDs, such as naproxen and ibuprofen have been evaluated and are confirmed not to interfere with efficacy.

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.

15 Treatment Discontinuation:

Surveillance monitoring for the detection of *Lm* will be initiated at the completion of study treatment according to the protocol or at the time of study discontinuation if earlier. This surveillance monitoring phase will consist of a 6 month course of oral antibiotics, obtaining a blood sample to monitor CBC, CMP, including CRP and ESR, and blood cultures at regular intervals. This testing will be performed on all subjects who have received at least one dose of ADXS11-001. It will occur at 3 months(+/- 3 weeks), 6 months(+/- 3 weeks), 9 months(+/- 1 Month), 12 months(+/- 1 Month), and every 4 months(+/- 1 Month) for 2nd and 3rd year beginning after the last dose of study treatment. If a persistent increase in CRP and/or ESR is observed with negative blood cultures for Listeria during this time the subject should be evaluated and treated, as appropriate, for another possible cause. In the event that a definite cause has not been identified then subjects must continue to be monitored closely, including another additional testing and a blood culture, for possible signs/symptoms of listeriosis. This testing may be performed at the investigative site or at another acceptable location following consultation with the Sponsor.

16.0 STATISTICAL CONSIDERATIONS

We will employ the Simon's two-stage design for the phase II clinical trials. (Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials 1989; 10: 1-10.) This trial design maximizes the chances of detecting a true difference in the HPV-specific T cell frequencies in pre- and post-vaccination samples while minimizing the number of patients treated if there is no difference. We plan to enroll and vaccinate up to 22 HPV+ OPC patients. In parallel we also plan to enroll and collect samples from up to 10 un-vaccinated HPVOPC patients in the observational group.

(a) Definition of primary outcome/endpoint:

We will determine the rate of vaccine-induced T cell responses at the time of surgical resection. For evaluation of immune responses in the peripheral blood, for each patient, a vaccine-induced T cell response will be considered:

1. Any post-vaccination E6- or E7-specific T cell response frequency (response to E6 or E7 peptide pool, minus nonspecific background, measured by ELISPOT or intracellular cytokine staining) equal or greater to two times the initial pre-vaccination baseline response. Where two baseline measurements are available, the mean value will be used.
2. When pre-vaccination E6/E7-specific T cell responses are not observed, any post-vaccination response at least two-fold greater than the average value of nonspecific background (for ELISPOT, approx. 10-15 spots/well) will be considered a positive response.

Toxicity will be scored using the NCI CTCAE 4.0 criteria, with any grade 3 or 4 toxicity considered as an adverse outcome.

(b) Definition of secondary outcomes/endpoints:

1. We will determine the rate of vaccine-induced T cell responses at various time-points after surgery (3W, 6W, 3M, 6M, 12M). Otherwise as per the primary outcome/endpoint

(c) Analytic plan for primary objective:

For each patient, the baseline mean frequency +/- SD of peptide-specific IFN-γ and/or TNFα expressing T cells in the peripheral blood will be determined by averaging the results of 3 replicate assays. At each subsequent blood draw, the mean frequency +/- SD of IFN- γ positive T cells will be determined in an identical fashion, and vaccine-induced T cell responses determined as described above. The response to each peptide will be evaluated separately. While the ADXS 11-001 vaccine contains the HPV E7 protein, cross-reactive responses to E6 and other HPV proteins can be observed following vaccination (personal communication, Robert Petit, Advaxis Pharmaceuticals) so we will monitor responses to E6, E7 and E2 peptides.

The trial is designed to conclude that ADXS 11-001 vaccination is likely highly immunogenic and worth further investigation if the overall rate of vaccine-induced T cell responses is 75% or more. ADXS 11-001 vaccination will be deemed not worthy of further investigation if the overall rate of vaccine-induced T cell responses is less than 50%.

For this trial, we propose a maximum sample size of 22 subjects, with 9 eligible subjects accrued in the first stage and 13 eligible subjects accrued in the second stage. One interim monitoring will be performed at the end of the first stage. The trial will be terminated early if it is evident that the T cell response rate is lower than the acceptable level. Since we do not yet know when the peak effect of vaccination will be, we will consider any responses at time of surgery or at 3 or 6 weeks after surgery to “count” as positive vaccine-induced T cell responses. If responses in five (5) or fewer patients are observed during the first stage, we will conclude that ADXS 11-001 vaccination does not merit continued investigation due to lack of immunologic efficacy. Otherwise accrual will continue until a total of 22 evaluable patients have been vaccinated and assessed for T cell response and toxicity.

If after completion of accrual more than fourteen (14/22) patients have vaccine-induced T cell responses, we will conclude that ADXS 11-001 vaccination is sufficiently active to justify recommendation for future studies.

(d) Analytic plan for secondary objectives:

The frequency of ELISPOT-positive T cells in the peripheral blood will be determined as described above for each patient at baseline and at different time points after surgery (3W, 6W, 3M, 6M, 12M). The average number of ELISPOT-positive T cells in the baseline and post-surgical samples will be compared, with $p<0.05$ by two-sided paired student’s T test considered a statistically significant difference.

(e) Sample size justification:

Using this design for the primary objective, the type-I error is 10%, and the power is 80.7%. If the T cell response rate is indeed only 50%, then the probability to stop the trial at the end of first stage is 75%, and the expected sample size of 13.

17.0 DATA AND PROTOCOL MANAGEMENT

The study chairperson, and IND holder Dr. Sikora, the site principal investigator and the site co-investigator will be jointly responsible for the management of the protocol. All patients will be treated according to the recommendations of the institutional Head and Neck tumor board, and the standard-of-care for their disease.

17.1 DATA CONFIDENTIALITY

All efforts will be made to ensure patient confidentiality and assurance of HIPAA compliance. Immediately after obtaining any specimens and microscopic images, subjects will be assigned a protocol specific unique code that will be used for all further data management. A list matching the patient medical record number to the protocol specific unique code will be kept in a locked cabinet in the office of the site PIs. The names of the patients will not be released to any outside organizations or to persons not involved with the study. They will not be revealed in written reports or publications detailing the research findings. All electronic data with patient identifying information will be maintained on password-protected computers at the respective institution. Use of portable media devices (external hard drives, USB drives, etc.) will be discouraged; if use of portable media devices is required for transfer of data, these devices are to be password protected and encrypted.

17.2 PROTOCOL COMPLIANCE

The primary research coordinator for this protocol will be responsible for accurate implementation of the protocol. Any deviations from the protocol will be reported to Dr. Sikora and the site PIs, who will determine the appropriate course of action.

17.3 PROCEDURE TO OBTAIN INFORMED CONSENT

Patients to undergo transoral surgical resection of oropharyngeal cancer will be identified by the study team at weekly tumor board conference, or on their initial visit to the clinic at each institution. Participation in the trial will be discussed with the patient by a study investigator or approved delegate prior to the initiation of treatment. Consent will be obtained by a study investigator or study coordinator. All patient confidentiality will be strictly maintained in accordance with HIPAA regulations.

The principal investigators, all co-investigators, and study coordinators have taken the NIH approved Institutional Instructional Course for Studies Involving Human Tissues and must have completed Human Research Protection Education requirements by their institution.

17.4 REPORTING REQUIREMENTS - ADVERSE EVENTS

17.4.1 Adverse event (AE): any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of study treatment (ADXS11-001 or Placebo) is also an AE.

A serious AE (SAE) is any AE occurring at any dose or during any use of Advaxis' product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

All adverse clinical experiences, whether observed by the investigator or reported by the patient, must be recorded, with details about the duration and intensity of each episode, the action taken with respect to the test drug, and the patient's outcome. The investigator must evaluate each adverse experience for its relationship to the test drug and for its seriousness.

The PIs, co-Investigator, or a delegate will query the patient about adverse event (AE) at each follow-up visit, and notify the PIs, who will be responsible for maintaining records of all AEs. Reporting will follow the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. Any unanticipated adverse events resulting from this study will be reported to the study chair. Additionally, unanticipated and SAEs should be reported to each site's institutional review boards as per their requirements and by the IND holder to the FDA according to the 21CFR312.32.

Post 30 day AE assessment and for 3 year safety observation period: It is required that sites submit all SAEs that are thought to be possibly related to ADSX11-001 or which have any potential relationship or correlation to Listeria. This requirement is applicable for the ENTIRE Lm follow-up period of 3 years. All other SAEs or AEs not related to ADXS11-001 or with no relation to, suspicion of or possibility of being late listeriosis, do not need to be reported.

To provide an enhanced opportunity to detect any adverse events, a 2 week delay (stagger) will be implemented between enrollment of patient #1 and #2 and patient #2 and #3.

17.4.2 Expected adverse events include:

1. Minor, self-limiting pain due to the injection.
2. Minor, self-limiting, localized injection-site reaction <2cm and lasting for less than 72 hours.
3. Fever (>100.9°F) with or without chills for up to 24 hours.
4. Anemia, Headache, Vomiting and Nausea
5. Tender cervical lymphadenopathy (possible).

Please refer to the ADXS11-001 IB for a current list of expected adverse events.

Progression of the cancer under study is not considered an AE unless it results in hospitalization or death.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset and end dates, severity (grade of the event), Investigator's opinion of the relationship to ADXS11-001 (see definitions below), treatment/action required for the AE, and information regarding resolution/outcome.

17.4.3 Reporting Abnormal Test Findings

The criteria for determining whether an abnormal test finding should be reported as an AE are as follows:

- Test result is associated with accompanying symptoms; and/or
- Test result requires additional diagnostic testing or medical/surgical intervention: and/or
- Test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatments, or other; and or
- Test result is considered to be an AE by the Investigator or sponsor

Merely repeating an abnormal test, in the absence of any of the above conditions does not constitute an AE. An abnormal test result that is determined to be an error does not require reporting as an AE.

17.4.4 Reporting Cytokine Release Syndrome

As per NCI CTCAE v 4.03, CRS is defined as a disorder characterized by nausea, headache, tachycardia, hypotension, rash, and shortness of breath. Subjects must have experienced ALL symptoms for an AE to be documented as CRS. Individual symptoms associated with CRS should not be reported as CRS, but should be reported as separate AEs.

17.5 Data/Safety Monitoring

AE reporting and data and protocol monitoring will be performed in accordance with the protocol-specific Data Monitoring and Reporting Plan and current Data Safety Monitoring Plan for investigator-initiated Phase I and II studies in the Dan L Duncan Cancer Center at Baylor College of Medicine.

18.0 REFERENCES

1. Parkin DM, Bray F, Ferlay J, et al. Estimating the world cancer burden: Globacan 2000. *Int J Cancer* 2001; 94:153-56.
2. American Cancer Society. Cancer Facts and Figures, 2007. Available at American Cancer Society, http://www.cancer.org.
3. Nasman A, Attner P, Hammarstedt L, Du J, Eri- ksson M, Giraud G, et al. Incidence of human papillomavirus (HPV) positive tonsillar carci- noma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? *Int J Cancer* 2009; 125:362–6.
4. Ringström, E. et al. 2002. Human papillomavirus type 16 and squamous cell carcinoma of the head and neck. *Clin. Cancer Res* 8, 3187-3192.
5. Fakhry, C. & Gillison, M.L. 2006. Clinical Implications of Human Papillomavirus in Head and Neck Cancers. *J Clin Oncol* 24, 2606-2611.
6. Fakhry, C. et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J. Natl. Cancer Inst* 100, 261-269 (2008).
7. Ang, K.K. et al. 2010 Human Papillomavirus and Survival of Patients with Oropharyngeal Cancer. *N Engl J Med* .doi:10.1056/NEJMoa0912217
8. Siddiqui, M.A.A. & Perry, C.M. 2006. Human papillomavirus quadrivalent (types 6, 11, 16, 18) recombinant vaccine (Gardasil). *Drugs* 66, 1263-1271; discussion 1272-1273.
9. Stanley, M. 2006. Immune responses to human papillomavirus. *Vaccine* 24 Suppl 1, S16-22.
10. Lehoux, M., D'Abramo, C.M. & Archambault, J. 2009. Molecular mechanisms of human papillomavirus-induced carcinogenesis. *Public Health Genomics* 12, 268-280.
11. de Vos van Steenwijk, P.J. et al. 2010. An unexpectedly large polyclonal repertoire of HPV-specific T cells is poised for action in patients with cervical cancer. *Cancer Res* 70, 2707-2717.
12. Albers, A. et al. 2005. Antitumor activity of human papillomavirus type 16 E7-specific T cells against virally infected squamous cell carcinoma of the head and neck. *Cancer Res* 65, 11146-11155.
13. Hoffmann, T.K. et al. 2002. T cells specific for HPV16 E7 epitopes in patients with squamous cell carcinoma of the oropharynx. *Int. J. Cancer* 118, 1984-1991 (2006).
14. Tindle, R.W. Immune evasion in human papillomavirus-associated cervical cancer. *Nat. Rev. Cancer* 2, 59-65.
15. Grulich, A.E., van Leeuwen, M.T., Falster, M.O. & Vajdic, C.M. 2007. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 370, 59-67.
16. Chaturvedi, A.K., Madeleine, M.M., Biggar, R.J. & Engels, E.A. 2009. Risk of human papillomavirus-associated cancers among persons with AIDS. *J. Natl. Cancer Inst* 101, 1120-1130.
17. Frumento, G., Piazza, T., Di Carlo, E. & Ferrini, S. 2006. Targeting tumor-related immunosuppression for cancer immunotherapy. *Endocr Metab Immune Disord Drug*

Targets 6, 233-237.

18. Kim, R., Emi, M., Tanabe, K. & Arihiro, K. 2006. Tumor-driven evolution of immunosuppressive networks during malignant progression. *Cancer Res* 66, 5527-5536.
19. Marigo, I., Dolcetti, L., Serafini, P., Zanovello, P. & Bronte, V. 2008. Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. *Immunol. Rev* 222, 162-179.
20. Gabrilovich DI, Nagaraj S. 2009. Myeloid-derived suppressor cells as regulators of the immune system, *Nat Rev Immunol* 9, pp. 162–174.
21. Almand, B., J. I. Clark, E. Nikitina, J. van Beynen, N. R. English, S. C. Knight, D. P. Carbone, D. I. Gabrilovich. 2001. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J. Immunol.* 166: 678-689.
22. Diaz-Montero, C. M., M. L. Salem, M. I. Nishimura, E. Garrett-Mayer, D. J. Cole, A. J. Montero. 2009. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol. Immunother.* 58: 49-59.
23. Sica, A., V. Bronte. 2007. Altered macrophage differentiation and immune dysfunction in tumor development. *J. Clin. Invest.* 117: 1155-1166.
24. Marigo, I., L. Dolcetti, P. Serafini, P. Zanovello, V. Bronte. 2008. Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. *Immunol. Rev.* 222: 162-179.
25. Haile, L. A., R. von Wasielewski, J. Gamrekelashvili, C. Kruger, O. Bachmann, A. M. Westendorf, J. Buer, R. Liblau, M. P. Manns, F. Korangy, T. F. Greten. 2008. Myeloid-derived suppressor cells in inflammatory bowel disease: a new immunoregulatory pathway. *Gastroenterology* 135: 871-881. *881.e1–5.*.
26. Delano, M. J., P. O. Scumpia, J. S. Weinstein, D. Coco, S. Nagaraj, K. M. Kelly-Scumpia, K. A. O'Malley, J. L. Wynn, S. Antonenko, S. Z. Al-Quran, et al 2007. MyD88-dependent expansion of an immature GR-1^{+}CD11b^{+} population induces T cell suppression and Th2 polarization in sepsis. *J. Exp. Med.* 204: 1463-1474.
27. Brys, L., A. Beschin, G. Raes, G. H. Ghassabeh, W. Noel, J. Brandt, F. Brombacher, P. De Baetselier. 2005. Reactive oxygen species and 12/15-lipoxygenase contribute to the antiproliferative capacity of alternatively activated myeloid cells elicited during helminth infection. *J. Immunol.* 174: 6095-6104.
28. Angulo, I., F. G. de las Heras, J. F. Garcia-Bustos, D. Gargallo, M. A. Munoz-Fernandez, M. Fresno. 2000. Nitric oxide-producing CD11^{b+}Ly-6G(Gr-1$^{)+}$CD31(ER-MP12$^{)+}$ cells in the spleen of cyclophosphamide-treated mice: implications for T-cell responses in immunosuppressed mice. *Blood* 95: 212-220.
29. Zhu, B., Y. Bando, S. Xiao, K. Yang, A. C. Anderson, V. K. Kuchroo, S. J. Khoury. 2007. CD11b^{+}Ly-6C^{hi} suppressive monocytes in experimental autoimmune encephalomyelitis. *J. Immunol.* 179: 5228-5237.
30. Kerr, E. C., B. J. Raveney, D. A. Copland, A. D. Dick, L. B. Nicholson. 2008. Analysis of retinal cellular infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations.*J. Autoimmun.* 31: 354-361.

31. Makarenkova, V. P., V. Bansal, B. M. Matta, L. A. Perez, J. B. Ochoa. 2006. $CD11b^{+}$/$Gr-1^{+}$ myeloid suppressor cells cause T cell dysfunction after traumatic stress. *J. Immunol.* 176: 2085-2094.

32. Kusmartsev, S., S. Nagaraj, D. I. Gabrilovich. 2005. Tumor-associated CD^{8+} T cell tolerance induced by bone marrow-derived immature myeloid cells. *J. Immunol.* 175: 4583-4592.

33. Xu W, Liu LZ, Loizidou M, Ahmed M, Charles IG. 2002. The role of nitric oxide in cancer. *Cell Res*; 12: 311^20.

34. Ambs S,MerriamWG,OgunfusikaMO, et al. 1998. p53 and vascular endothelial growth factor regulate tumor growth ofN OS2-expressing human carcinoma cells. *NatMed*; 4:1371^ 6.

35. Kao J, Packer S, Vu HL, Schwartz ME, Sung MW, Stock RG, Lo YC, Huang D, Chen SH, Cesaretti JA. 2009. Phase 1 study of concurrent sunitinib and image-guided radiotherapy followed by maintenance sunitinib for patients with oligometastases: acute toxicity and preliminary response. *Cancer*; 115(15):3571-80.

36. Thomas KA. 1996. Vascular endothelial growth factor, a potent and selective angiogenic agent. *J Biol Chem*; 271(2):603–6.

37. F. Riedel, K. Gotte, J. Schwalb, H. Wirtz, W. Bergler and K. Hormann. 2000. Serum levels of vascular endothelial growth factor in patients with head and neck cancer,*Eur Arch Otorhinolaryngol* 257**,** pp. 332–336.

38. Ambs S, Bennett WP, Merriam WG, et al. 1998. Vascular endothelial growth factor and nitric oxide synthase expression in human lung cancer and the relation to p53. *Br J Cancer*;78(2):233.

39. Marrogi AJ, Travis WD,Welsh JA, Khan MA, Rahim H, Tazelaar H, et al. 2000. Nitric oxide synthase, cyclooxygenase 2, and vascular endothelial growth factor in the angiogenesis of non-small cell lung carcinoma. *Clin Cancer Res*; 6(12):4739–44.

40. Brown LE, Berse B, Jakman RW, et al. 19. 3 Expression of vascular permeability factor (vascular endothelialgrowth factor) and its receptors in adenocarcinomas of gastrointestinal tract. *Cancer Res*; 53:4727–35.

41. NakamuraY, Yasuoka H,Tsujimoto M, et al. 2003. Clinicopathological significance of vascular endothelial growth factor-C in breast carcinoma with long-term follow-up. *Mod Pathol*; 16:309^14.

42. Mazzoni, A., V. Bronte, A. Visintin, J. H. Spitzer, E. Apolloni, P. Serafini, P. Zanovello, D. M. Segal. 2002. Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. *J. Immunol.* 168: 689-695.

43. Kusmartsev, S., D. I. Gabrilovich. 2002. Immature myeloid cells and cancer-associated immune suppression. *Cancer Immunol. Immunother.* 51: 293-298.

44. Bunt, S. K., L. Yang, P. Sinha, V. K. Clements, J. Leips, S. Ostrand-Rosenberg. 2007. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. *Cancer Res.* 67: 10019-10026.

45. Liu, W.M., Fowler, D.W., Smith, P. & Dalgleish, A.G. 2010. Pre-treatment with chemotherapy can enhance the antigenicity and immunogenicity of tumours by promoting adaptive immune responses. *Br. J. Cancer* 102, 115-123.

46. Nowak, A.K., Lake, R.A. & Robinson, B.W.S. 2006. Combined chemoimmunotherapy of solid tumours: improving vaccines? *Adv. Drug Deliv. Rev* 58, 975-990.

47. Vu, H.L., Sikora, A.G., Fu, S. & Kao, J. 2010. HPV-induced oropharyngeal cancer, immune response and response to therapy. *Cancer Lett* 288, 149-155.

48. Smeets SJ, Hesselink AT, Speel EJ, Haesevoets A, Snijders PJ, Pawlita M, Meijer CJ, Braakhuis BJ, Leemans CR, Brakenhoff RH. 2007. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. *Int J Cancer.* 121(11):2465-72.

49. Kenter, et al. 2009 Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. *NEJM* 361:1838-1847

50. Genden et al. 2011 Transoral robotic resection and reconstruction for head and neck cancer. *Laryngoscope*. 121(8): 1668-74.

51. Eisenhauer et al. 2009 New response evaluation criteria in solid tumors: revised RECIST guideline (version1.1). *Eur J Cancer.* 45(2) 228-47.

52. Maciag, P.C., S. Radulovic, and J. Rothman, *The first clinical use of a live-attenuated Listeria monocytogenes vaccine: a Phase I safety study of Lm-LLO-E7 in patients with advanced carcinoma of the cervix.* Vaccine, 2009. **27**(30): p. 3975-83.

53. Beebe, J.L. and E.W. Koneman, *Recovery of uncommon bacteria from blood: association with neoplastic disease.* Clin Microbiol Rev, 1995. **8**(3): p. 336-56.

54. Le Monnier, A., et al., *Listeria monocytogenes: a rare complication of ventriculoperitoneal shunt in children.* J Clin Microbiol, 2011. **49**(11): p. 3924-7.

55. Dominguez, E.A., A.A. Patil, and W.M. Johnson, *Ventriculoperitoneal shunt infection due to Listeria monocytogenes.* Clin Infect Dis, 1994. **19**(1): p. 223-4.

56. Winslow, D.L. and M.L. Steele, *Listeria bacteremia and peritonitis associated with a peritoneovenous shunt: successful treatment with sulfamethoxazole and trimethoprim.* J Infect Dis, 1984. **149**(5): p. 820.

57. Charlier, C., et al., *Listeria monocytogenes-associated joint and bone infections: a study of 43 consecutive cases.* Clin Infect Dis, 2012. **54**(2): p. 240-8.

58. http://www.cdc.gov/listeria *accessed September 23, 2015.*

59. Chosewood LC, W.D., editors, *Biosafety in microbiological and biomedical laboratories (BMBL) 5th ed. US Department of Health and Human Services.* http://www.cdc.gov/biosafety/publications/bmbl5/index.htm, 2009.

60. Lorber, B., *Listeria monocytogenes*, in *PRINCIPLES AND PRACTICE OF INFECTIOUS DISEASES*, B. Mandell, and Dolan, Editor. 2000. p. 2208-14.

61. Grupp, S.A., et al., *Chimeric antigen receptor-modified T cells for acute lymphoid leukemia.* N Engl J Med, 2013. **368**(16): p. 1509-18

62. Lee, D.W., et al., *Current concepts in the diagnosis and management of cytokine release syndrome.* Blood, 2014. **124**(2): p. 188-95

63. Winkler, U., et al., *Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8).* Blood, 1999. **94**(7): p. 2217-24

64. Herceptin, *Package Insert.* Genentech, Inc., 2015

APPENDIX A

ADXS11-001 Drug Ordering

Drug Ordering and Distribution: ADXS11-001 will be supplied by Advaxis and distributed by Almac Clinical Services LLC. No supplies will be shipped to any site until regulatory approval has been obtained. There will **NOT** be an initial drug supply forwarded to all investigational sites until initial regulatory approval.

Initial and Subsequent Requests: The designated person(s) from each participating hospital will complete the Drug Order Request Form and email the completed form to Almac. Study drug is not patient specific and you may order multiple vials of study drug to accommodate more than one patient depending upon the enrollment expectations at your site and storage capacity at -80 ± 10°C.

ADXS11-001 will be shipped from Almac Clinical Services directly to the institution. Shipments will be made Monday through Thursday for Tuesday through Friday delivery per the table below. To ensure ample time for delivery, drug requests must be received by Almac before 12:00 pm Eastern Standard Time (EST) Monday through Wednesday. If the drug request is received prior to 12 noon, that will be considered Day 1. Study drug will be shipped Day 2 to arrive on site Day 3 before 10:30 am local time. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Orders in (by 12 PM EST)	Shipment Out	Expected Delivery
Monday	*Tuesday*	*Wednesday*
Tuesday	*Wednesday*	*Thursday*
Wednesday	*Thursday*	*Friday*
Thursday	*Monday*	*Tuesday*
Friday	*Monday*	*Tuesday*

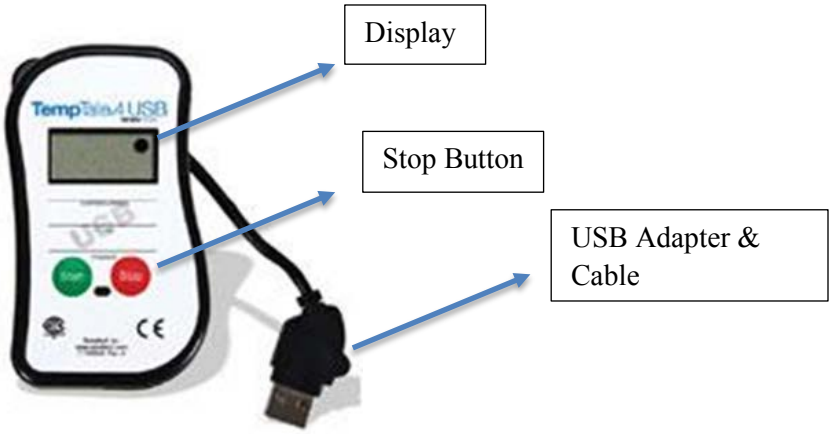
EST = Eastern Standard Time

Complete and email the Drug Reorder Request Form to:

ATTN: palogistics.clinicalservices@almacgroup.com
CC: Larry.haines@almacgroup.com
logistics@advaxis.com

Cold Chain Verification: Cold chain verification must be completed immediately before unpacking the shipper, by carefully following the instructions below:

TempTale USB4 Temperature Recorder Review Instructions



Stopping the Monitor:

On receipt of the shipment, the TempTale USB4 temperature recorder should be stopped. For this the **RED stop button is held down firmly for 3 seconds**. A hexagonal "STOP" icon appears in the upper right hand corner of the display.

Alarm:

A small blinking "BELL" icon is on the display after the device has been stopped. This means that during the transport a significant temperature excursion occurred. Please follow steps 1-6.

1) Plug the USB cable into your computer.
2) Copy both files from the USB device into an email.
3) Specify the subject header with **Protocol Name, Site Name/No. and Shipment No.**
4) Please send an e-mail with both attachments to Almac and Advaxis via the following e-mail addresses:
   - coldchainteampa@almacgroup.com
   - logistics@advaxis.com
5) The medication contained in this shipment **must not be dispensed to patients** until a positive written feedback from Advaxis is available.
6) Please store the affected medication separately and wait until confirmation of supply usability is received.

No Alarm:

If the device has been stopped and no blinking "BELL" icon is visible, verification that the medication was shipped under good conditions is complete. No further actions from your side are necessary. Please discard the temperature logger. You can use the medication immediately.

No study drug should be administered to any patients unless the TempTale USB4 has been reviewed and the shipment has been verified or authorized for clinical use.

For questions about TempTale USB4 and drug shipments, please e-mail the following:

Almac: palogistics.clinicalservices@almacgroup.com
CC: larry.haines@almacgroup.com
logistics@advaxis.com

Drug Accountability: All study drug must be accounted for by using the NCI Drug Accountability Form during the course of this study. The pharmacist or qualified research staff member will maintain inventory records. The records will include details of materials received, the date dispensed, the patient identification number and initials of patient receiving the dose, and documentation of drug destruction following notification from the Sponsor or completion/termination of the study. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

Drug Destruction and Return: **Opened and unopened vials** can be destroyed according to the site's guidelines for Biohazard Waste Disposal or by using the steps shown below for ADXS11-001 destruction.

The used or unused vials of ADXS11-001 can be treated with a 10% bleach solution for disinfection. Use a 10% solution of Clorox☐ (or any similar commercial chlorine bleach solution containing 5.25% sodium hypochlorite (NaClO) for disinfection.

1. Treat unopened or opened vials(s) with a minimum volume of 0.25 mL of bleach solution to sterilize its contents.
2. Empty the disinfected chemical solution in drain, run water to remove residual materials from drain and discard empty vials into designated biohazardous waste container, as applicable.
3. The residual IV bags or other components used for preparing the drug product should also be inactivated with bleach and discarded in similar manner as explained in steps 1 and 2.

If ADXS11-001 is destroyed at the site, it will be the investigator's responsibility to ensure that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures and that:

- BrUOG has been alerted to drug destruction and has been sent hospital destruction policy
- written authorization for disposal/destruction has been granted by Advaxis
- arrangements have been made for the disposal
- appropriate records of the disposal have been documented

A separate NCI Drug Accountability Form must be completed for Drug Destruction.

Accidental Spills: All accidental spills shall be handled in compliance with applicable site safety procedures or following the guidance below:

1. In the event there is an accidental spill of ADXS11-001, isolate the area and notify others in the vicinity.
2. Put on appropriate personal protective equipment (PPE) if not already worn (e.g. gown or lab coat, gloves and loose fitting mask with eye shield or goggles).
3. Remove any broken glass or sharps and place them into sharps container.
4. Place paper tower over the spill.
5. Saturate the paper-towel(s) with bleach starting at the outside of the spill and working towards the center. Allow the 10% bleach solution to remain on area for approximately 10 min.
6. Dispose the paper towel(s) in a biohazardous waste container.
7. Clean the remaining disinfectant with additional paper towels, as needed.
8. Discard all materials including PPEs, in the designated biohazardous waste container(s).
9. Inform the Principal Investigator (PI) and other appropriate personnel, e.g. Research Manager, Pharmacy Manager.

Exposure to ADXS11-001: All exposure incidences shall be handled in compliance with applicable site safety procedures or following the guidance below:

1. In the event of an accidental exposure, remove and dispose of contaminated PPEs or clothing into the designated biohazardous waste containers.
   a. For skin contamination: thoroughly wash the affected area immediately with soap and water.
   b. For needle stick injury: wash the affected area thoroughly with soap and water and cover the area with a sterile gauze dressing. Notify the PI who will determine appropriate medical actions to be taken.
2. For eye contamination: immediately and thoroughly rinse the affected area for up to 15 minutes using an eyewash; making the water flow across the affected eye from the nose to the outer corner of the eye. If only one eye is contaminated, avoid contaminating the other eye (position your head so the affected eye must be below the other eye). Notify PI who will determine appropriate medical actions to be taken.

It will be the investigator's responsibility to ensure that procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures and that:

- the coordinating institution has been alerted to drug destruction and has been sent hospital destruction policy, if applicable
- arrangements have been made for the disposal
- appropriate records of the disposal have been documented

A separate (NCI) Drug Accountability Form must be completed for drug destruction.