

**A Phase I/II Evaluation of ADXS11-001, Mitomycin,
5-fluorouracil (5-FU) and IMRT for Anal Cancer**

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1.0 OBJECTIVES

1.1 Primary Objectives

1.1.1 To evaluate the safety of the addition of ADXS11-001 to standard chemoradiation for patients with anal cancer.

1.1.2 To evaluate the 6-month clinical complete response rate for patients with anal cancer treated with ADXS11-001 mitomycin, 5-FU and IMRT.

1.2 Secondary Objectives :

1.2.1 To evaluate progression-free and overall survival for patients with anal cancer treated with ADXS11-001, mitomycin, 5-FU and IMRT.

1.2.2 To correlate HPV type with 6-month clinical complete response, and progression-free and overall survival, after treatment with ADXS11-001 and standard chemoradiation.

2.0 BACKGROUND

2.1 Anal Cancer:

There are about 5,290 new cases of anal cancer each year. A higher incidence is associated with female gender, infection with human papillomavirus (HPV), lifetime number of sexual partners, anal intercourse, and infection with human immunodeficiency virus.¹ Substantial progress has been made in the management of anal cancer.²⁻⁴ Randomized trials have shown that chemoradiation improves local control compared with radiotherapy alone, and that radiotherapy and concurrent 5-fluorouracil (5-FU) and mitomycin-C (MMC) improves disease-free survival and colostomy-free survival, compared with radiotherapy and concurrent 5-FU.²⁻⁴ The RTOG 98-11 trial demonstrated that patients treated with induction 5-FU and cisplatin followed by concurrent 5-FU/cisplatin and radiotherapy have a higher colostomy rate than patients treated with concurrent 5-FU/MMC and conventional radiotherapy.⁵ Preliminary results from the UK ACT II study show that maintenance chemotherapy with 5-FU/cisplatin is not effective after chemoradiation at decreasing cancer recurrence.⁶

Need for Further Improvement in Outcomes:

While many patients treated with chemoradiation for anal canal cancer have adequate outcomes, there remains considerable room for improvement.^{5,7,8} In RTOG 98-11, patients in the radiotherapy and concurrent 5-FU/MMC arm had a 3-year disease-free survival rate of only 68%.⁵ Five year outcomes of the RTOG 98-11 trial per individual tumor and nodal stage, presented at 2011 ASCO GI, demonstrated that disease-free survival is 40% or less for patients with T4N0 and/or node positive disease.⁸ An M.D. Anderson retrospective study showed that higher T stage ($P = 0.023$) and higher N stage ($P = 0.030$) independently predicted for a higher rate of locoregional failure.⁷ The 3-year rate of locoregional control was 90% for T1/Tx, 86% for T2, 77% for T3 and 63% for T4 tumors. Therefore, trials examining more aggressive and innovative regimens in anal canal cancer are warranted in patients at high-risk for treatment failure in order to improve outcomes.

HPV and Anal Cancer:

There is a close association between human papilloma virus and squamous cell cancer (SCC) of the anal canal.⁹⁻¹¹ HPV DNA has been isolated from 80 to 100 percent of in situ and invasive SCCs of the anus.⁹⁻¹¹ While multiple HPV types can be found in the anal canal, the subgroup that appears to be the most common high-risk type is HPV subtype 16 (HPV-16) which is present in approximately 80% of anal cancer cases.^{12,13} Expression of viral E6 and E7 oncoproteins inactivate the tumor-suppressor proteins p53 and the retinoblastoma protein (pRb), respectively.^{14,15} In SCC of the head and neck, the RTOG has established a strong relationship between tumor HPV status, as determined by in situ hybridization, and survival following chemoradiation (Ang 2010). The RTOG has also correlated the expression of p16, an established biomarker for the function of the HPV E7 oncoprotein, to outcome after treatment for head and neck cancer.¹⁶

2.2 HPV Vaccines

Similar to anal cancer, squamous cell carcinoma of the cervix is largely the result of persistent infections with high-risk types of human papillomavirus.¹⁷ Approximately 95-99% of cervical cancers have detectable amounts of HPV DNA.¹⁷ Squamous cell carcinoma of the cervix requires the constitutive expression of the HPV oncoproteins E6 and E7.¹⁸ Thus, the E6 and E7 antigens have been an intense focus of cancer immunotherapies using a variety of vaccine vectors. Because of the intra-cellular localization of these antigens, these therapies are mostly directed at cellular immune responses. The ability to deliver antigen to the cytosolic compartment of APCs in order to develop HLA class I presentation to induce CD8⁺ cytotoxic T cell responses is widely believed to be necessary for developing an effective therapeutic anti-tumor vaccine.¹⁹

The literature provides many references to support the idea that many HPV species may respond to therapeutic agents based upon HPV-16 E7.²⁰⁻²² A phase II study, conducted by the New York Phase II Consortium (an NCI-sponsored consortium), immunized 31 healthy women with biopsy-proven Cervical Intraepithelial Neoplasia (CIN) III with three, monthly subcutaneous vaccinations with 500 mcg of SGN-00101 [Heat Shock Fusion Protein-Based Immunotherapy (HspE7)]. Of the 31 evaluable patients, 32% (10/31) had a complete pathologic response; 39% (12/31) had a partial response and 29% (9/31) had stable disease. The overall response rate was 71% (22/31, 95% C.I.=55-87%). No patients progressed. Fifty-five percent (17/31) were HPV-16 positive prior to vaccination and one patient had HPV-16 subsequently detected. These results suggest many cervical cancer patients (not just those who are HPV-16 positive) could potentially benefit from the vaccine targeted at HPV-16 E7. Further, it supports the idea that HPV epitopes are sufficient similar to allow efficacy in other HPV types. Research completed by Nventa, formerly Stressgen, reports extremely high efficacy using the HPV-16 E7 antigen even in non-HPV-16 positive patients; which demonstrates the antigen has immuno-therapeutic activity across HPV types.²³ Davidson et al. reported cross reactivity to the E7 antigen and responses in patients with other HPV types in a trial of a vaccinia based HPV-16 E7 vector tested in the clinical treatment of high grade vulval intraepithelial neoplasia (VIN).²⁴ Luxton et al, reported that 22% of responding women treated with HPV-16 E7 peptide epitopes for the treatment of cervical dysplasia or neoplasia were negative for HPV-16 DNA.²⁵ Slevy et al. reported that B cell epitopes of HPV-16 E7 are cross reactive with HPV-18.²⁶ Krchnak et al, found while investigating HPV-16 E7 epitopes that of the 9 overlapping peptides that were made to map the immunogenic domains, some epitopes were type-specific and others were cross reactive with other HPV types.²⁷ Additional support for the cross reactivity of HPV-16 E7 with other HPV types has been demonstrated in human HLA-A2 transgenic mice.²⁸ It further appears that certain HPV-16 E7 epitopes are conserved across genera, as Nilges et al demonstrated that "...cross-reactivity represents the inherent nature of the T-cell repertoire" with cross reactivity between HPV-16 E7 and Coronavirus protein OC43 NS2.²⁹

External Beam Radiation Therapy May Enhance Vaccines: Tumor cells by themselves do not express the complete repertoire of molecules necessary to render them maximally susceptible to immune responses induced either endogenously or by immunotherapy.³⁰ Evolving knowledge on the 'abscopal effect' (also called the 'bystander effect'), wherein local radiation has cell mediated antitumor effect on tumors distant from the sites of radiation, has created interest in radiation as a possible modulating agent for immune responses towards tumor cells. Radiation induces expression of molecules that add specificity to tumor cell responses induced by immunotherapy. Potentially, such effects can also be exploited at tumor sites distant from radiation sites. For example, dead tumor cells from radiation provide signals for dendritic cell activation.³¹ Radiation damage causes phenotypic changes such as upregulation of FAS,³² MHC Class-I,³³ and expression of calreticulin³⁴ on tumor cell surface. In murine models of metastatic renal cell cancer, combination therapy with radiation and IL-2 achieved greater tumor reduction than either modality alone.³⁵ Similarly, addition of 8Gy of local radiation to therapeutic vaccination for CEA expressing subcutaneous murine tumors increased immune response.³⁶ In a phase I study by Chi et al, the combination of 8 Gy of radiation and intratumoral injection of autologous immature dendritic cells in refractory hepatoma was well tolerated.³⁷ A phase II study by Okawa et al assigned women with carcinoma cervix to 15-30Gy of radiation with or without an immune adjuvant LC9018 (a bacterial

extract).³⁸ The study showed a significant effect on tumor reduction with the combination compared to XRT alone. Gulley et al randomized men with localized prostate cancer to XRT with or without vaccinia-based prostate cancer vaccine.³⁹ Thirteen of 17 patients in the combination treatment arm had increases in PSA specific T cells of at least 3-fold.⁴⁰ Slovin et al reported a phase I/II trial of ipilimumab alone and in combination with radiation in both chemotherapy naïve and post-chemotherapy, hormone refractory prostate cancer patients.⁴¹ Preliminary results show no increase in immune related adverse events in all subgroups when compared to ipilimumab in melanoma. Addition of 10mg/kg ipilimumab to prostate radiation was well tolerated.^{40,41}

2.3 Recombinant *Listeria monocytogenes* (Lm) as a Vaccine Vector

The advantages that *Listeria monocytogenes* possesses as a vaccine vector are rooted in its biology. It is a beta hemolytic gram-positive facultative intracellular bacterium that has been used to study cell mediated immunity for decades.⁴² *Listeria* preferentially infects APC, and unlike other intracellular bacteria, escapes into the cytoplasm of the host cell by disrupting the phagosomal membrane. Since *Listeria* is a gram positive organism it does not release endotoxin. Humoral immunity does not play a major role in combating *Listerial* infections because *Listeria* quickly leaves the circulation becoming an intracellular infection, and because it replicates in the cytoplasm. Peptides derived from *L. monocytogenes* in the phagolysosome and the cytosol can be presented by both the MHC Class I and Class II molecules, inducing both CD4+ and CD8+ T-cell responses. *Listeria* has other useful properties, such as stimulating monopoiesis, stimulating the differentiation and maturation of APC, and the generation of a particularly strong innate immune response.⁴³

Advaxis builds upon the unique attributes of *Listeria* by engineering a highly attenuated strain to release HPV-E7 antigen as a fusion protein to a non-hemolytic fragment of the *Listeria* protein Listeriolysin O (LLO) within the cytoplasm of the APC, resulting in more potent and broad CTL responses to the target antigen. The HPV tumor antigen proteins E6 and E7 are constitutively expressed in the nucleus of HPV associated tumors. Most therapies targeting these antigens are directed at cellular immune responses. The ability to deliver antigen to the cytosolic compartment of APCs in order to develop HLA Class I presentation to induce CD8+ cytotoxic T cell responses appears to be an important aspect of developing an effective therapeutic anti-tumor vaccine.

LLO is a virulence factor of Lm as it enables the bacterium to escape from the phagolysosome and thus become virulent. As such, it appears to have evolved as a target of the immune system and stimulates many potentially therapeutic immune responses by itself, independent of the microbe. By using it as part of a fusion protein along with an antigen this vaccine secretes a combined antigen-adjuvant directly within both the phagolysosome and cytoplasm of APC.

There is evidence that Lm also gets into tumors, probably carried by infected macrophages and neutrophils. Live *Listeria* vaccines that secrete an LLO-antigen fusion, but not those that secrete only an antigen, are able to diminish regulatory T cells within the tumor, but not in the spleen or normal peripheral tissues.^{44,45}

2.4 *Listeria monocytogenes* Expressing HPV 16-E7 (ADXS11-001 Immunotherapy)

ADXS11-001 is a live attenuated *Listeria monocytogenes* (Lm) 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.

ADXS11-001 is manufactured for Advaxis, Inc. by Cobra Biomanufacturing PLC in the UK at EU and FDA certified facilities, and with open Drug Master Files in place on both continents. Clinical materials have been manufactured under GMP conditions and in compliance with GMP regulations, and are so documented.⁴⁶⁻⁵⁹ Please refer to Advaxis Inc IND#13712.

2.5 ADXS11-001 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.

2.6 Non clinical Studies

(The following studies are described in depth in the investigator brochure for ADXS11-001.)

Advaxis chose the Balb/C strain of mice in order to test the toxicity of ADXS11-001 in the most stringent manner and to assess the worst case. Balb/C mice are deficient in their ability to kill or inhibit the growth of *Lm* organisms. Thus, the use of Balb/C mice provides the most stringent and rigorous assessment of the toxicologic properties of ADXS11-001 due to low level of cell mediated immunity response.

2.6.1 Acute Single Dose Toxicity

The purpose of this study was to assess the maximum tolerated dose (MTD) of ADXS11-001 administered subcutaneously (SC) and intravenously (IV) to female Balb/C mice and to compare that tolerability to Wild Type *Lm* and Lm-SIV-Env (an early HIV construct that was found to be a more virulent *Lm* construct than ADXS11-001). A dose of 0.1 ml was administered on the day of dosing and all animals were observed once a day for seven days thereafter for mortality and signs of toxicity. Body weights were taken day of dosing and at day of termination. All surviving mice were sacrificed on Day 7 and gross necropsies were performed with all lesions recorded. Based on data obtained during the in-life phase and gross pathology at necropsy, it was found that MTD for IV administered Lm-LLO-E7 to be 2.8×10^7 and SC administered Lm-LLO-E7 to be 2.8×10^9 . The IV administered Lm-LLO-E7 at 2.8×10^9 resulted in a slight spleen enlargement in two mice, which while an acceptable adverse event in the treatment of cancer, prevented this dose from being classified as the MTD by definition. There were no clinical signs during the in-life phase or abnormalities at necropsy in the saline IV or SC groups of mice. The MTD for IV administered WT Lm was 10^3 and for the SC group the MTD was not observed as all

five mice in the 10^3 exhibited slight piloerection from day 2 to termination. At higher doses there were deaths, severe clinical signs of toxicity during in-life phase and pathology reported in the necropsies. The MTD for IV Lm-SIV-Env was 10^4 and no MTD was established for the SC dose group. In the higher dose groups for both IV and SC Lm-SIV-Env, there were clinical signs of toxicity during the in-life phase observations and gross pathology at necropsy. One mouse died on day 6 in the SC 10^8 treatment group.

2.6.2 Multi-dose 28 day Toxicity Study

Advaxis assessed the toxicity of ADXS11-001 when administered once weekly for four weeks either SC or IV to female Balb/C mice. On days 0, 7, 14, and 21, 0.1 ml of ADXS11-001 or saline control was administered to ten mice per group. All animals were observed twice daily for mortality and clinical signs of toxicity. Body weights were taken on days -1, 7, 14, and 28. Blood samples were taken on day 28 from five animals per group for serum chemistry and five animals for hematology. The shipment with hematology samples had delays in transit to the laboratory so there is no hematology data. All surviving mice were sacrificed on day 28 and gross necropsies were performed with all lesions recorded. A total of 26 tissues were collected and sent to Biotechnics, Inc. for histopathologic analysis. This study demonstrated in-life phase observations for clinical signs of toxicity indicated weight loss that averaged 1.2 grams in only one group IV 2.8×10^9 . Two mice, one from IV 2.8×10^7 and one from IV 2.8×10^9 , died immediately following the intravenous injection on Day 7 apparently unrelated to *Lm*. The attending veterinarian recorded the death as possibility being from volume shock. There were no other mortalities in the other groups or at later dates. General health observations for toxicity reported no observable abnormalities in the saline group, IV 10^5 , IV 10^7 , SC 10^5 and SC 10^7 . All mice receiving IV 10^9 reported piloerection lasting for a couple days following injections on day 14 and 21. All animals in SC 10^9 reported piloerection following injections on days 2-3, 14-17 and 23-28. Also, lumps developed at the SC dose sites on all group 7 mice on day 14-17 and 21-28. Overall, there was an increase in the liver enzymes (ALT, AST and ALP) in the ADXS11-001 treated mice over the saline controls and there was an increase in globulin levels in the SC and IV high dose groups. Necropsies reported gross pathological finding mostly in the SC and IV high dose groups and the most common finding was enlarged spleens and in the SC group lumps at the injection site. All histopathologic findings in the ten saline-treated mice were considered incidental. All IV 2.8×10^9 mice demonstrated histologic alterations within the liver and kidneys. The liver exhibited multifocal, random accumulations of mixed inflammatory cells including macrophages, lymphocytes, and neutrophils. Infiltration was minimal to occasionally mild but was typically disseminated, with numerous foci (>25 per section in 8 mice and <10 in one mouse) scattered throughout the hepatic parenchyma. Kidneys were variably affected and bore tubular alterations consisting of dilatation, epithelial attenuation, and regeneration. Active neutrophilic pyelonephritis was present in several mice. The numerous inflammatory foci disseminated throughout the liver and the tubular alterations in the kidney are indicative of an infectious state. The majority of IV 2.8×10^5 mice demonstrated histologic alterations within the liver. The liver exhibited multifocal, random accumulations of mixed inflammatory cells including macrophages, lymphocytes, and neutrophils. Infiltration was minimal, with approximately 5-20 cells per foci and <10 foci per section in 6 mice and 10-20 foci per section in 2 mice.

2.6.3 Toxicity of Four Doses given One Month Apart with Ampicillin Treatment and Recovery Period

Study 110607-07 was designed to evaluate the safety of 4 vaccinations of ADXS11-001 given at 1-month intervals in Balb/c mice. The study incorporated a prior treatment with cyclophosphamide one day before ADXS11-001 and oral ampicillin for 10 days starting 5 days after each vaccine dose in order to mimic the proposed patient populations and clinical regimen. Cyclophosphamide was given as an immuno-conditioning agent to deplete aspects of the immune system (regulatory T cells).

Fifty female mice were divided into two groups of 20 to receive 7.6×10^7 or 7.6×10^6 CFU ADXS11-001 given IV and a Control group of 10 mice to receive saline. Cyclophosphamide at 30mg/kg was given IV to all animals on day 0. Groups were vaccinated on days 1, 31, 61, and 91. All mice received a 10-day course of oral ampicillin given by gavage at 50mg/kg/day starting on the fifth day after each vaccination.

Animals were observed for mortality and pharmacologic/toxicologic effects. Ten mice from each group were sacrificed for necropsy, histology, blood chemistry and hematology on day 111. Following an additional 21-day recovery period, the remaining (20) mice in the vaccinated groups were sacrificed on day 132. Body weight gains were approximately the same in all groups. There were no observable abnormalities noted during post-dose observations on days of vaccination. In each group approximately 10% of mice showed decreased activity, emaciation, piloerection, and/or hunched posture, but this were not considered to be related to test article (or placebo) administration. One animal died by drowning in Group 1 (day 25), due to malfunction of the watering device. One animal in Group 2 was found dead on day 28. Neither death was considered due to test article. There were no observable abnormalities at necropsy on either day 111 or day 132. The weight of lungs/trachea/pharynx/larynx complex was significantly lower in the saline group. There were no significant differences in any other organ weights on day 111 or day 132. Serum chemistry results showed low albumin/globulin ratio for Group 1 high dose at day 111. Amylase was significantly high in this group at this time point. All other serum chemistry values were comparable to the saline group. At day 132, Group 1 high dose showed elevated ALT, AST and inorganic phosphorus; Group 2 low dose showed elevated AST. On day 111, WCB counts were significantly lower in the high dose group. Day 132 results are not available due to poor blood sample quality. Histopathology results showed mild inflammatory lesions in the livers of some mice, both test article-treated and saline groups. Most mice in all groups had moderate hemorrhagic lesions in the lungs. There were no histopathologically important

2.6.4 Overall Conclusions from *in vivo* studies.

No deaths attributable to ADXS11-001 were seen at any dose used in these studies. A total of three deaths in the 10^7 and 10^9 dosage groups were reported in one study, the 28 day toxicity study (42), and all deaths occurred within minutes of administration; a time course consistent with volume shock and not with infectious disease or the toxicity of ADXS11-001, which was the ascription made by the test laboratory. An equivalence calculation reveals that 0.1 ml in a mouse is the equivalent of 300 ml in a human, and the injection was administered over a 3-5 second interval. Thus, the potential for volume shock existed. No other deaths were observed in any study.

The highest dose used in the panel of studies reported here was 2.8×10^9 , which was used consistently in all studies, except in the SCID mouse trial. This dosage resulted in behavioral toxicity as evidenced by piloerection, modest weight loss, and histopathologic changes noted above. This dosage equates with a human dose of either 7.8×10^{12} if equated by mass, or 7.45×10^{11} if equated by surface area. Thus, in a species of mouse with a known deficit in its immunologic ability to respond to *Lm* infection, toxicity was only seen at doses that are significantly higher than those proposed in the initial clinical protocol. It should be noted to the clinical investigator that IV administration in these *Lm* susceptible mice differed from the experience of IP administration that was commonly used in research studies in more robust species of mice. This route of administration appeared to result in a longer subclinical infection in the absence of antibiotics, although it appears as though the microbe would clear to sterility given sufficient time. This is believed to result from the increased delivery of the construct via this route to all target tissues, including the liver, and spleen where *Lm* is known to preferentially colonize. Also, the immune environment of the peritoneal cavity is known to minimize the infectivity of the micro-organism when compared to IV administration. Ampicillin administration, as described above, completely eradicated the microbe in all tissues tested.

The major conclusions that can be reached from this body of data include:

- A dose of 2.8×10^7 was determined as the maximally tolerated dose, which corresponds to a human dose of 7.84×10^{10} by weight or 7.45×10^9 by surface area. This range consistent with the clinical doses proposed for the initial clinical trial. However, in the context of cancer therapy a certain amount of toxicity is tolerated to obtain maximal efficacy. Thus, the proposed dosages for the human study are safe, and probably below the dose that will eventually be used to treat disease.

- The persistence of the construct in the liver of animals not receiving antibiotic indicates that the clinician should assess hepatic toxicity routinely. This was already known as a site of *Lm* toxicity, and our work re-confirms this finding.
- Ampicillin, as well as 9 of the 11 tested antibiotics, is an effective antibiotic capable of eradicating the construct in the event of a toxic response.

2.7 Summary of previous clinical studies

Refer to the Investigator Brochure (IB) for detailed preclinical and clinical data.

2.7.1 Lm-LLO-E7-01: Phase 1 Experience

In the initial Phase 1 dose escalation study (Lm-LLO-E7-01), ADXS11-001 was assessed in 15 subjects with previously treated metastatic, refractory or recurrent invasive cervical cancer [60]. ADXS11-001 was administered as an intravenous (IV) infusion at doses of 1×10^9 to 1×10^{10} CFU followed by a second dose 3 weeks later. Overall, ADXS11-001 infusion was safely administered and well tolerated in end-stage subjects who had failed multiple prior therapies.

No pre-medications were given in this study and all subjects experienced flu-like symptoms and/or symptoms associated with CRS. The most commonly reported AEs were pyrexia (100%), vomiting (60%), chills, headache and anemia (53.3% each), nausea and tachycardia (46.7% each), and musculoskeletal pain (26.7%). At all doses, AEs were acute and transient in most subjects, and responded to non-prescription symptomatic treatment with no observed listeriosis or *Lm* shedding. A total of 9 (60%) subjects experienced Grade 3 AEs, and in 6 (40%) of those subjects AEs were considered treatment-related (pyrexia in 3 subjects, fatigue in 1 subject, and increased gamma-glutamyltransferase [GGT] levels in 2 subjects). No Grade 4 AEs were observed. The highest dose of 1×10^{10} CFU led to a dose-limiting toxicity (DLT) of Grade 2 diastolic hypotension. Under typical interpretation of a DLT (as defined per the protocol) in Phase 1 clinical studies in refractory cancers, this AE would not have met the criteria for DLT. Two deaths occurred during the study period and were considered unrelated to ADXS11-001 administration. Although not designed to assess efficacy, stable disease (SD) was reported in 7 of 13 (54%) evaluable subjects, and 1 subject (8%) had an unconfirmed partial tumor response. Based on the AE profile, all subsequent studies incorporate the administration of NSAIDs and antiemetic medications pre- and post-infusion to help reduce the incidence and severity of AEs related to cytokine release. As an additional precaution, a course of oral antibiotics is initiated on Day 3 post-dosing of ADXS11-001 to assist in clearance of the *Lm*.

2.7.2 Lm-LLO-E7-15: Phase 2 Study of ADXS11-001 vs ADXS11-001 and Cisplatin in Recurrent, Refractory Cervical Cancer

Lm-LLO-E7-15, was a randomized, multicenter, actively controlled Phase 2 study evaluating the safety and efficacy of ADXS11-001 alone compared with ADXS11-001 + cisplatin in 110 subjects with advanced cervical cancer that recurred after prior cytotoxic therapy, including chemotherapy and/or radiotherapy. Eligible subjects were randomized 1:1 to ADXS11-001 or ADXS11-001 + cisplatin. Subjects in the ADXS11-001 group received 1 cycle (3 doses of 1×10^9 CFU) of ADXS11-001 at 28-day intervals. Subjects in the ADXS11-001 + cisplatin group received a single dose of ADXS11-001, followed 28 days later by 5 weekly doses of cisplatin (40 mg/m²), followed 28 days later by 1 cycle of ADXS11-001; as a precautionary measure, a course of oral antibiotics was given to ensure clearance of the *Lm*. After first dosing, subjects were followed at 3, 6, 9, 12, and 18 months; 109 subjects received 264 doses of ADXS11-001.

The primary endpoint of the study was OS. Of 109 enrolled subjects, 89 (81.7%) subjects at 3 months, 70 (64.2%) at 6 months, 49 (45%) at 9 months, 35 (32%) at 12 months, and 24 (22%) at 18 months were alive, suggesting that ADXS11-001 is an active agent in recurrent cervical cancer. There was no statistically significant difference in the median duration of OS between ADXS11-001 and ADXS11-001 + cisplatin ($p = 0.9993$), nor was there any statistically significant effect of disease status, prior therapy,

or Eastern Cooperative Oncology Group (ECOG) performance status on OS between treatment groups.

Of 69 subjects in the efficacy population, 10% of subjects had an objective tumor response. Complete response (CR) was recorded as the best response in 5 subjects (3 ADXS11-001; 2 ADXS11-001 + cisplatin), partial response (PR) in 6 subjects (3 ADXS11-001; 3 ADXS11-001 + cisplatin), SD in 31 subjects (16 ADXS11-001; 15 ADXS11-001 + cisplatin), and progressive disease (PD) in 27 subjects (13 ADXS11-001; 14 ADXS11-001 + cisplatin). Tumor response at 3, 6, 9, 12, and 18 months was comparable between treatment groups. The disease control rate was 39% (42/109). Results of the safety profile are shown in Table 1.

2.7.3 GOG-0265: Phase 2 Evaluation of ADXS11-01 in Recurrent, Refractory Cervical Cancer

GOG 0265 is an ongoing study being conducted by the GOG, through sponsorship by the NCI Cancer Therapy Evaluation Program (CTEP). It is a 2-stage study designed to evaluate ADXS11-001 (3 doses of 1×10^9 administered every 4 weeks) in subjects with advanced, metastatic, or recurrent squamous or non-squamous carcinoma of the cervix who have had at least 1 prior systemic chemotherapeutic regimen in the metastatic setting. The primary objectives of the study are to evaluate:

1. The tolerability, safety, nature and degree of toxicity of ADXS11-001 by the numbers of subjects with DLTs and AEs as assessed by CTCAE v 4.03
2. To assess the activity of ADXS11-001 for subjects with persistent or recurrent carcinoma of the cervix by the frequency of subjects who survive for at least 12 months after initiating therapy

Twenty-nine subjects were enrolled to Stage 1 of the study, of which 3 were never treated with ADXS11-001. Among the 26 subjects, 8 (30%) had 1 prior therapy, 14 (54%) had 2 prior therapies and 4 (16%) had 3 prior therapies for treatment of their metastatic disease. The study is being conducted according to the GOG's standard Simon 2-stage design (for recurrent/refractory cervical cancer). A Stage 1 efficacy bar of 20% 12-month survival was established as a predetermined criterion (by a logistic regression model from 17 studies conducted by GOG in over 500 subjects with recurrent/refractory cervical cancer) to be met in order for the study to proceed to Stage 2. Based on the historical data in subjects with recurrent/refractory cervical cancer, the expected 12-month survival rate is 10-15%.

As of May 2015, Stage 1 of GOG-0265 is complete with 26 subjects receiving at least 1 dose of ADXS11-001. The 12 month survival rate from Stage 1 was 38% (10/26) with a 7.7 month median survival. Stage 2 of the study opened on March 11, 2015 and will enroll approximately 37 subjects. The study has been amended to allow for treatment with ADXS11-001 until disease progression as per RECIST 1.1, and to limit the number of prior therapies in the metastatic setting to no more than 1 prior therapy.

2.7.4 Updated previous clinical experience:

As of January 1, 2015, 573 doses of ADXS11-001 have been administered to 229/230 enrolled subjects at doses of 5×10^7 , 3.3×10^8 , 1×10^9 , 3.3×10^9 , and 1×10^{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 230 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. There have been no persistent symptoms or cumulative toxicity observed in subsequent doses. Although subjects have manifested cytokine release symptoms, no single subject has manifested all of the

following symptoms collectively: nausea, headache, tachycardia, hypotension, rash, and shortness of breath per NCI CTCAE v 4.03 that defines cytokine release syndrome (CRS).

A summary of AEs in >5% of patients by MedDRA system organ class and preferred term is presented in Table 1. There have been no Grade 3-4 drug related AEs observed in over 5% of the patient population (see Table 2).

Table 1 Number (%) of Subjects with Adverse Events >5% Incidence (Safety Population, N=230)

System Organ Class (SOC),n % [1]	Grades 1-4 (N=230)	Grade 3-4 (N=230)
Blood And Lymphatic System Disorders		
Anaemia	80 (34.8 %)	35 (15.2 %)
Cardiac Disorders		
Tachycardia	13 (5.7 %)	-
Gastrointestinal Disorders		
Abdominal Pain	34 (14.8 %)	4 (1.7 %)
Constipation	29 (12.6 %)	1 (0.4 %)
Diarrhoea	16 (7.0 %)	3 (1.3 %)
Nausea	64 (27.8 %)	2 (0.9 %)
Vomiting	46 (20.0 %)	4 (1.7 %)
General Disorders And Administration Site Conditions		
Chills	89 (38.7 %)	2 (0.9 %)
Disease Progression	-	-
Fatigue	41 (17.8 %)	2 (0.9 %)
Oedema Peripheral	14 (6.1 %)	-
Pain	19 (8.3 %)	3 (1.3 %)
Pyrexia	71 (30.9 %)	3 (1.3 %)
Investigations		
Alanine Aminotransferase Increased	12 (5.2 %)	2 (0.9 %)
Aspartate Aminotransferase Increased	14 (6.1 %)	2 (0.9 %)
Blood Alkaline Phosphatase Increased	15 (6.5 %)	2 (0.9 %)
Blood Creatinine Increased	16 (7.0 %)	6 (2.6 %)
Gamma-Glutamyltransferase Increased	14 (6.1 %)	3 (1.3 %)
Haemoglobin Decreased	12 (5.2 %)	2 (0.9 %)
White Blood Cell Count Decreased	20 (8.7 %)	7 (3.0 %)
Metabolism And Nutrition Disorders		
Decreased Appetite	21 (9.1 %)	-

System Organ Class (SOC),n % [1]	Grades 1-4 (N=230)	Grade 3-4 (N=230)
Hypoalbuminaemia	28 (12.2 %)	9 (3.9 %)
Hypocalcaemia	13 (5.7 %)	2 (0.9 %)
Hypokalaemia	15 (6.5 %)	4 (1.7 %)
Hyponatraemia	19 (8.3 %)	9 (3.9 %)
Musculoskeletal And Connective Tissue Disorders		
Back Pain	17 (7.4 %)	3 (1.3 %)
Nervous System Disorders		
Dizziness	12 (5.2 %)	-
Headache	41 (17.8 %)	-
Respiratory, Thoracic And Mediastinal Disorders		
Dyspnoea	14 (6.1 %)	1 (0.4 %)
Vascular Disorders		
Hypotension	16 (7.0 %)	2 (0.9 %)
Note: [1] Percentage is calculated using column header count as denominator for percentage calculation.		

Table 2 Number (%) of Subjects with Adverse Events >5% with Respect to Relationship (Possibly Related, Probably Related and Related) (Safety Population, N=230)

System Organ Class (SOC),n % [1]	Grades 1-4 (N=230)	Grade 3-4 (N=230)
Blood And Lymphatic System Disorders		
Anaemia	18 (7.8 %)	2 (0.9 %)
Gastrointestinal Disorders		
Nausea	47 (20.4 %)	-
Vomiting	31 (13.5 %)	2 (0.9 %)
General Disorders And Administration Site Conditions		
Chills	78 (33.9 %)	2 (0.9 %)
Fatigue	27 (11.7 %)	1 (0.4 %)
Pyrexia	57 (24.8 %)	3 (1.3 %)
Nervous System Disorders		
Headache	36 (15.7 %)	-
Vascular Disorders		
Hypotension	15 (6.5 %)	2 (0.9 %)

System Organ Class (SOC),n % [1]	Grades 1-4 (N=230)	Grade 3-4 (N=230)
Note: [1] Percentage is calculated using column header count as denominator for percentage calculation.		

2.7.5 Delayed/Late Listeria Infection:

ADXS11-001 has been attenuated over 4 logs more in comparison to the wild-type (wt)-*Lm*. ADXS11-001 is cleared by SCID mice lacking a functioning cellular immune system and 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 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 (1×10^{10} CFU) [60].

wt-*Lm* is known to form and persist within biofilms especially on medical devices despite antibiotic treatment [69]. Although rare, medical device-related infections such as ventriculo-peritoneal shunt infection, peritoneovenous shunt infection, and prosthetic joint infection have been reported [70-73]. ADXS11-001 is highly sensitive to antibiotics such as ampicillin and sulfamethoxazole/trimethoprim which can be an effective treatment regimen for listeria infection. 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 September 2015, approximately 263 subjects have received approximately 675 doses of ADXS11-001. During the course of treatment in subjects with various cancers, there has been one reported case of Grade 3 listeriosis (0.38%). 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 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 patient refused. Two weeks later, the subject presented with acute respiratory distress caused by her metastatic disease and died. The investigator ruled the cause of death was due to disease progression (metastatic cervical cancer). It is hypothesized that dosing of attenuated *Lm* soon after her bone graft likely resulted in biofilm formation, which protected the organisms 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. Genomic sequencing confirmed by PCR later revealed that the *Lm* was avirulent, and therefore incapable of spreading cell-to-cell. The *Lm* isolate also remained highly susceptible to multiple antibiotics.

2.8 Six-Month Clinical Complete Response As Efficacy Endpoint In Anal Cancer After Chemoradiation:

The optimal time for performing a post treatment proctoscopy and biopsy in anal cancer is controversial. Results from the ACT II trial, a 940 patient anal cancer clinical trial, demonstrated that clinical complete response at 6 months is predictive of progression free survival.⁶² Therefore an efficacy endpoint for this trial will be the rate of clinical complete response as determined by evaluation by proctoscopy at 6 months.

2.9 Protocol Rationale:

Novel treatments are needed in anal cancer. An important percentage of patients with locally advanced anal cancer will have persistent loco-regional disease or develop systemic metastases. Virtually all cases of anal cancer are related to infection by HPV. Anal cancer cells infected with HPV have the tumor associated antigen HPV E7. ADXS11-001 causes antigen presenting cells to be stimulated to facilitate immune cells to attack cancer cells expressing HPV E7. ADXS11-001, at the phase II dose of 1×10^9 CFU, has been shown to be safe in patients with advanced cervical cancer which also is caused by HPV infection. Anti-tumor activity and safety have been demonstrated in cervical cancer to single agent ADXS11-001 and the combination of ADXS11-001 and cisplatin chemotherapy. Data presented at ASCO 2012 ADXS11-001 is currently being evaluated in women in the United States with cervical intraepithelial neoplasia. Radiation may augment the activity of ADXS11-001 increasing the exposure of tumor related antigens thereby increasing the chance for loco-regional disease eradication and preventing systemic recurrence. Therefore, ADXS11-001 may increase complete response, prevent recurrence disease and increase disease-free and overall survival in anal cancer. This protocol will develop sufficient preliminary safety and efficacy data to facilitate the investigation of ADXS11-001 in anal cancer within “NRG”, the newly formed cooperative group based on the merger of the RTOG, NSABP and GOG.

As described above, Phase I studies and preliminary data from phase II studies have demonstrated that ADXS11-001, 1×10^9 CFU, can be safely administered as a single agent and in combination with chemotherapy. For example in over 200 patients treated at the dose of 1×10^9 CFU there have been no cases of severe listeria bacteremia or grade 3 cardiopulmonary toxicity. However, since ADXS11-001 has not previously been administered with radiation, we will confirm safety and investigate effectiveness with the primary objective of this study being to establish the safety of the addition of ADXS11-001 to chemoradiation for anal cancer.

- Treatment Schedule: The first dose will be given 6-14 days prior to the initiation of chemoradiation. The 2nd-4th dosages of ADXS11-001 will not be until after completion of all chemoradiation. The second dosage of ADXS11-001 will not be administered until a minimum of 10 days (10- 28 days) after completion of chemoradiation, ANC > 1,000 cells/mm³, serum creatinine < 1.5 mg/dl and all toxicities from chemoradiation have resolved to grade 2 or less. *If in the investigators opinion a delay for vaccine # 2 is necessary post chemo/RT it is allowable for vaccine # 2 to be given with a + 2 week window but reason and delay must be documented and submitted to BrUOG.* The subsequent third and fourth treatment with of ADXS11-001 will be administered at 28 day intervals (+7 days). Standard treatment with mitomycin, 5-FU and radiation for anal cancer has substantial toxicity. In RTOG 9811, 74% of patients had grade 3/4 nonhematologic toxicity and 61% of patients had grade 3 or grade 4 hematologic toxicity from this regimen. Therefore, the toxicities of standard chemoradiation with mitomycin, 5-FU and radiation are well above the conventionally accepted parameters in a phase I study even prior to adding ADXS11-001. However, it is critical that the addition of ADXS11-001 does not compromise the delivery of potentially curative standard chemoradiation for anal cancer.

Therefore we define toxicities for patient removal as:

- Grade 4 treatment related toxicities lasting greater than 7 days
- Febrile neutropenia lasting > 7 days.

- Inability to administer full dose of intended radiation within a period of 14 weeks due to treatment related toxicities.
- Persistence of listeria bacteremia as documented by positive blood cultures for > 3 days (> 72 hours).
- Documented bacterial meningitis
- Clinical sepsis requiring pressors.

2.10 Protocol Coordination:

The protocol will be centrally coordinated by the Brown University Oncology research Group. The principal investigator, Dr. Howard Safran, will hold the IND. The primary contract for the clinical management of the study will be between Advaxis and Rhode Island Hospital, the principal teaching hospital of the Brown University Oncology research Group. Rhode Island Hospital will coordinate subcontracts with all other participating institutions. The Brown University Oncology research Group central office will be responsible for clinical, data and regulatory oversight of all participating sites. (See section 15.4.1 for more information on protocol coordination.)

2.11 Phase I Data and Initiation of Phase II Component of Study:

The Brown University Oncology Research Group initiated this phase I study with the goal of evaluating two different treatment schedules of ADXS11-001 with standard mitomycin, 5-FU and concurrent radiation. Patients with newly diagnosed anal cancer with a primary tumor > 4cm or lymph node involvement, without distant metastases were eligible. Patients received 2 courses of mitomycin, 5-FU with concurrent radiation (54 Gy in 30 fractions by IMRT). Patients were to receive 4 treatments of ADXS11-001, 1×10^9 colony forming units intravenously once approximately every 28 days. In treatment schedule #1, the first dose was given before chemoradiation and the 2-4th doses were given every 28 days after completion of radiation. In treatment schedule #2, the second dose of ADXS11-001 was to be administered currently during chemoradiation on day 21 (the week prior to the second course of chemoradiation).

The goal of the Brown University Oncology Research Group study was to establish the safety of each treatment cycle to acquire the necessary preliminary data to evaluate ADXS11-001 with chemoradiation in the cooperative groups. Four patients have been treated with ADXS11-001 and chemoradiation on treatment schedule #1. The median age was 57. Two patients had N3 disease, one patient had N2 disease, and one patient had a T4 tumor with a fistula requiring a diverting colostomy prior to starting treatment. There has been no evidence of enhanced hematologic or non-hematologic toxicity from the addition of ADXS11-001. One patient developed grade 3 neutropenia. There have been no DLTs observed, and, as originally defined by the protocol, it has been determined that Treatment Schedule #1 is safe. However, of the four patients treated on Treatment Schedule #1, three had significant symptoms following infusion of ADXS11-001 including temporary rigors requiring 2-3 courses of IV meperidine. Patients also experienced fever and myalgia (lasting approximately 24 hours). One of these patients required overnight admission. These were expected toxicities and not of severity to characterize as dose limiting toxicities. However, the Brown University Oncology Research Group has decided not to evaluate Treatment Schedule #2. The intensity of the symptoms following infusion, are not unexpected in patients with a good performance status without exposure to prior chemotherapy, who are exposed to Listeria. These symptoms may be difficult to manage in a cooperative group setting on Treatment Schedule #2, when the ADXS11-001 infusion is given during chemoradiation, just prior to the second cycle of mitomycin and 5-FU. Fever and rigors produced by administration of day 21 ADXS11-001 could be quite problematic. Therefore, this amendment modifies the current clinical study to become a phase II trial to further evaluate treatment schedule #1. There will only be one treatment schedule assessed.

3.0 PATIENT ELIGIBILITY

3.1 Conditions for Patient Eligibility

- 3.1.1 Histologically-proven, invasive primary squamous, basaloid, or cloacogenic carcinoma of the anal canal;
- 3.1.2 AJCC 2009 TN Stage: T1N1-N3, T2(< 4cm)N1-N3, T2(\geq 4cm)N0,T3N0-3, T4N0-3;based upon the following minimum diagnostic workup:
- 3.1.2.1 History/physical examination within 14 days prior to registration;
- 3.1.2.2 Within 42 days prior to registration, the patient must have an anal examination with documentation of primary anal lesion size and distance from the anal verge. The preference is by any of the following: colonoscopy, sigmoidoscopy, or rigid proctoscopy, however if patient underwent anoscope this can be used for documentation. If patient is unable to tolerate any scope, reason must be documented and MRI can be used for disease measurements, distance and monitoring of disease response. Documentation of primary anal lesion size and distance from the anal verge to be sent regardless of type of scope or imaging.
- 3.1.3 Groin examination (clinical) within 42 days prior to registration with documentation of any groin adenopathy and lymphadenopathy (location: right vs. left; medial vs. lateral; mobile vs. fixed; and size);
- 3.1.4 X-ray (PA and lateral), CT scan, or PET/CT scan of the chest within 42 days prior to registration;
- 3.1.5 CT scan, MRI, or PET/CT of the abdomen and pelvis within 42 days prior to registration;
- 3.1.6 Zubrod Performance Status 0-1;
- 3.1.7 Age \geq 18;
- 3.1.8 Laboratory data obtained \leq 14 days prior to registration on study, with adequate bone marrow, hepatic and renal function defined as follows:
- Absolute neutrophil count (ANC) \geq 1,500 cells/mm³;
 - Platelets \geq 100,000 cells/mm³;
 - Hemoglobin \geq 8.0 g/dl (Note: The use of transfusion or other intervention to achieve Hgb \geq 8.0 g/dl is acceptable.);
 - Serum creatinine \leq 1.5 mg/dl;
 - Bilirubin $<$ 1.4mg/dl;
 - ALT/AST $<$ 3 x ULN;
 - Negative serum pregnancy test for women of child-bearing potential;
- 3.1.9 Women of childbearing potential and male participants must agree to use a 2 forms of medically effective means of birth control (such as a condom and spermicide) throughout their participation in the treatment phase of the study and for 90 days post last dose of study drug. To be documented.
- 3.1.10 Patients must sign a study-specific informed consent prior to study entry.
- 3.1.11 Patients with a history of clinically significant pulmonary disease must have PFTs demonstrating a DLCO \geq 40%. This testing is considered standard of care prior to mitomycin, 5-FU and radiation.
- 3.1.12 Patients with a history of clinically significant cardiac disease must have a LVEF \geq 30% by ECHO. (MUGA scan may also be used to determine LVEF) This testing is considered standard of care prior to mitomycin, 5-FU and radiation.
- 3.1.13 Patients must be able to swallow pills.

3.2 Conditions for Patient Ineligibility

- 3.2.1 Prior invasive malignancy (except non-melanomatous skin cancer), unless disease free for a minimum of 2 years;
- 3.2.2 Prior systemic chemotherapy for anal cancer;
- 3.2.3 Prior allergic reaction to the study drugs involved in this protocol.
- 3.2.4 Prior radiotherapy to the pelvis that would result in overlap of radiation therapy fields;
- 3.2.5 Severe, active co-morbidity, defined as follows:
- 3.2.5.1 Patients with uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris and cardiac

arrhythmia are ineligible. Furthermore, patients with unstable angina and/or congestive heart failure requiring hospitalization within the past 6 months are ineligible;

- Patients with active infection requiring systemic therapy (oral or IV) or those currently receiving antibiotics that cannot discontinue prior to dosing are ineligible. (Subjects who discontinue an antibiotic prior to dosing must wait at least 5 half-lives after the last dose of antibiotic before receiving any ADXS11-001 infusion- documentation required to be sent to BrUOG to confirm eligibility)
- 3.2.5.2 Transmural myocardial infarction within the last 6 months;
- 3.2.5.3 Acute bacterial or fungal infection requiring intravenous antibiotics at the time of registration;
- 3.2.5.4 Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of registration;
- 3.2.5.5 Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects;

3.2.6 Patients known to be seropositive for HIV and/or active hepatitis, even if liver function studies are in the eligible range.

3.2.7 Other immunocompromised status (e.g., organ transplant or chronic glucocorticoid use). If patient has diagnosis of immunodeficiency, is dependent on or has received systemic steroids therapy or any form of immunosuppressive therapy within 7 days prior to the first dose of ADXS11-001 they are ineligible. Topical corticosteroid or occasional inhaled corticosteroids are allowed.

3.2.8 Women who are pregnant or lactating are ineligible because the treatment involved in this study may be significantly teratogenic and there is the potential for transmission of listeria to the infant.

3.2.9 Patients allergic to or with sensitivity to penicillin, ampicillin, trimethoprim-sulfa *and* quinolones (including history of rash or anaphylaxis).

3.2.10 Patients allergic to naproxen.

3.2.11 Patients receiving oral or IV antibiotics

3.2.12 Patients with a prior history of a splenectomy and/or sickle cell trait/disease

3.2.13 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. Sponsor must be contacted prior to consenting any subject who has any other device and/or implant. Site is required to submit to BrUOG ALL surgical implants patient has ever had in their medical history and ALL surgeries regardless of link to this cancer diagnosis.

3.2.14 Patients who are receiving or may receive future treatment with PI3K or TNF α inhibitors. To be confirmed by treating medical oncologist in writing

3.2.15 Has 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: if patient underwent surgery > 6 weeks from start of ADSX11-001, all toxicities and/or complications must have recovered to baseline or Grade 1 prior to the initiation of ADXS11-001 study therapy and this must be documented for confirmation to BrUOG prior to registration.

3.2.16 Patient not being willing to have new infusion line placed for each infusion of ADXS11-001 as existing or newly placed central venous catheter or infusion ports are not allowed to be used for ADXS11-001 administration. Must be confirmed as discussed with patient and that they agreed.

3.2.17 Patient not willing to comply with requirement of central venous catheter or infusion port **must not be used** for 72 hours following the completion of the ADXS11-001 infusion **and** the patient receives the first post-treatment dose of oral antibiotics. Must be confirmed as discussed with patient and that they agreed.

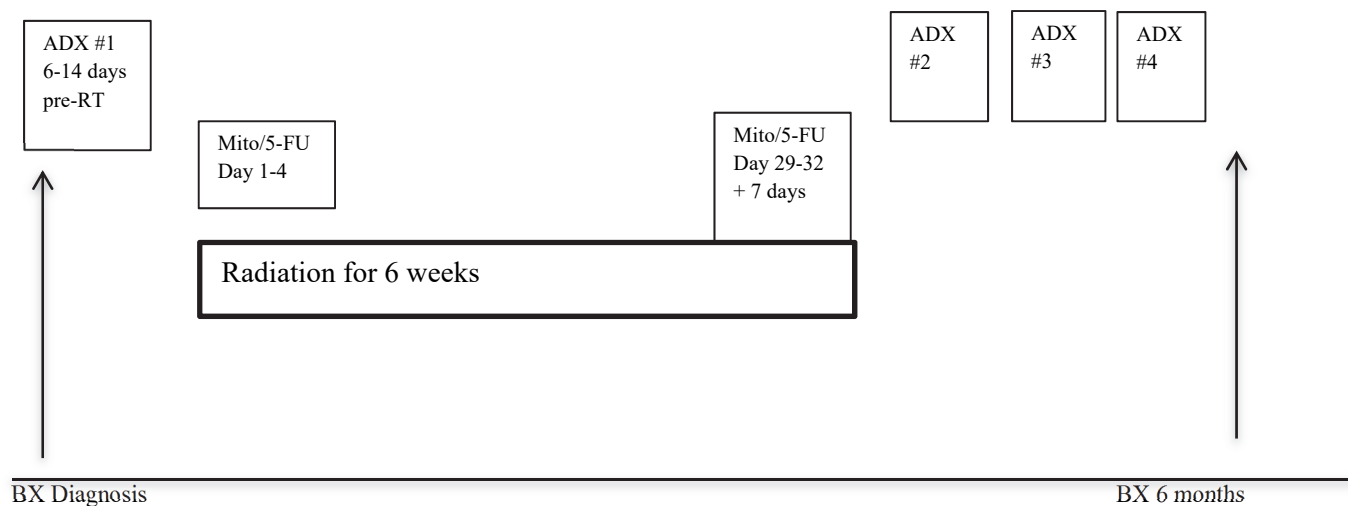
3.2.18 Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza 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. All recent vaccines (within 30 days) to be listed on conmed log and submitted to BrUOG.

3.2.19 Patient has a history of listeriosis or prior ADXS11-001 therapy.

4.0 TREATMENT

Treatment Schedule



Treatment Schedule: The first dose will be given 6-14 days prior to the initiation of chemoradiation. The 2-4th dosages of ADXS11-001 will not be until after completion of all chemoradiation. The second dosage of ADXS11-001 will not be administered until a minimum of 10 days (10-28 days) after completion of chemoradiation, ANC > 1,000 cells/mm³, Platelets ≥ 50,000 cells/mm³, serum creatinine < 1.5 mg/dl and all non-hematologic toxicities from chemoradiation have resolved to grade 2 or less. *If in the investigators opinion a delay for vaccine # 2 is necessary post chemo/RT it is allowable for vaccine # 2 to be given with a + 2 week window but reason and delay must be documented and submitted to BrUOG.* The subsequent third and fourth treatment of ADXS11-001 will be administered at 28 day intervals (+7 days). The second cycle of chemotherapy may be +7 days.

- The biopsy post chemoradiation will be performed at approximately 6 months after day #1 of the initiation of chemoradiation if there is residual suspicious tissue seen on examination.
- In addition, all subjects enrolled with amendment # 16 will participate in a 3 year *Lm* surveillance period.

Patients experiencing any the following toxicities will be removed from the study and should not receive any additional ADXS11-001 unless review of a specific case with clinical rationale provides support for patient to remain on study (information must be submitted to BrUOG with enough time for extensive review of the case):

- Grade 4 treatment related toxicities lasting greater than 3 weeks
- Febrile neutropenia lasting > 7 days.
- Inability to administer full dose of intended radiation within a period of 14 weeks due to treatment related toxicities.
- Persistence of listeria bacteremia as documented by positive blood cultures for ≥ 3 days (> 72 hours).
- Document bacterial meningitis
- Clinical sepsis requiring pressors.

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 patient; 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. BrUOG 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.

On study, surgeries and/or procedures must be scheduled following the completion of 7 day course of prophylactic antibiotics post ADXS11-001 infusion. In addition, “recommended antibiotic prophylaxis” per institution standards for respective surgeries/and or procedures must be followed.

BrUOG must be made aware of any surgery prior to it happening for review and please see section 11.1 for SAE definitions and contact BrUOG to confirm if surgery needs to be reported as a SAE

4.1 ADX

ADXS11-001 will be given at a dose of 1×10^9 cfu intravenously as per the treatment schedules described in Section 4.0. All doses of ADXS11-001 will be 1×10^9 cfu. The drug will be given as a 250ml infusion over 60 minutes.

ADXS11-001 **must be administered** 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.

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.

The following criteria must be met to receive ADXS11-001:

- $\text{ANC} > 1,000 \text{ cells/mm}^3$,
- $\text{Platelets} \geq 50,000 \text{ cells/mm}^3$,
- $\text{Serum creatinine} < 1.5 \text{ mg/dl}$
- All non-hematologic treatment related toxicities from chemoradiation have resolved to grade 2 or less.

PRETREATMENT PROPHYLACTIC REGIMEN:

- Mild to moderate flu-like symptoms and 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 30 minutes)

Premedication Regimen:

- Antihistamine- PO or IV (e.g., diphenhydramine 25mg or equivalent), once
- Naproxen, per schedule in section 4.1.2
- Antiemetic- PO or IV (e.g., promethazine or ondansetron), once
- Histamine H2-receptor antagonist- PO or IV (e.g., famotidine 20 mg or equivalent), once

Pretreatment medication should be given on the day of dosing and completed approximately 30 minutes prior to the start of the assigned study treatment infusion. Additional NSAID doses and antiemetic administration should be given per label or package insert post initial administration on Day 1 and Day 2, 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.

4.1.1 Antibiotics: At approximately 72 hours following each dose a 7 day regimen of oral antibiotics will be administered. Subjects will receive a 7 day course of either oral 80mg trimethoprim / 400 mg sulfamethoxazole once daily (x 7 days) or double strength, 160 mg trimethoprim / 800 mg sulfamethoxazole (DS) three times over the course of the 7 days. Subjects with known allergy to sulfa drugs may receive ampicillin 500 mg four times daily for 7 days beginning on Day 4 (approximately 72 hours) after each ADXS11-001 infusion. If calculated creatinine clearance is 10-50 mL/min, change frequency of oral ampicillin to TID. If calculated creatinine clearance is <10 mL/min, change frequency of oral ampicillin to BID.

Post Study Treatment - All subjects who enroll to this study with or after amendment # 16, will receive a 6 month course of oral trimethoprim/sulfamethoxazole or ampicillin for subjects with sulfa allergies to be initiated approximately 72 hours following the last dose of study treatment or at the time of study discontinuation. 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.

4.1.2 Naproxen: Patients will take naproxen beginning the day prior to each ADXS11-001 treatment. The dose of naproxen will be 440mg x 1 (first dose), followed by 220mg every 12 hours. The patient will receive a minimum of 4 doses of naproxen with each cycle (the day prior to dosing: 440mg in the morning and 220mg in the evening; Day 1: 220mg in the morning and 220mg in the evening). On the day of dosing with ADXS11-001, patients will take the AM dose of naproxen prior to arrival to the study site. Do not substitute acetaminophen or other NSAID for naproxen.

4.1.3: Antiemetics:

On the day of receiving ADXS11-001, patients will receive an oral or intravenous antiemetic/anticholinergic, such as promethazine (Phenergan), prochlorperazine or ondansetron . If an oral regimen such as promethazine is chosen, two doses of oral promethazine (25 mg) are to be

administered. One dose will begin just prior to the onset of the infusion at approximately 7:30 am, and a second dose will be given to the patient to take 8-12 hours after the first dose to mitigate possible side effects. An alternative antiemetic can be substituted at the discretion of the treating physician. On the date of receiving ADXS11-001, patients will also receive naproxen (as noted in section 4.1.2), an antihistamine, oral or IV, once, and a histamine H2-receptor antagonist, oral or IV, once. Pretreatment medication should be given on the day of dosing and completed approximately 30 minutes prior to the start of the assigned study treatment infusion.

Patients should be advised to continue naproxen prn (per the OTC label instructions) following each dose of ADXS11-001 dose until any symptoms subside.

4.1.4 Patient Monitoring and Assessment with ADXS11-001:

Treatment is to be done on an outpatient basis. Patients will be observed in the outpatient area for 4 hours following the ADXS11-001 infusion. Vital signs will be taken pre-dose, 30 minutes, 1 hour, 90 minutes, 2 hours, 2.5 hours, 3 hours, 3.5 hours and 4 hours after dose (+/- 15 minutes given for all time points except 4 hours post which is only +15 minutes). Patients will return on Day 2 for toxicity assessment and vital signs 24 hours (+/- 2 hours) after dose. Patients will return 72 hours after dosing for toxicity assessment and vital signs.

Day 1 of each treatment: At 4 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. If a patient's baseline (medical history) HR, blood pressure, respiration are not within normal limits but are within their normal medical history limits, documentation is to be made to note baseline medical history values and treating investigator to document that vital signs are not clinically significant or related and rather per patient's medical history.

A subject who experiences a fever (CTCAE Grade 1 or greater) 24 hours following the completion of ADXS11-001 infusion should be started on NSAIDs, hydration and other appropriate measures to treat the fever. In the event that the fever persists or worsens 48 hours following the completion of ADXS11-001 infusion then oral or broad spectrum IV antibiotics should be considered based on the subject's medical condition. If the fever remains unresponsive to oral/IV antibiotics 72 hours following the completion of the infusion then a blood culture should be obtained to evaluate for listeremia and determine the appropriate treatment course for the subject.

In the event a patient experiences a persistent fever at 72 hours after receiving ADXS11-001 the oral regimen below will be preceded by an IV dose of 500 mg of ampicillin. If calculated creatinine clearance is <10 mL/min, change dose of IV ampicillin to 250mg.

Patients should be monitored carefully for symptoms of bacterial meningitis, i.e., fever, stiff neck, headaches and altered mental status. Neurology and Infectious Disease consultation should be obtained if appropriate. All patients when stable for discharge should complete a regimen of antibiotics (beginning on 72 hours after administration of study drug, if not already started) for 7 days to ensure resolution of the infection.

Hypotension grade 1 will be defined, using CTCAE criteria, as a blood pressure (BP) for which intervention is not needed and where the patient is asymptomatic. At the baseline/registration appointment, the patient's BP is to be assessed based on their medical history and normal value. Per CTCAE hypotension is defined as: a disorder characterized by a blood pressure that is below the normal expected for an individual in a given environment. For all changes (drops) in blood pressure, for which

the patient is asymptomatic and no intervention is given, the treating MD is to document if the drop is expected for the patient in their environment. If the answer is no, then sites must document hypotension grade 1 on AE log.

See CTCAE for grade 2-4 criteria.

Sites are to use institutional standard practices to manage any adverse events that occur during or after vaccine administration (see table for management guidelines). Sites may also use institutional practices prior to vaccine administration, such as hydration to abate adverse events. **It is required however that the volume of the drug not be changed and the drug volume be 250mL. It is also required that the pre-medications be given per protocol section 4.1. Documentation on what patient was given to alleviate and manage events to be documented and submitted to BrUOG on CRF.**

Antibiotics post each dose ADXS11-001:

All subjects will receive a 7 day course of either oral 80mg trimethoprim / 400 mg sulfamethoxazole once daily or 160 mg trimethoprim / 800 mg sulfamethoxazole (DS) three times over the course of the 7 days. Subjects with known allergy to sulfa drugs may receive ampicillin 500 mg four times daily for 7 days. Antibiotics will begin on Day 4 (approximately 72 hours) after each ADXS11-001 infusion. Antibiotics post last dose of ADXS11-001 or off study: All subjects who were consented to amendment # 16 (or a later amendment) will be followed for 3 year *Lm* surveillance. The surveillance period will begin following the last dose of study treatment or at the time of study discontinuation. This period is intended to help ensure the eradication of *Lm* bacteria. This surveillance will include a 6 month course of either trimethoprim/sulfamethoxazole which will be initiated approximately 72 hours after the completion of the last dose of ADXS11-001 or immediately following study discontinuation.

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. In subjects with a sulfa allergy, the 6 month course should consist of ampicillin 500 mg four times daily for 6 months, initiated approximately 72 hours following the last dose of study treatment or anytime upon discontinuation of study treatment.

4.1.5 *Lm* Monitoring:

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 (for patients who sign consent with amendment # 16 or later). This period is intended to help ensure the eradication of *Lm* bacteria.

This surveillance monitoring phase will consist of the following:

- 6 month course of oral antibiotics,
- CBC, chemistries, including CRP and ESR, every 4 months (+/- 2 weeks)
- Blood cultures every 4 months (+/- 2 weeks)

This testing will be performed on all subjects who have received at least one dose of ADXS11-001 and who consented to amendment #16 (or later versions). Surveillance will occur every 4 months (± 2 weeks) for 3 years beginning after the last dose of study treatment (ADXS11-001 or chemotherapy/radiation, whichever comes last). Institutional practice is to be followed for blood culture guidelines, but blood cultures are required to be drawn and results of cultures and all labs are required to be submitted to BrUOG. It is required that site submit location from where blood culture sample was drawn.

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.

4.1.6 SUPPORTIVE CARE GUIDELINES:

The major safety findings with ADXS11-001 occurring in >5% of subjects, as of January 2015 (n=230) and being possibly, probably or definitely related include anemia (7.8%), chills (33.9%), fatigue (11.7%), fever (24.8%), nausea (20.4%), vomiting (13.5%), headaches (15.7%), and hypotension (6.5%). Most are Grade 1-2 in severity. Subjects should receive appropriate supportive care measures as deemed necessary by the treating Investigator including but not limited to the items outlined below:

4.1.7 CYTOKINE RELEASE SYMPTOMS:

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, 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. The symptoms are caused by an increase in cytokines such as TNF α , IFN γ 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 rare instances (~1%) 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 hypotension⁶⁴⁻⁶⁷ and are therefore recommended for cases of severe hypotension refractory to supportive care (e.g., fluids and/or pressors).

Recommended Management Guidelines for Adverse Events Associated with Cytokine Release

Toxicity	NCI CTCAE Grade or Severity	Treatment	Modification for Subsequent infusions
All other cytokine release symptoms (e.g. chills, rigors, fever, nausea, vomiting)	1	<ul style="list-style-type: none"> Supportive care 	<ul style="list-style-type: none"> No modification
Hypotension	2 Moderate	<ul style="list-style-type: none"> 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) 	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Appropriate supportive care measure 	<ul style="list-style-type: none"> Extend infusion time to 2 hours. Consider increasing doses of prophylactic medications
Hypotension	3 Severe	<ul style="list-style-type: none"> 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 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. 	<ul style="list-style-type: none"> Follow institutional standard of care for management
All other cytokine release symptoms (e.g. chills, rigors, fever, nausea, vomiting)	3	<ul style="list-style-type: none"> Appropriate supportive care measures 	<ul style="list-style-type: none"> Extend infusion time to 2 hours. Consider increasing doses of prophylactic dose of NSAID, or antiemetic as appropriate

Toxicity	NCI CTCAE Grade or Severity	Treatment	Modification for Subsequent infusions
Hypotension/ Organ toxicity, mechanical ventilation	4 Life threatening	<ul style="list-style-type: none"> • 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) 	<ul style="list-style-type: none"> • 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.[65-67]

4.1.8 Listeriosis and Listeria Infection - Identification and Management

A person with wild type 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.[68] 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 patients, 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. *Listeria monocytogenes* 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. [68]

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.

4.1.9 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, such as ampicillin. 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 (who consent to this study with amendment # 16 or later versions) will receive a 6 month course of an oral antibiotic regimen as a prophylactic measure 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 for all patients who are enrolled with amendment #16 (or later versions). This monitoring will include obtaining a blood sample for CBC, Chemistries, including CRP and ESR, and blood cultures for the detection of listeria. Institutional practice is to be followed for blood culture guidelines, but blood cultures are required to be drawn and results of cultures and all labs are required to be submitted to BrUOG. Testing will be performed on all subjects who have received at least one dose of ADXS11-001 and occur every 4 months (± 2 weeks) for 3 years. The 3 year time period will commence after the last study treatment patient receives on study (whether that be ADXS11-001 or chemotherapy/radiation)

Should a diagnosis of listeriosis be made at any point after treatment with ADXS11-001 and the 6 month course of oral antibiotics are 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 patients should be prepared and educational training performed.

4.1.10 Concurrent therapies and procedures: All prescription and nonprescription medication (excluding vitamins, nutritional supplements and hormone replacement therapy) taken by the patient from 30 days prior to screening and up to and including 30 days after the last administration of ADXS11-001 (or chemotherapy or radiation whichever is given last) will be recorded in the medical record and on the concomitant CRF. Any addition, deletion, or change in the dose of these medications will also be recorded during the study and 30 days post the last dose of ADXS11-001 or the last treatment with chemotherapy or radiation, whichever is last. 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 that are required per Lm monitoring are required to be captured on the CRF. If patients receive PI3K and TNF α inhibitors it is required that this be added to the concomitant medication log. As a reminder these drugs are prohibited.

Study subjects should be reminded that acetaminophen should not be used for pretreatment prophylaxis associated with the foreseeable adverse events related to the study drug 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 of this trial:

- Anti-cancer systemic chemotherapy or biological therapy
- Surgical treatment as per consultation with the sponsor
- PI3K and TNF α 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. Pneumonia vaccine is 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.

4.2 Chemotherapy

5-FU: 1 gm/m²/day x 96 hours beginning on day 1-4 and day 29-32 + 7 days

Mitomycin: 10 mg/m², day 1 and 29 (day 29 can be + 7 days), dose should be capped at 20mg for patients with higher BSA's

Second cycle of chemotherapy (Day 29-32) (5-FU, Mitomycin) will not be administered unless ANC is \geq 1,500 and platelets are \geq 100,000

Chemotherapy will be administered as per institutional standard procedure. Chemotherapy for cycle 2 can not be held for more than 4 weeks for toxicity. Once radiation is completed no chemotherapy is to be given, therefore the second dose of chemotherapy may need to be omitted depending on toxicity. Chemotherapy is given concurrent with radiation.

4.3 Radiation (IMRT)

Note: Intensity Modulated RT (IMRT) Is Mandatory. Tomotherapy Is Allowed.

(For specific questions or concerns regarding this section please contact Dr. Lisa Kachnic at Boston University Medical Center by email: lisa.kachnic@bmc.org or telephone: 617-638-7070)

An anorectal atlas and guidelines to aid in contouring of clinical target structures can be found at http://www.rtog.org/pdf_file2.html?pdf_document=AnorectalContouringGuidelines.pdf

Protocol treatment must begin within 21 days after protocol registration.

4.3.1 Dose Specifications

- 4.3.1.1** Radiation treatment will be delivered once daily, 5 fractions per week. All targets will be treated simultaneously. The treatment plan used for each patient will be based on an analysis of the volumetric dose, including dose-volume histogram (DVH) analyses of the PTV and critical normal structures. An “inverse” planning method using dose-objective-based computerized optimization shall be used. The treatment aim will be the delivery of dose to the PTVs and the exclusion of noninvolved tissue. As in RTOG 0529, review of all DP-IMRT volume contouring and planning directives will be undertaken (this will be done retrospectively). Please send the plan to BrUOG via email at kayla_rosati@brown.edu, via fax at 401-863-3820 or via mail at the address below. Please make sure to email Kayla Rosati when plans are to be sent.

BrUOG

Division of Biology & Medicine
Brown University
Box GR001
Providence, RI 02912

4.3.1.2 Target prescription dose: The prescription dose scheme shall depend on staging as follows:

4.3.1.3 For N0 disease: The primary tumor PTV (PTVA) will receive 54 Gy in 30 fractions at 1.8 Gy per fraction. The nodal PTVs will receive 45 Gy in 30 fractions at 1.5 Gy per fraction.

- PTVA will receive 54 Gy in 30 fractions at 1.80 Gy per fraction.
- PTV45 will receive 45 Gy in 30 fractions electively at 1.5 Gy per fraction and will include all nodal regions (mesorectum, presacral, obturators, internal iliac, external iliac and inguinal per the RTOG anorectal atlas, Myerson 2009).

4.3.1.4 For N+ disease: The primary tumor PTV (PTVA) and involved nodes PTV (PTV54) will receive 54 Gy in 30 fractions at 1.8 Gy per fraction.

For example, if the right groin nodes are positive and the left groin and pelvic nodes are clinically negative, then the respective PTV doses are 54, 45, and 45 Gy in 30 fractions

- PTVA will receive 54 Gy in 30 fractions at 1.8 Gy per fraction.
- PTV45 will receive 45 Gy in 30 fractions electively at 1.5 Gy per fraction and will include all uninvolved nodal regions.
- PTV54 will receive 54 Gy in 30 fractions at 1.8 Gy per fraction and will include all nodal regions containing involved nodes > 1cm in greatest dimension by CT/MRI or PET maximum standardized uptake value (SUV_{max}) uptake of 2.5 or greater.

**** At 54 Gy, a further boost of 5.4 Gy to persistent and bulky gross disease plus 1.5 cm CTV and 0.5 cm PTV margin may be provided at the discretion of the treating radiation oncologist, while maintaining the normal tissue radiation dose constraints provided in **Section 4.3.1.6**.

4.3.1.5 Treatment schedule: Treatment will be delivered once daily, 5 fractions per week (except for holiday weeks). All targets will be treated simultaneously. Breaks in treatment should be minimized.

4.3.1.6 Dose specifications:

The prescription isodose surface will encompass at least 90% of the primary and involved nodal PTVs, and at least 85% of the uninvolved nodal PTVs, reflecting the inherent difficulty of covering the shallow portions of these targets.

- No more than 5% of any PTV will receive < 95% of the prescription dose.
- No more than 2% of any PTV will receive < 90% of the prescription dose.
- No more than 2% of the primary PTV will receive > 115% of the prescription dose.

4.3.1.7 Planning priorities:

Tumor prescription goals followed by critical normal structure constraints are the most important planning goals.

4.3.2 Technical Factors

Megavoltage equipment capable of delivering static intensity modulation with a multileaf collimator or dynamic intensity modulation (using a multileaf collimator or tomotherapy) is required. Inverse-planned IMRT is required; conventional 3D CRT methods and forward-planned IMRT are excluded.

4.3.2.1 Localization, simulation and immobilization

4.3.2.2 A custom immobilization device (such as Alpha Cradle for supine patients and an Alpha Cradle with bowel displacement device for prone patients) is suggested to minimize set-up variability. Simulation will be done with the patient in the supine "arms up" or prone "arms up" position using a CT-simulator with a slice thickness ≤ 5 mm. It is strongly urged that patients be simulated in the prone position using bowel displacement techniques similar to those used for rectal cancer. On RTOG 0529, all cases with major small bowel violation were planned in the supine position. Oral and IV CT contrast is recommended, and an anal marker/wire at the verge or at the inferior extent of the tumor. Of note, daily

portal imaging is strongly encouraged for all patients. Additionally, consideration should be given to using the lightest couch top available to avoid unnecessary bolusing. Extra bolus on the inguinal skin is not typically necessary. For patients with macroscopic skin involvement, simulation should include placement of radio-opaque wires at the edge of visible/palpable skin involvement. Bolus (either auto-bolus or external bolus) must extend at least 2 cm beyond macroscopic disease. If the tumor does not extend on the perianal skin, check TLDs (4.3.3.12) to see if any bolus is warranted.

4.3.2.3 Treatment planning CT scans will be required to define gross target volume, and clinical target volumes. The treatment planning CT scan should be acquired with the patient in the same position and using the same immobilization device as for treatment. A description of the immobilization system used by each institution, patient position (supine or prone) and data regarding the range of positioning errors (if data exists) should be provided.

4.3.2.4 All tissues to be irradiated must be included in the CT scan. CT scan thickness should be 0.5 cm or smaller slices through the region that contains the primary target volumes. The regions above and below the target volume may be scanned also with slice thickness of 0.5 cm.

4.3.2.5 The GTV and CTV, and normal tissues must be outlined on all CT slices in which the structures exist.

4.3.3 Treatment Planning/Target Volumes

The definition of volumes will be in accordance with the 1993 ICRU Report #50: Prescribing, Recording and Reporting Photon Beam Therapy.

4.3.3.1 The Gross Tumor Volume (GTV) is defined as all known gross disease determined from CT (and MRI or PET if performed), clinical information, digital exam, endoscopic findings and biopsy. There will be three GTVs:

4.3.3.2 GTV_A includes the gross primary anal tumor volume (as documented by digital exam, and as seen on CT, and PET or MRI if performed). To help define the cephalad extent of palpable disease it is strongly recommended that, at simulation, a thin flexible tube be placed either in the rectum or vagina with the tip of the tube at the level of the cephalad extent of palpable disease.

4.3.3.3 GTV_{N54}, including all nodal regions (as documented by biopsy or radiograph) which will receive 54 Gy.

4.3.3.4 The Clinical Target Volume (CTV) is defined as the GTV plus areas considered to contain potential microscopic disease. Three different CTVs will be defined, namely CTV_A for the primary anal tumor volume; CTV₄₅ for the elective nodal regions; and CTV₅₄ for positive nodal regions.

4.3.3.5 CTV_A includes the gross primary anal tumor volume, the anal canal, and a 2 cm expansion into soft tissue and mesorectum, 1-2 cm into bladder, prostate, and gynecologic structures and none into bone and air.

4.3.3.6 CTV₄₅, CTV₅₄ includes the nodal regions (respectively uninvolved, involved); uninvolved nodal regions should receive a 7 mm expansion around the vessels, while involved nodes should receive a 1.5 cm expansion (except into uninvolved bone, genitourinary structures, muscles, or bowel).

4.3.3.7 Nodal regions include:

- a. Mesorectal (including peri-rectal and presacral)
- b. Right and left inguinal
- c. Right and left external iliac
- d. Right and left internal iliac

Examples of the definitions of the appropriate nodal groups can be found on

http://www.rtog.org/pdf_file2.html?pdf_document=AnorectalContouringGuidelines.pdf and in Ng M, Leong T, Chander S, Chu J, Kneebone A, Carroll S, Wiltshire K, Ngan S, **Kachnic L**: Australasian Gastrointestinal Trials Group (AGITG) Contouring Atlas and Planning Guidelines for intensity-Modulated Radiotherapy in Anal Cancer. Int J Radiat Oncol Biol Phys 2012; 83(5): 1455-62.

4.3.3.8 The Planning Target Volume (PTV) will provide a margin around the CTV to compensate for the variables of treatment set-up and internal organ motion. A minimum of 5 mm around the CTV is required in all directions to define each respective PTV; 1 cm is recommended if daily pre-treatment image guidance with kV or cone beam is not performed. A nodal PTV should not be allowed to overlap with the primary PTVA, provided that their dose objectives are different, so that the maximum dose to the nodal PTV can be controlled in the optimization.

4.3.3.9 Target volumes: Target tumor volumes are delineated slice by slice on the treatment planning CT images. The PTVs should spare non-target skin surfaces (manually or automatically trimmed to 3-5 mm within the skin surface).

4.3.3.10 Critical normal structures: In addition, surrounding critical normal structures, including the femoral heads (right and left), bladder, external genitalia, iliac crest, small bowel, large bowel outside the CTVs, and perianal skin should be outlined. Bowel should be drawn as individual loops without the intertwining mesentery. The normal tissues will be contoured and considered as solid organs. The tissue within the skin surface and outside all other critical normal structures and PTVs is designated as unspecified tissue.

4.3.3.11 Heterogeneity corrections: All dose distributions shall include corrections for tissue heterogeneities. The method used for heterogeneity calculations shall be reported.

4.3.3.12 TLDs (thermoluminescent dosimeters) or diodes should be placed at the anal verge for at least one full treatment fraction to verify the planned dose to that region within 20% accuracy. If TLDs are used, a set of at least 3 chips should be placed.

4.3.4 Critical Structures

4.3.4.1 Critical normal structures: DVHs must be generated for all critical normal structures.

NOTE: Effort should be made to achieve the listed dose constraints to normal tissues below. The dose constraints are listed in order from most to least important. Major violations include > 5cc small bowel above 50 Gy, any point dose small bowel > 54 Gy, and > 5% femoral heads above 44 Gy. All other dose constraint deviations are considered minor violations but are acceptable for treatment. BrUOG and the Radiation Chair must be made aware of any violations in writing prior to radiation being administered if site believes they are unable to abide by radiation guidelines per protocol for a patient.

4.3.4.2 Small bowel:

- No more than 200 cc above 30 Gy
- No more than 150 cc above 35 Gy
- No more than 20 cc above 45 Gy
- No more than 5 cc above 50.4 Gy
- Point dose > 54 Gy

4.3.4.3 Femoral heads:

- No more than 50% above 30 Gy
- No more than 35% above 40 Gy
- No more than 5% above 44 Gy

4.3.4.4 Iliac crests:

- No more than 50% above 30 Gy
- No more than 35% above 40 Gy
- No more than 5% above 50 Gy

4.3.4.5 External genitalia:

- No more than 50% above 20 Gy
- No more than 35% above 30 Gy
- No more than 5% above 40 Gy

4.3.4.6 Bladder:

- No more than 50% above 35 Gy
- No more than 35% above 40 Gy
- No more than 5% above 50 Gy

4.3.4.7 Large bowel:

- No more than 200 cc above 30 Gy
- No more than 150 cc above 35 Gy
- No more than 20 cc above 45 Gy

4.3.5 Radiation Adverse Events

4.3.5.1 Interruption of radiation during the treatment program is discouraged. However, a rest period of ≤ 7 days will be allowed for grade 4 skin reactions. If radiation is held for > 7 days, the radiation Study Chair, Dr. Kachnic, or Dr. Christopher Crane, must be notified.

4.3.5.2 Radiation therapy must be held/interrupted for the following indications:

- $ANC < 500/mm^3$ and/or platelets $< 50,000$: Radiation will resume when $ANC \geq 500$ and $PLT \geq 50,000$.
- \geq Grade 3 diarrhea (≥ 7 stools/day above baseline): Resume radiation when diarrhea is \leq grade 2 (< 7 stools/day above baseline). Obtain electrolytes and creatinine
- Grade 4 dermatitis: Radiation therapy can be resumed when dermatitis is \leq grade 3. **If RT is held for > 7 days, BrUOG, and the radiation Study Chairs, Dr. Kachnic, or Dr. Christopher Crane, must be notified.** Ulceration deemed to be due to tumor regression, rather than radiation dermatitis, is not a mandatory condition for holding treatment.
- Hold radiation therapy for \geq grade 3 vomiting: Resume radiation therapy at \leq grade 2.
- If localized or generalized infection develops secondary to an area of confluent moist desquamation, radiation therapy will be held. In this setting, radiation therapy will be resumed after there has been complete resolution of infection.

Please note as an example: If radiation is held on day 29, chemotherapy on day 29 will also be held until radiation can be resumed.

5.0 TOXICITIES, DOSE MODIFICATIONS, AND MANAGEMENT

Toxicities will be recorded as adverse events on the Adverse Event case report form and must be graded using The National Cancer Institute's Common Toxicity Criteria (CTCAE) version 4.0 (Appendix C).

5.1 Dose Modifications (Radiation, Mitomycin-C, 5-FU)

(These dose modifications were utilized in the anal cancer study RTOG 0529)

Note: Once reduced, drug doses must not be re-escalated to the original dose.

Toxicity	Parameters	Agent	Modification ^{c,d}
Diarrhea	Grade 3	1)5-FU 2) RT	1) Decrease dose 50% ^a 2) Hold RT until diarrhea is ≤ grade 2
	Grade 4	1)5-FU 2)RT	1) Decrease dose 50% ^{a, b} 2) Hold RT until diarrhea is ≤ grade 2
Mucositis/Stomatitis	Grade 3	1)5-FU	1)Decrease dose 50% ^a
	Grade 4	2)5-FU	2)Decrease dose 50% ^{a, b}
Rash: Dermatitis associated with radiation	Grade 3	1)5-FU 2)RT	1)Decrease dose 50% 2)No required interruption of RT
	Grade 4	1)5-FU 2)RT	1)Do not give the next cycle 2) Hold RT until ≤ grade 3
ANC or Platelets	Grade 3 (ANC<1000) (Platelets<50,000)	1)5-FU and mitomycin-C 2)RT	1)Decrease dose 50% ^e 2)Hold RT; resume when ANC ≥ 500 and PLT ≥ 50,000
	Grade 4 (ANC<500) (Platelets <25,000)	1)5-FU and mitomycin-C 2)RT	1)Decrease dose 50% ^b 2)Hold RT; resume when ANC ≥ 500 and PLT ≥ 50,000
NOTE: Second cycle of chemotherapy (5-FU, Mitomycin) will not be administered unless ANC is ≥ 1,500 and platelets are ≥ 100,000. Chemotherapy will not be held for more than 4 weeks secondary to toxicity.			

- Obtain electrolytes and creatinine according to good medical practice, if not performed in previous 24 hours, and correct any electrolytic, renal, or volume abnormalities. These should have returned to baseline prior to any further chemotherapy being administered.
- Obtain CBC for any non-hematologic toxicity (including sepsis, fever, and bleeding) other than rash. In the presence of **any** non-hematologic toxicity, use the most severe hematologic toxicity observed during cycle 1 (including during drug infusion, interval, incidental, or pre-treatment) in considering dosages for the second cycle as follows:
Responses to modifications of 5-FU dosage are unpredictable due to genetic polymorphism (Dihydropyrimidine dehydrogenase, or DPD). If, in the investigator's opinion it would *not* be in the best interest of the patient to continue with 5-FU after a grade 4 adverse event because of the risk of repeated severe toxicity, further 5-FU should not be administered. Assessment of the patient should be based on the patient's *overall* condition including consideration of each individual toxicity encountered. Consideration must include the duration of the toxicity in addition to its *severity*. In a study of patients experiencing severe toxicity from 5-FU, a correlation was shown between the sum of all the toxicity grades for mucositis, neutropenia, thrombocytopenia, diarrhea, and neurotoxicity (always graded 4 as neurotoxicity is a strong predictor for DPD deficiency) and measured DPD levels.²⁸ Patients with any neurotoxicity or several concurrent grade 4 toxicities should not receive further 5-FU without a DPD assay. However, there is no absolutely safe level, and all patients require careful individual assessment. Women are more likely to have DPD deficiency than men. A thorough discussion should be conducted with the patient as to the possible risks and benefit of receiving further 5-FU.
- If a grade 4 toxicity occurs during the 96-hour infusion of 5-FU, the 5-FU must immediately and permanently be discontinued for that cycle.
- The second cycle of chemotherapy will not be administered until ANC is ≥ 1,500 and platelets are ≥ 100, 000.
- Based on pre-treatment counts, not interval counts.

5.2 ADXS11-001 Dose Modifications

5.2.1 ADXS11-001 dose will not be reduced.

The following criteria must be met to receive ADXS11-001:

- ANC > 1,000 cells/mm³,
- Platelets ≥ 50,000 cells/mm³,

- Serum creatinine < 1.5 mg/dl
- All non-hematologic treatment related toxicities from chemoradiation have resolved to grade 2 or less.

Patients experiencing any the following toxicities will be removed from the study and should not receive any additional ADXS11-001 unless review of a specific case with clinical rationale provides support for patient to remain on study (information must be submitted to BrUOG with enough time for extensive review of the case):

- Grade 4 treatment related toxicities lasting greater than 3 weeks
- Febrile neutropenia lasting > 7 days.
- Inability to administer full dose of intended radiation within a period of 14 weeks due to treatment related toxicities.
- Persistence of listeria bacteremia as documented by positive blood cultures for ≥ 3 days (> 72 hours).
- Document bacterial meningitis
- Clinical sepsis requiring pressors.

5.2.2 Additional specific toxicities for ADXS11-001:

5.2.2.1 Cardiovascular Toxicity:

Patients with persistent grade 2-4 hypotension and/or grade 2 or 3 tachycardia (greater than 24 hours) with or without associated symptoms (e.g., syncope, dizziness, etc) despite optimal medical management may require a delay in subsequent therapy until recovered to grade 1.

5.2.2.2 Persistent Listeria Infection: Patients who become persistently listeremic (defined by a positive blood culture for Listeria at greater than or equal to 72 hours after dosing), or for whom symptomatic treatment of the adverse event fails, will remain hospitalized until the infection has resolved with antibiotics. IV ampicillin is recognized as first line therapy for Listeria infection.

Trimethoprim/sulfamethoxazole is second line, for penicillin-allergic patients. In the event any patient fails to respond to ampicillin (or trimethoprim/sulfamethoxazole if allergic to penicillin), ciprofloxacin should be considered. As a third line, oral amoxicillin or oral augmentin may be tried. Infectious Disease consultation may be helpful. Patients with persistent listeria bacteremia documented by persistently positive blood cultures for > 72 hours will be removed from study treatment.

5.2.2.3 Hypersensitivity/Anaphylaxis/Infusion Site Extravasation: Grade 3 (or greater) toxicity requires prompt cessation of drug infusion and treatment of associated symptoms including bronchospasm, urticaria, hypotension, ulceration, and/or necrosis. Patients with this type of reaction will be removed from the study. Patients with such reactions occurring from treatment initiation with ADXS11-001 until 28 days post radiation will be replaced. Patients will be added to the same treatment schedule.

6.0 Schedule of Events For new patients who sign on to the study with amendment # 16 (or later versions)	Prior to study entry/registration	Prior to each tx with ADXS11- 001 (within 72 hours prior to dosing)	0-3 days pre- RT	Weekly during RT ^f	1-2 weeks after completion of RT	2-6 weeks after 4 th tx of ADXS11-001 or if off study prior to 4 th ADXS11-001, at time study discontinuation	6 months post day 1 chemo/RT	Follow-up b
Informed Consent	X							
Medical History including all past surgeries	≤ 14 days							
Concomitant medications (see section 4.1.10)	<u>Inclusive of 30 days prior to drug</u>	X				X		X
Physical ^e	≤ 14 days	X	X	X	X	X		X
Groin Exam _e	≤ 42 days							
Diagnostic Biopsy	anytime							
X-ray or CT or PET/CT of Chest	≤ 42 days					X		X ^K
CT or MRI or PET/CT of Abdomen & Pelvis	≤ 42 days					X		X ^K
ECOG performance status	≤ 14 days	X	X	X	X	X		X
CBC w/ diff, Platelets and ANC	≤ 14 days	X ^d	X	X	X ^d	X		X ^L
Serum creatinine	≤ 14 days	X ^d	X	X	X ^d	X		
Electrolytes(Na, K, Cl, Co2)	≤ 14 days	X ^d	X	X	X ^d	X		X ^L
ALT/AST/bilirubin	≤ 14 days	X ^d	X	X	X ^d	X		
Serum pregnancy test (if applicable)	≤ 14 days							
PFTs with DLCO if history of clinically significant pulmonary disease	≤ 6 weeks							
Cardiac echo for LVEF if history of clinically significant cardiac disease	≤ 6 weeks							
HIV test	≤ 14 days							
Adverse event evaluation ^a	X	X	X	X	X	X		X ^a
Tumor evaluation by colonoscopy, sigmoidoscopy or proctoscopy	≤42 days ^H						X (Bx if clinically indicated)	12 months (6 months post 6 months scope) then annually for 5 years. (Bx if clinically indicated)B
Bx after chemo radiation and 4 th tx of ADXS11-001							6 months ^G	
Dispense/Prescribe Oral Prophylactic Antibiotics J		X				X		X
Disease and survival status								X
Lm Surveillance Monitoring								X ^L

A Patients will be followed for AE/SAE for 1 month (30 days) post last study drug (treatment on study). Post treatment assessment should not be done earlier than 30 days but may be done up to 1 week post 30 days. See section 11 for more details

B Patients will be followed every 4 months (+/-2 weeks) for 3 full years post the last dose of ADXS11-001 or the final study treatment with chemotherapy/radiation (whichever is last) for Lm surveillance and follow-up assessments. The follow-up portion will commence once patient comes off study (for patients who come off prior to receiving all 4 vaccines) or post the 2-6 week post the 4th treatment time point/visit. (For example if a patient comes off post vaccine #2, they will have the off study visit then commence follow-up 4 months later. If they complete study and all 4 vaccines, they will undergo an assessment 2-6 weeks post the 4th vaccine and then will start follow-up 4months later.) After 3 years, follow-up to be done annually year 4 and year 5 (year 4 should be approximately 1 year after final 3 year post appointment and 5 year should be approximately 1 year after year 4 appointment). Follow-up is not required for patients who were registered then withdrew prior to any study treatment with

ADXS11-001. Tumor evaluation is not required for patients who came off study for disease progression or for patients who progress in follow-up.

C clinical documentation of any groin adenopathy and lymphadenopathy (location: right vs. left; medial vs. lateral; mobile vs. fixed; and size)

D Labs prior to ADXS11-001 are to be done within 72 hours of administration. If it is a Monday holiday an additional day is provided. Labs from screening can be used for ADXS11-001 #1 if within the 14 day window.

E Physical exam may include anal exam. Physical exam, PS and AE evaluation from screening may be used for pre ADXS11-001 #1 time point if done within the 14 day window.

F Labs during weekly radiation must be done weekly. D29-32(+ 7 days) chemotherapy: labs will correspond with weekly radiation labs and a 72 hour window is also allowed prior to dosing. If there is a holiday an additional day is provided but this is to be documented.

G If at 6 month time point (6 months post day 1 of chemo-radiation) there is no residual suspicious tissue or if thought by physician to not be in the best interest of patient to biopsy, documentation of examination with reason for no biopsy to be sent to BrUOG to provide data for this time point.

H If patient underwent anoscope this can be used for documentation. If patient is unable to tolerate any scope, reason must be documented and MRI can be used for disease measurements, distance and monitoring of disease response. Documentation of primary anal lesion size and distance from the anal verge to be sent regardless of type of scope or imaging at baseline.

I Sigmoidoscopy, proctoscopy, colonoscopy to be used for assessment. Anoscope is allowed but is not preferred. Unlike follow-up assessments, tumor evaluations are to be based on start date of chemotherapy/radiation. The 6 months tumor evaluation (and potential biopsy, see subscript H), is to be done 6 months post day 1 of chemo/RT. After this assessment, scopes are to be ordered as per institutional standard of care approximately 1 year (6 months post initial 6 month scope with bx if applicable) and then annually from last scope (with bx if applicable) for a total of 5 years. Therefore, if patient's day 1 chemo/RT was January 1 2015, the 6 month scope would be due in July 2015. The 1 year scope would then be due 6 months later in January 2016. After this scope, the patient should be due annually. If more frequent scopes are ordered, BrUOG is to receive the results and information.

J. All Subjects will receive a 37-day course of oral antibiotic therapy starting 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 mg sulfamethoxazole administered daily for 7 days. All subjects will also receive an additional 6 month oral antibiotic course to be initiated 72 hours following the last dose of study treatment or anytime upon discontinuation of study treatment. This course will consist of 80 mg trimethoprim/160 mg sulfamethoxazole once daily or 160 mg trimethoprim/800 mg sulfamethoxazole (DS) tablet three times a week for 6 months. In subjects with 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 and the 6 month course should consist of ampicillin 500 mg four times daily initiated approximately 72 hours following the last dose of study treatment or upon discontinuation of study treatment.

Antibiotics are required to be documented on the conmed log as well as the treatment form.

K. For patients who come off study without progression, they will be assessed via CT or MRI or PET/CT of Abdomen & Pelvis and X-ray or CT or PET/CT of Chest every 6 months (+/- 1 month) until progression or up to 5 years.

L. *Lm* Surveillance Monitoring will include a 6-month course of oral antibiotics and routine monitoring of CBC with differential and PLT, Chemistries (Na, K, Cl, Co2, serum creatinine, ALT/AST/bilirubin), CRP, ESR, and blood cultures. Following completion of the last dose of study treatment or following study discontinuation all assessments will be performed every 4 months (+/-2 weeks) for 3 years.

Patient must begin trial within 21 days of registration

Subject study participation:

Each subject may participate in the study for up to 6 years from the time informed consent is signed through the final study contact. This includes a study treatment phase, a 3 year post-ADXS11-001 treatment *Lm* surveillance period which includes a prophylactic oral antibiotic administration phase (6 months) and *Lm* surveillance monitoring approximately every 4 months for 3 years. All patients will be followed for disease and overall survival for 5 years post treatment as well. It is expected that a subject will participate in the study until the completion of the full 3 year *Lm* surveillance phase. However, following the completion of the 6 month oral antibiotic treatment, a subject will be eligible to participate in other investigational clinical studies.

Reminder that patients may not and should not receive treatment with PI3K or TNF α inhibitors post this study treatment. If a patient is known to receive treatment with a PI3K or TNF α inhibitor BrUOG must be made aware via the concomitant medication log..

For patients who were previously consented and treated with ADXS11-001 on BrUOG 276 prior to amendment # 16 please see table below for follow-up schedule. It is very important that previously treated patients be re-consented via the consent addendum to ensure they are informed of the new information regarding possible delayed and late Listeria infection. Re-consent must be clearly documented and submitted to BrUOG and also kept in the patient shadow chart in the research office, to ensure compliance with study guidelines.

Schedule of Events for patients who were consented and treated prior to amendment # 16	At months 6, 12, then annually for 5 years^a
Physical	X
X-ray or CT or PET/CT of Chest	X
CT or MRI or PET/CT of Abdomen & Pelvis	X
ECOG performance status	X
Adverse event evaluation ^a	X
Tumor evaluation by colonoscopy, sigmoidoscopy or proctoscopy	^B 6 and 12 months then annually for 5 years. (Bx if clinically indicated)

A. Patients will be followed for AE/SAE for 1 months post last study drug (treatment on study).

Patients will be followed every 6 months for year 1 then annually for a total of 5 years. The follow-up portion will commence once patient comes off study or post the 2-6 week post the 4th treatment time point/visit. (For example if a patient comes off post vaccine #2, they will have the off study visit then commence follow-up 6 months later. If they complete study and all 4 vaccines, they will undergo an assessment 2-6 weeks post the 4th vaccine to commence follow-up 6 months later.) For patients who come off study without progression, they will be assessed via CT or MRI or PET/CT of Abdomen & Pelvis and X-ray or CT or PET/CT of Chest every 6 months (+/- 1 month) until progression or up to 5 years. Assessment time periods will not be adjusted based on out of window assessments. For example if a patient is due for imaging in January and then again July and the January imaging is not done until February, the next assessment is still due per study calendar in July.

B. Sigmoidoscopy, proctoscopy, colonoscopy to be used for assessment. Anoscope is allowed but is not preferred. Unlike follow-up assessments, tumor evaluations are to be based on start date of chemotherapy/radiation. The 6 months tumor evaluation (and potential biopsy, see subscript H), is to be done 6 months post day 1 of chemo/RT. After this assessment, the 1 year scope (and bx if applicable) is to be done 6 months later. The patient will then undergo annual scopes (and bx if applicable). Therefore, if patient's day 1 chemo/RT was January 1 2015, the 6 month scope would be due in July 2015. The 1 year scope would then be due 6 months later in January 2016. After this scope, the patient would be due annually. Efforts to be made to keep patient on correct schedule and late or early assessments should not "reset" time frames for when scopes are due.. If more frequent scopes are ordered, BrUOG is to receive the results and information. Once a patient progresses additional scopes are not required per study.

Schedule of vital sign monitoring

	Pre-Dose	30 minutes	1 hour post Dose	1.5 hours	2 Hours Post Dose	2.5 hours	3 hours Post Dose	3.5 hours	4 Hours Post Dose ^b	24 hours +/- 2 hours	72 hours +/- 2 hours
ADXS11-001	VS ^a	VS	VS	VS	VS	VS	VS	VS	VS	VS + Toxicity Assessment	VS + Toxicity Assessment

^a VS: Vital signs to include temperature, heart rate, blood pressure, and respiration

^b the patient must have a temperature less than 38.5 degrees C/ 101.3 degree F 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. See section 4.1.4

(+/- 15 minutes given for all time points except 4 hours post which is only +15 minutes as patients must to be assessed a full 4 hours post vaccine)

6.1 Laboratory Correlative Studies:

Laboratory correlative studies may be performed on formalin-fixed, paraffin-embedded tumor specimens obtained during standard of care biopsies to evaluate for markers of immune response. Potential markers may include immunohistochemistry for T cell infiltration.

Formalin-fixed, paraffin-embedded tumor specimens may be evaluated for HPV-16 DNA with the use of the in situ hybridization–catalyzed signal-amplification method for biotinylated probes (GenPoint, Dako). HPV-16–negative tumors may be further evaluated for 12 additional oncogenic HPV types (18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) by means of a biotinylated-probe cocktail (GenPoint, HPV Probe Cocktail, Dako). An HPV-positive tumor will be defined as a tumor for which there was specific staining of tumor-cell nuclei for HPV in either analysis. Expression of p16 is an established biomarker for the function of the HPV E7 oncoprotein. Tumor p16 expression may be evaluated by means of immunohistochemical analysis with a mouse monoclonal antibody (MTM Laboratories) visualized with use of an autostainer (Ventana XT, Ventana) and a one-view secondary detection kit (Ventana). Positive p16 expression will be defined as strong and diffuse nuclear and cytoplasmic staining in 70% or more of the tumor cells.

7.0 Patient Assessments

7.1 Criteria For Evaluation And Endpoint Definitions

7.2 Measurement of Local Tumor

Clinical complete response will be assessed by no disease on evaluation. Biopsy not required if physician does not feel there is any suspicious tissue to biopsy, however examination/scope to be completed and documentation no biopsy done secondary to MD decision for no suspicious tissue to be submitted to BrUOG.

7.3 Other Response Parameters

7.3.1 Progressive Disease:

RECIST criteria for progressive disease: At least a 20% increase in the sum of the longest diameter (LD) of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more lesions

Local or regional progression by RECIST criteria must assessed with caution in patients with complete response by proctoscopy and biopsy since immunologic therapy may cause local inflammation or adenopathy. In these cases serial imaging is recommended prior to definitive determination of disease progression.

7.3.2 Second Primary Failure: The appearance of second primary tumor

7.3.3 Disease-Free Survival (DFS): Local-regional failure, appearance of distant metastases, appearance of a second primary and death due to any cause

7.3.4 Colostomy Failure: For patients entering the study without a diverting colostomy, failure is a colostomy for any reason. For patients entering the study with a diverting colostomy, failure is defined as one of the following:

- a. If the colostomy is reversed within 1 year from study entry, failure is a subsequent colostomy for any reason;
- b. If the colostomy is not reversed within 1 year, then it will be considered a colostomy failure at that time.

7.3.5 Colostomy-Free Survival: Failure is a colostomy or death due to any cause.

7.3.6 Overall Survival: Failure is death due to any cause.

8.0 PATIENT REGISTRATION

All patients will be registered through the Brown University Oncology Research Group Central Office. Eligibility Checklist with supporting documentation, and the signed Patient Consent Form must be faxed to the BrUOG Central Office, Fax: (401) 863-3820, at the time of registration and prior to patient treatment.

Details of patient's study participation should be documented in clinic/file notes. Registrations should be faxed to:

Kayla Rosati
Brown University Oncology Research Group,
Fax: 401-863-3820
Phone: 401-863-3000
Kayla_Rosati@brown.edu

All support data must be sent in with the corresponding BrUOG forms.

It is the treating physician's responsibility to review all data submitted to the BrUOG Central Office for accuracy and completeness and he/she must sign the off study form. Documentation and confirmation to be submitted to BrUOG to confirm all inclusion and exclusion criteria and for all assessments for registration in the schedule of evaluation table (section 6).

9.0 PHARMACEUTICAL INFORMATION

9.1 ADXS11-001 (IND #13,712)

9.1.1 Description:

ADXS11-001 for infusion 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 $-80 \pm 10^{\circ}\text{C}$.

9.1.2 How Supplied, Storage and Stability:

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

ADXS11-001 will be supplied by Advaxis and distributed by Almac Clinical Services LLC. 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 $-80 \pm 10^{\circ}\text{C}$. 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**

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 $-80 \pm 10^{\circ}\text{C}$. 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.**

- ADXS11-001 must be received frozen on dry ice and immediately stored at $-80 \pm 10^{\circ}\text{C}$.
- 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.[1] 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.
- Clinical supplies may not be used for any purpose other than that stated in the protocol.

9.1.3 **Safety Precautions:**

ADXS11-001 is classified a BSL-2, minimally pathogenic. Precautions as stated in the BMBL 5th Edition include [74]:

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 [50]. 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[75]. 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 (1×10^{10} CFU)[76].

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 Edition⁵⁰ 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 *Listeria monocytogenes* 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).

9.1.4 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± 10⁰ C and thaw the vials at room temperature for approximately 5-10 minutes. Gently agitate mildly by hand to ensure that the drug is in suspension. Prepare the dose and infusion volume of ADXS11-001 as described below.

ADXS11-001 (Drug Product concentration of 1.9x10⁹ cfu/mL)

To make a dose of 1.0x10⁹ cfu: Thaw 1 vial of 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 approximately 250 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 in the source document (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). **NOTE: The 60 minute ADXS11-001 infusion at room temperature must be completed within 6 hours from product vial removal from the freezer.**

9.1.5 Adverse Effects:

ADXS11-001 is a live attenuated bioengineered nonpathogenic strain of *Lm*, and one that is known to stimulate a strong innate immune response characterized by high levels of cytokine release from immune cells into the general circulation. This pattern of side effects is consistent with other immunotherapy agents.

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). These symptoms usually present within 2-4 hours after the completion of infusions and are often mild to moderate and transient in nature or respond 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.

Please reference the ADXS11-001 IB for complete information regarding AEs.

9.1.6 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.

9.1.6.1 Initial Requests:

The designated person(s) from each participating hospital will complete the Drug Order Request Form and email the completed form to Almac and cc BrUOG. 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 $-80 \pm 10^{\circ}\text{C}$.

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
<i>Monday</i>	<i>Tuesday</i>	<i>Wednesday</i>
<i>Tuesday</i>	<i>Wednesday</i>	<i>Thursday</i>
<i>Wednesday</i>	<i>Thursday</i>	<i>Friday</i>
<i>Thursday</i>	<i>Monday</i>	<i>Tuesday</i>
<i>Friday</i>	<i>Monday</i>	<i>Tuesday</i>

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
 Kayla Rosati at: Kayla_Rosati@brown.edu

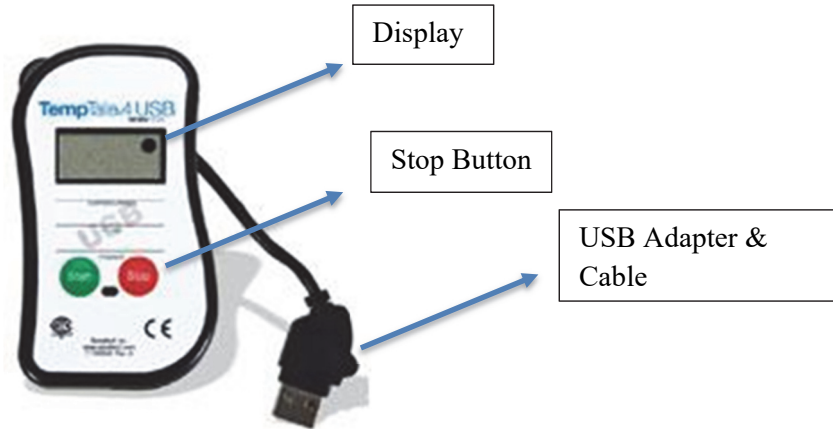
9.1.6.2 Subsequent Requests:

If additional drug is needed, please email a “Drug Order Request Form” to Almac as noted above.

Please note that study drug is shipped at -70°C and has a TempTale4 (temperature monitor) included in the box. It is imperative that all study drug shipments be stored at - 80 ± 10°C immediately after being removed from the shipping container.

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

Kayla_rosati@brown.edu

9.1.7 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.

9.1.8 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 and submitted to BrUOG.

9.1.9 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 towel 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.

9.1.10 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.

9.2 **5-Fluorouracil (5-FU): Refer to package insert for additional information**

9.2.1 Dose Formulation: 5-FU is a marketed drug available as 50 mg/ml in 10, 50 or 100 ml vials as a colorless to faint yellow preservative free aqueous solution, with pH adjusted to approximately 9.0 with sodium hydroxide. Compatible with NS, D5W. Administration of 5-FU should be only by the intravenous route taking care to avoid extravasation. Direct administration is over 1-2 minutes, or intermittently in 50-100 ml minibag over 20-30 minutes, or by continuous infusion (in 1 L normal saline or ambulatory infusion pump) which has the best therapeutic index. Vein pigmentation and thrombophlebitis may be seen distal to infusion sites. For continuous infusion a central venous access device is preferred. Compatible with heparin and leucovorin but not with ondansetron.

9.2.2 Pharmacology:

It is a fluorinated pyrimidine antimetabolite. 5-FU resembles the natural uracil molecule in structure, except that a fluorine atom has been substituted for a hydrogen atom in the 5 position. $T_{1/2}$ is 8-13 minutes. It is distributed in all body water by passive diffusion and crosses the placenta. There is evidence that the metabolism of fluorouracil in the anabolic pathway blocks the methylation reaction of deoxyuridylic acid to thymidylic acid. 5-FU generates fluorinated nucleotides (FdUMP, FUTP), which interfere with the synthesis of DNA and to a lesser extent inhibit the formation of ribonucleic division and growth by incorporation into RNA. The effect of fluorouracil may be to create a thymidine deficiency which provides unbalanced growth and death of the cell. Catabolism is via hepatic and extrahepatic routes via DPD to dehydrofluorouracil (DHFU), which is subsequently metabolized to fluoro- β -alanine (FBAL). Excreted as respiratory CO_2 , only 5% being excreted unchanged in the urine. Administration should be avoided in the presence of hepatic dysfunction.

9.2.3 Supplier:

5-FU is available commercially. Generic forms are available.

9.2.4 Storage:

Although 5-FU solution may discolor slightly during storage, the potency and safety are not adversely affected. Store at room temperature (49°-86°F) and protect from light. If a precipitate occurs due to exposure to low temperatures, resolubilize by heating to 140°F (60°C) with vigorous shaking; allow to cool to body temperature before using. Discard after 8 hours once vial is opened. Discard if yellow coloration is marked since this indicates decomposition. Stable in D5W for 30 days in plastic syringes at room temperatures. In portable infusion pump reservoirs 5-FU is stable for up to 7 days but precipitation may occur at low temperatures.

9.2.5 Side Effects and Toxicities:

Immediate side effects include mild nausea and vomiting (common), lacrimation, conjunctivitis, angina, arrhythmia, radiation recall and anaphylaxis (rare). Early onset effects include stomatitis and esophagopharyngitis (which may lead to sloughing and ulceration), diarrhea with cramping and/or bleeding, and anorexia. Leukopenia usually follows every course of adequate therapy with fluorouracil. The lowest white blood cell counts are commonly observed between the 9th and 14th days after the first dose, although uncommonly the maximal depression may be delayed for as long as 20 days. By the 30th day the count has usually returned to the normal range. Megaloblastosis may occur. Alopecia (usually mild) and dermatitis may be seen. The dermatitis most often seen is a pruritic maculopapular rash usually appearing on the extremities and less frequently on the trunk. Other side effects include myocardial ischemia, lethargy, malaise, headache, allergic reactions, neurotoxicity (disorientation, confusion, euphoria, dizziness, incoordination-acute cerebellar syndrome, encephalopathy), visual changes, photosensitivity (eyes and skin), nail changes including loss of nails, skin thickening, cracking, dryness or sloughing, biliary sclerosis, or acalculous cholecystitis. Hand foot syndrome (palmar plantar erythrodysesthesia) is more common with continuous infusion. Late effects may include tear duct fibrosis and neurotoxicity. See 7.4.1 for more information on decreased tolerance 5-FU and the management of toxicity.

9.3 Mitomycin-C

Refer to package insert for additional information

9.3.1 Dose Formulation:

Each vial contains either mitomycin-C 5 mg and mannitol 10 mg or mitomycin-C 20 mg and mannitol 40 mg. To administer, add sterile water for injection, 10 ml or 40 ml respectively to a concentration of 0.5 mg per ml. Shake to dissolve. If product does not dissolve immediately, allow to stand at room temperature or shake vial under warm tap water for 2 minutes to assist dissolution. Reconstituted mitomycin is a clear blue or purple solution. If reconstituted to higher concentrations (1-2 mg/ml) avoid low temperatures because crystals may form.

Mitomycin-C should be given intravenously only, using care to avoid extravasation (severe, see Appendix V). Give as slow push through sidearm of free flowing i.v. at 1.5 mg/3 ml per minute. May be mixed in 50-100 ml minibag over 10-30 minutes.

9.3.2 Pharmacology:

Mitomycin-C is an antibiotic with alkylating agent properties cross-linking guanine and cytosine, which selectively inhibits the synthesis of deoxyribonucleic acid (DNA) and degrades preformed DNA. This results in nuclear lysis and formation of giant cells. At high concentrations of the drug, cellular RNA and protein synthesis are also suppressed. Non-phase specific, but has maximum effects in late G1 and S.

In humans, mitomycin, which acts as a pro-drug is rapidly cleared from the serum after intravenous administration. It is distributed in kidneys, muscles, heart, lungs, intestines and ascites; and enters breast milk. The time required to reduce the serum concentration by 50% after a 30 mg bolus injection is 17

minutes. After injection of 30 mg, 20 mg, or 10 mg i.v., the maximal serum concentrations were 2.4 mcg/ml, 1.7 mcg/ml, and 0.52 mcg/ml, respectively. Clearance is affected primarily by metabolism in the liver, but metabolism occurs in other tissues as well. The rate of clearance is inversely proportional to the maximal serum concentration likely due to saturation of the degradative pathways.

Approximately 10% of a dose of mitomycin-C is excreted unchanged in the urine. Since metabolic pathways are saturated at relatively low doses, the percent of a dose excreted in urine increases with increasing dose. Small amounts are also found in the bile and feces.

Administer with caution in the presence of impaired liver or renal function.

9.3.3 Supplier: Mitomycin-C is available commercially. Generic forms are available.

9.3.4 Storage:

1. Unreconstituted: Mitomycin-C is stable for the lot life indicated on the package. Store at room temperature. Avoid excessive heat (over 40°C) and keep away from light.
2. Reconstituted Mitomycin-C is stable for 14 days refrigerated or 7 days at room temperature (up to 30°C). Higher concentrations than 0.5 mg/ml may degrade and precipitate if stored.
3. Compatible with D5W, NS and Ringer's lactate Diluted in various i.v. fluids at room temperature, to a concentration of 20 to 40 micrograms per ml:

IV Fluid	Stability
5% Dextrose Injection	3 hours
0.9% Sodium Chloride Injection	12 hours
Sodium Lactate Injection	24 hours

9.3.5 Side Effects and Toxicity

Immediate effects may include vein irritation, mild nausea and vomiting (1-2 hours) and bronchospasm (4-12 hours).

Early toxicities include thrombocytopenia and leukopenia which are cumulative. Nadir occurs at 24-28 days, with recovery at 42-56 days. Deaths due to septicemia have been reported. Stomatitis is frequent but mild, alopecia is rare. Rashes are rare and blue bands may be seen in the nails. Mitomycin may also cause dyspnea with cough and radiographic evidence of pulmonary infiltrate. A few cases of adult respiratory distress syndrome (interstitial pneumonitis) have been reported; also fever, anorexia, fatigue, blurred vision, amenorrhea, edema, thrombophlebitis, hematemesis, elevated LFTs or diarrhea may occur. CNS effects may include syncope and rarely acute encephalopathy.

Delayed toxicities (weeks to months) include chronic pulmonary fibrosis, erythema and/or ulceration occurring either at or distant from the injection site, rising creatinine, microangiopathic hemolytic anemia, and renal failure.

Note: Mitomycin is a vesicant and cellulitis, necrosis, ulceration and consequent sloughing of tissue may result if the drug is extravasated during injection (see Appendix V).

10.0 Agent Accountability

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from manufacturer using a Drug Accountability Record Form.

10.1 Treatment compliance

Records of study medication used, dosages administered, and intervals between visits will be recorded during the study. Drug accountability will be noted.

All drugs will be administered to eligible patients under the supervision of the investigator or identified sub-investigator(s). The pharmacist will maintain records of drug receipt drug preparation, and dispensing, including the applicable lot numbers. Any discrepancy between the calculated dose and dose administered and the reason for the discrepancy must be recorded in the source documents.

11.0 Adverse Drug Reaction (ADR) Reporting

U.S. regulations require that a sponsor reports Serious Adverse Events (SAEs) occurring with use of its product in a clinical trial if it is unexpected, and felt to be related to use of the drug. During the conduct of Investigator Sponsored Trials (ISTs), where the investigator holds the Investigational New Drug (IND) application, all SAEs that occur will be evaluated by the investigator for reportability to FDA. An Adverse Event should be identified, Serious Adverse Event and Expectedness determined and causality assessed by the investigator using the definitions that follow.

Intensity for each adverse event will be scored using CTCAE Version 4.0. A copy of the CTCAE Version 4.0 can be downloaded from the CTEP homepage (<http://ctep.info.nih.gov>). All appropriate treatment areas have access to a copy of the CTCAE Version 4.0.

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. All AEs and SAEs will be recorded from the time the consent form is signed through the 3-year *Lm* surveillance period of the study and at each examination on the AE CRF as described below. The reporting timeframe for AEs meeting any serious criteria is described in Section 11.1 below.

11.1 Definitions

An **Adverse Event** is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have to have a causal relationship with the treatment. For marketed products in the U.S., a **Serious Adverse Event** is an adverse event occurring at any dose that results in any of the following outcomes:

- Death
- Life-threatening¹
- Persistent or significant disability/incapacity²
- In patient hospitalization or prolongation of existing hospitalization: Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Congenital anomaly/birth defect

An event may not meet any of the above seriousness criteria but still be judged as medically serious. That is, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes (a-e) listed above. Some examples of this type of event are:

- blood dyscrasia without inpatient hospitalization
- convulsions without inpatient hospitalization
- intensive treatment in an emergency room or at home for allergic bronchospasm without inpatient hospitalization
- development of drug dependency
- drug abuse

¹ The term "life-threatening" in the definition of "serious" refers to an event in which in the view of the initial reporter the patient was at immediate risk of death from the adverse experience as it occurred; it does not refer to an event which had it occurred in a **more** severe form, might have caused death.

² A substantial disruption of a person's ability to conduct normal life functions.

- overdose with an associated serious event, or required intervention to prevent impairment/damage

An **Unexpected Adverse Event** is not listed in the current US Package Insert (USPI) or an event that may be mentioned in the USPI, but differs from the event because of greater severity or specificity.

Causality is a determination of whether there is a reasonable possibility that the drug may have caused or contributed to an adverse event. It includes assessing temporal relationships dechallenge/rechallenge information, association (or lack of association) with underlying diseases, and the presence (or absence) or a lack of one or more likely causes.

The Investigator must determine if an adverse event is in some way related to the use of the study drug. This relationship should be described as follows:

Unlikely: The event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the effect of a concomitant medication, a new condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug.

Possible: The event follows a reasonable temporal sequence from administration of the study drug or the event follows a known response pattern to the study drug *BUT* the event could have been produced by an intercurrent medical condition which, based on the pathophysiology of the condition, and the pharmacology of the study drug, would be unlikely related to the use of the study drug or the event could be the effect of a concomitant medication

Probable: The event follows a reasonable temporal sequence from administration of the study drug and the event follows a known response pattern to the study drug *AND* the event cannot have been reasonably explained by an intercurrent medical condition *or* the event cannot be the effect of a concomitant medication

Definite: The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug and based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug

Unknown: Based on the evidence available, causality cannot be ascribed

For both serious and non-serious adverse events, the investigator or sub-investigator must determine both the intensity of the event and the relationship of the event to drug administration.

Any serious adverse event occurring after the patient has provided informed consent and until 4 weeks after the patient has stopped study participation (last date of treatment) must be reported. This includes the period in which the study protocol interferes with the standard medical treatment given to a patient (e.g. treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication).

For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer by the investigator

11.2 Recording and Reporting: Serious Adverse Events Reportable to the FDA

In the context of an Investigator Sponsored Trial being conducted under an IND, CFR 312.32 states the FDA needs to be informed of unexpected and related SAEs (SUSARs) as soon as possible or within 15 calendar days. In addition, the investigator must notify the FDA of any unexpected, fatal or life-threatening experience associated with the use of the drug as soon as possible, or within 7 calendar days by telephone or facsimile.

When the principal investigator has determined that a Serious Adverse Event requires reporting to the FDA (unexpected and possibly related to study drug), the following actions must be completed:

Actions towards FDA:

Sites: All events must be reported, by FAX to Brown University Oncology Research Group.

BrUOG: The BrUOG Central Office will notify by phone and/or fax all drug reaction reports to the FDA, Advaxis, the Principal Investigator, and the participating sites (who will in turn notify their local IRBs) who will report all SAEs to Advaxis regardless of relationship with any study drug or expectedness **within 24 hours of learning of its occurrence but no later than 7 days.** The BrUOG Central Office will report all unexpected and related SAEs (SUSARs) to Advaxis and their Pharmacovigilance CRO **within 24 hours of learning of its occurrence but no later than 7 days.**

Sites: All sub-investigators must report all SAEs to the investigator-sponsor so that the investigator-sponsor can meet his/her foregoing reporting obligations. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences.

BrUOG: Serious unexpected adverse events will be reported as an amendment to the IND within 15 days of sponsor notification. They will all receive a simultaneous copy via facsimile of all adverse events filed with the FDA. A copy of the form will be kept by the BrUOG Central Office.

Sites: All deaths during treatment or within 30 days following completion of active protocol therapy (treatment) must be reported within 5 working days of being made aware of the event. For SAEs that are thought to be related to drug or for deaths thought to be even possibly related to drug, within 24 hours of being made aware of the event (when possible), but no later than 5 business days.

30 days post last dose of treatment: All deaths or SAEs that are thought to be potentially related to the ADXS11-001 drug or SAEs with any potential link to Listeria are to be reported to BrUOG within 5 days of the site being made aware of the event. AEs that are not serious during this time frame do not need to be reported to BrUOG. See 11.2.1 #5

11.2.1 Site responsibility when reporting an FDA action

The principal investigator is also responsible for providing all Serious Adverse Events (on the OBA form) to the BrUOG office who will submit to the FDA and Advaxis on behalf of the site. The BrUOG office will in return send a formal letter to all sites participating regarding each event. It is then the responsibility of each participating site to submit to the IRB on record as per their local IRB policy. This applies to initial and follow-up information.

1. **Telephone report:** For SAE's, both initial and follow-up, contact BrUOG Central Office (401) 863-3000 within 24 hours of being made aware of the event and PRIOR to submitting any reports to BrUOG. For SAEs BrUOG will contact Advaxis within 24 hours of being made aware of the event, but no later than 7 days with the completed SAE report.
2. **Complete the OBA Adverse Event Page (Appendix H)**

OBA Reporting Guidelines:

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description of the OBA form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics

- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication (ADXS11-001, Mitomycin, 5-FU, radiation)
3. **Attach the photocopy of all applicable examinations**, medical notes and records related to the Serious Adverse Event and document the dates these were made. For laboratory results, include the laboratory normal ranges. For hospitalizations, Admission H&P, Discharge Summary, Consultative reports, etc. could be very helpful. In the case of a Fatal event, provide an autopsy report, when it becomes available
 4. **Sites to send the completed OBA Serious Adverse event page to the BrUOG office no later than 5 business days from site being made aware of the event. For SAEs that are thought to be related to drug or for deaths thought to be even possibly related to drug, within 24 hours of being made aware of the event (when possible), but no later than 5 business days. 30 days post last dose of treatment: All deaths or SAEs that are thought to be potentially related to the ADXS11-001 drug or SAEs with any potential link to Listeria are to be reported to BrUOG within 5 days of site being made aware of the event.**
 5. **Post 30 day AE assessments all patients & for 3 year safety observation period (for patients who enroll to amendment # 16 or later versions): It is required that sites submit all SAEs that are thought to be possibly related to ADXS11-001 or which have any potential relationship or correlation to Listeria. This requirement is applicable for all patients including 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 BrUOG or any other authority.**

Brown University Oncology Research Group (BrUOG) Central Office,
 Phone: (401) 863-3000,
 Fax: (401) 863-3820
 Cell: (401) 413-5844

Kayla_Rosati@brown.edu

11.2.2 BrUOG responsibility for reporting:

1. To review all materials received from sites
2. Telephone the FDA immediately (day of awareness), in the case of reportable death or life-threatening events
3. Fax completed OBA form to the FDA and mail when appropriate within the timelines mentioned above

FDA CBER

10903 New Hampshire Avenue, Building 71, G112, Silver Spring, MD 20993

Fax division fax: 1-301-595-1303 and OBA fax at 301-496-9839

As well as sending the SAE to Center for Biologics Evaluation and Research as an amendment to the IND)

BrUOG will also send the OBA SAE form to OBA via their requirements.

The Brown University Oncology Research Group will provide a copy of the information sent to FDA by email within 24 hours, but no later than 7 days, after being made aware of the SAE to the following Advaxis responsible parties:

- Advaxis PV CRO (for SUSARs only)- serious unexpected and related to ADXS11-001: i3drugsafetyPV@inventivhealth.com or FAX: 866-880-9343
- Advaxis Pharmacovigilance (for all SAEs including SUSARs): APV_BrUOG276@advaxis.com

In addition, all SAEs shall be sent to Advaxis on an ongoing basis.

Results of any relevant complementary exams performed to obtain the final diagnosis of any SAE (e.g. hospital discharge summary, autopsy report, consultations, etc.) will be made available to Advaxis upon request.

11.2.3 Follow-up Reports

Additional Info may be added to a previously submitted report by any of the following methods.

- Adding to the original OBA report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original OBA form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report).
- The investigators should take all appropriate measures to ensure the safety of the subjects. Notably they should follow-up to determine the outcome of any Adverse Events (clinical signs, laboratory values or other, etc.) until the patient has recovered, abnormal values have returned to normal or until progression has been stabilized. This may imply that follow-up will continue after the subject has left the study and that additional investigations may be necessary.
- In the case of a Death or a Life-threatening event, the investigator should provide a follow-up report, with positive or negative findings within **5 business days** of the awareness of the initial observation.
- Any reportable Serious Adverse Events brought to the attention of the Investigator at any time after cessation of the trial and considered by him/her to be reasonably associated with medication administered during the period should also be submitted to the FDA. A copy should be provided to Advaxis, if an Advaxis product was involved or the appropriate manufacturer of another product within 24 hours after FDA submission (see above).

The OBA form, which will provide follow-up information about a reportable SAE must be submitted to the BrUOG office who will then send all information to the appropriate parties (FDA, Advaxis, the Principal Investigator, and the participating sites (who will in turn notify their local IRBs).

11.3: Safety Reporting for IND Holders

In accordance with 21 CFR 212.32, Sponsor-Investigator of the study conducted under an IND must comply with following safety-reporting requirements:

11.3.1: Expedited IND Safety Reports:

7 Day calendar Telephone or Fax Report: On behalf of the Sponsor-Investigator BrUOG is required to notify the FDA of any that is serious, unlisted/unexpected and assessed by the investigator to be possibly related to the use of study drug(s). An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be telephoned or faxed by the sites to Brown University Oncology Research Group (BrUOG) who will then fax the information to the FDA as well as report to Advaxis as soon as possible but no later than 7 calendar days of first learning of the event.

**Brown University Oncology Research Group (BrUOG) Central Office,
Fax: (401) 863-3820**

BrUOG will then fax the information received to the number below: it will be directed to the FDA division fax in the Center for Biologics Evaluation and Research at

**FDA fax number for IND/OBA Safety Reports:
FDA: 1-301-595-1303 and OBA fax at 301-496-9839**

All written IND/OBA Safety Reports submitted to the FDA by the Sponsor-Investigator must also be sent to Advaxis.

AND: Report of Adverse Events to the Institutional Review Board

The principal Investigator is required to notify his/her Institutional Review Board (IRB) of a serious adverse event according to institutional policy.

11.4: Adverse event updates/IND safety reports

Brown University Oncology Research Group, and Advaxis shall notify the Investigator via an IND Safety Report of the following information:

- Any AE associated with the use of study drug in this study or in other studies that is both serious and unexpected.
- Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

The Investigator shall notify his/her IRB/EC promptly of these new serious and unexpected AE(s) or significant risks to subjects.

11.5: IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the Sponsor-Investigator shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report. All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Brown University Oncology Research Group, Advaxis and assigned Clinical Science Liaison.

12.0 REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. Extraordinary medical circumstances or withdrawal of consent by the patient: If, at any time, the constraints of this protocol are detrimental to the patient's health, and/or the patient no longer wishes to continue protocol therapy, the patient shall be withdrawn from protocol therapy. Patients will also be withdrawn from study for the following reasons:

- **Disease Progression:** Any patient with disease progression should be removed from study. Details and tumor measurements should be documented on flow sheets.

Considerations about tumor-measurement and disease progression in immunotherapy:

Because response to immunotherapy involves inflammation of lesions and infiltration by lymphocytes, it is common for lesions that are responding to immunotherapy to become physically larger and inflamed before tumors begin to shrink in size. In addition small previously occult lesions may become clinically detectable once they are inflamed after immunotherapy so the appearance of small new lesions should not automatically be considered disease progression without confirmation. It takes time for a cellular immune response to develop after immunotherapy and it's best to wait 9-12 weeks after immunotherapy to begin assessment of potential response. Similarly, apparent tumor progression based on lesion measurements should be confirmed by another evaluation (or biopsy if possible) after 3-4 weeks unless accompanied by other signs of clinical deterioration. A biopsy largely devoid of lymphocyte infiltration is consistent with tumor progression, whereas the biopsy of a lesion showing extensive lymphocyte infiltration may be a sign of inflammation that could lead to subsequent response.

- Patient is unable to tolerate the toxicity resulting from the study treatment, even with optimal supportive care, in the opinion of the Treating Physician. Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug.
- The physician feels it is in the best interest of the patient to stop the treatment.

- Inter current illness that would, in the judgment of the Investigator, affect assessment of clinical status to a significant degree or require discontinuation of study treatment
- Non protocol chemotherapy or immunotherapy is administered during the study
- Noncompliance with protocol or treatment—major violation
- Suspected Pregnancy
- Patient is lost to follow-up
- Patient refuses to continue treatment (patient will continue to be followed for disease-free survival and overall survival)
- Patient request
- General or specific changes in the patient's condition unacceptable for further treatment in the judgment of the investigator
- Progressive disease at any time
- At the time of withdrawal, all study procedures outlined for the End of Study visit should be completed. The primary reason for a patient's withdrawal from the study is to be recorded in the source documents.
- Study closure
- Death
- Subject has major surgery or implanted device that poses a high risks of 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)). 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

In this event notify:

Brown University Oncology Research Group (BrUOG) Central Office,

Phone: (401) 863-3000,

Fax: (401) 863-3820

Kayla_Rosati@brown.edu

The BrUOG Central Office will in turn notify the Principal Investigator.

*Document the reason(s) for withdrawal on flow sheets. Follow the patient for survival with follow-up forms as dictated by the protocol

13.0 FOLLOW-UP

All Subjects that discontinue treatment early for any reason as well as patients who complete therapy will be followed for survival. See section 6 for details. At treatment discontinuation, subjects will undergo adverse event evaluation and again approximately 30 days post the last dose of study drug (treatment). In addition off study evaluations will be done when treatment is discontinued -Section 6.0. Reminder for patients who enroll to amendment # 16 or later versions: patients may not and should not receive treatment with PI3K or TNF α inhibitors post this study treatment. If a patient is known to receive treatment with a PI3K or TNF α inhibitor BrUOG must be made aware via the concomitant medication log. Following the completion of the 6 month oral antibiotic treatment, a subject will be eligible to participate in other investigational clinical studies.

14.0 REGULATORY CONSIDERATIONS

14.1 Protection of Human Subjects

The Investigator must ensure that patients or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB.

14.2 Compliance with the Protocol and Protocol Revisions:

The study must be conducted as described in this approved protocol.

All revisions to the protocol must be provided to Brown University Oncology Research Group, and Advaxis. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC and Advaxis of an Amendment, except where necessary to eliminate an immediate hazard(s) to study patients.

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to Brown University Oncology Research Group, and Advaxis. If the revision is an Administrative Letter, Investigators must inform their IRB(s)/IEC(s).

The Investigator must ensure that patients or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB.

After the study has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

14.3 Protocol amendments or changes in study conduct:

Any change or addition (excluding administrative) to this protocol requires a written protocol amendment that must be reviewed and approved by Brown University Oncology Research Group, Advaxis and the investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study require additional approval by the IRB at each study center. A copy of the written approval of the IRB must be provided to Brown University Oncology Research Group, and Advaxis.

Examples of amendments requiring such approval are:

- increases in drug dose or duration of exposure of subjects,
- significant changes in the study design (e.g. addition or deletion of a control group),
- increases in the number of invasive procedures,
- addition or deletions of a test procedure required for monitoring of safety.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by Brown University Oncology Research Group and Advaxis in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons Brown University Oncology Research Group and Advaxis must be notified and the IRB at the center must be informed immediately.

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB must be kept informed of such administrative changes.

Examples of administrative changes not requiring formal protocol amendments and IRB approval include:

- changes in the staff used to monitor trials (Advaxis considers a change in Principal Investigator or the addition of sub-site(s) to be substantial and requires Advaxis approval prior to implementation)
- Minor changes in the packaging or labeling of study drug.

15.0 DATA MONITORING / QUALITY ASSURANCE/ RECORD RETENTION

15.1 Good Clinical Practice:

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and in the US Code of Federal Regulations. The investigator will be thoroughly familiar with the appropriate use of the drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

15.2 Patient Confidentiality:

In order to maintain patient privacy, all data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number. The investigator will grant monitor(s) and auditor(s) from Advaxis or its designees and regulatory authority (ies) access to the patient's original medical records for verification of data gathered on the data capture records and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

15.3 Protocol Compliance:

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB/IEC and the appropriate regulatory authority (ies). Changes to the protocol will require approval from Advaxis and written IRB/IEC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB/IEC may provide, if applicable regulatory authority (ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB/IEC. The investigator will submit all protocol modifications to Advaxis and the regulatory authority (ies) in accordance with the governing regulations.

Any departures from the protocol must be fully documented in the source documents.

15.4 On-site Audits:

Regulatory authorities, the IEC/IRB and/or Advaxis clinical quality assurance group may request access to all source documents, data capture records, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

15.4.1 INSTITUTIONAL AUDITS

It is the intent that audits will be completed per the BrUOG audit Manual. Audits will be scheduled more frequently if required. Auditors will follow procedures established by BrUOG SOP's. Instructions for preparing for the audit will be sent to sites in advance of the audit date. With these instructions, the auditors will specify which case records will be reviewed during the audit. Auditors will review on-site records against the submitted form, and they will record their findings on specially prepared questionnaires. Major discrepancies will be forwarded to the appropriate oversight body within BRUOG and the institution. IRB procedures, approvals, and consent forms will also be reviewed at the audit.

To help sites prepare for audits and assure that clinical research associates to maintain records appropriately, the BrUOG data management and auditing department will offer training. This training will cover all aspects of data collection, but will include special instructions for finding and filing the kinds of source documentation needed to verify the accuracy of submitted data for this trial.

15.5 Institutional Review Board:

Sites must obtain local IRB initial approval. Prior to subject registration, a copy of the IRB approval letter for the protocol and the informed consent form must be sent to BrUOG, along a copy of the IRB approved

informed consent form. Investigator will provide a copy(s) of IRB approval letter(s) for any amendment(s), and copy(s) of annual renewal(s).

15.6 Drug Accountability:

Accountability for the drug at all study sites is the responsibility of the principal investigator. The investigator will ensure that the drug is used only in accordance with this protocol. Drug accountability records indicating the drug's delivery date to the site (if applicable), inventory at the site (if applicable), use by each patient, and return to Advaxis for disposal of the drug (if applicable and if approved by Advaxis) will be maintained by the clinical site. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers.

15.7 Use and Completion of Case Report Forms (CRF'S) and Additional Request:

It is the responsibility of the Investigator to maintain adequate and accurate CRFs (according to the technology used) designed by the Sponsor to record (according to Sponsor instructions) all observations and other data pertinent to the clinical investigation. All CRFs should be completed in their entirety to ensure accurate interpretation of data. Should a correction be made, the corrected information will be updated with the initials and date of the person modifying the data and initial information. An audit trail will allow identifying the modification. Data will be available within the system to the sponsor pharmaceutical company as soon as they will be entered in the CRF.

15.9 Source Documents:

Source data are found in all information, original records of findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source documents represent the first recording of any observations made or data generated about a study participant while he or she is enrolled in a clinical trial. Source documents for each study participant substantiate the data that are submitted to BrUOG. Source documents must verify the eligibility criteria and data submitted on all CRFs. If an item is not mentioned in the source documents (e.g., history and physical with no mention of a psychological condition), it will be assumed it is not present. Research records for each case should contain copies of the source documents for the data reported to BrUOG. If data is abstracted from medical charts that are not filed at the investigative sites (e.g. hospital charts), copies of these records should be filed in the research chart. However, every attempt must be made to obtain all records/charts that were used to abstract any study data for this protocol at the time of the audit visit. This will prevent any discrepancies and the inability to verify the document and the data reported.

15.10 Record Retention:

The investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s).

The Brown University Oncology Research Group, as coordinator of this study, is responsible for ensuring proper conduct of the study with regard to protocol adherence and the validity of the data recorded on the case report forms. The Principal Investigator (Howard Safran, M.D.) and the Brown University Oncology Research Group will monitor this study. Efforts will be made to review the case report forms against the submitted documents for accuracy, completeness, adherence to the protocol and regulatory compliance.

U.S. FDA regulations (21CFR312.62[c]) require all records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consents forms, laboratory test results and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the investigation is discontinued and the FDA and the applicable local health authorities are notified. Advaxis will notify the Principal Investigator if an application is filed.

15.11 Premature Closure of the Study: This study may be prematurely terminated, if in the opinion of the investigator or Advaxis, there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the investigator or Advaxis by the terminating party. Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients
- Failure to enter patients at an acceptable rate
- Insufficient adherence to protocol requirements
- Insufficient complete and/or evaluable data
- Plans to modify, suspend or discontinue the development of the drug

16.0 DATA SAFETY AND MONITORING BOARDS

All trials initiated by the Brown University Oncology-research Group (BrUOG) are subject to oversight by the Data Safety Monitoring Board (DSMB). This board meets two times per year with any additional meetings scheduled when needed. The responsibilities are as follows:

- Familiarize themselves with the research protocol (s)
- The DSMB reviews trial performance information such as accrual information.
- Review interim analyses of outcome data and cumulative toxicity data summaries to determine whether the trial should continue as originally designed, should be changed, or should be terminated based on these data.
- The DSMB also determines whether and to whom outcome results should be released prior to the reporting of study results.
- All adverse events are reviewed by the committee, with assurances that these have been in fact sent for review to all pertinent IRBs.
- Review of reports of related studies to determine whether the monitored study needs to be changed or terminated.
- Review major proposed modifications to the study prior to their implementation (e.g., termination, dropping an arm based on toxicity results or other reported trial outcomes, increasing target sample size).

Following each DSMB meeting, provide the study leadership with written information concerning findings for the trial as a whole related to cumulative toxicities observed and any relevant recommendations related to continuing, changing, or terminating the trial. The study leadership will provide information on cumulative toxicities and relevant recommendations to the local principal investigators to be shared with their IRB's.

17.0 STATISTICS

17.1 Primary objective of this phase II study is to assess the clinical complete response at the 6 month biopsy. The unacceptable complete response rate is $\leq 50\%$. The regimen will be considered promising if the complete response rate is $\geq 80\%$. Progression-free and overall survival will be determined from the time of study enrollment for all patients that received the study treatment.

Let 'pR' be the probability of response to treatment, 'pT' the probability of unacceptable toxicities with treatment

$$H_0: pR \leq pR_0 \text{ OR } pT \geq pT_0$$

$$H_A: pR \geq pR_0 \text{ AND } pT \leq pT_0$$

Simon's two-stage design⁶³ will be used. The null hypothesis that the true response rate is 50% (p_0) will be tested against a one-sided alternative. In the first stage, 16 patients will be accrued. If there are 10 or fewer responses in these 16 patients, the study will be stopped early for futility. Otherwise, 9 additional patients will be accrued for a total of 25. The null hypothesis will be rejected if 18 or more responses are observed in 25 patients. This design yields a type I error rate of 0.019 and power of 86% when the true response rate is 80% (p_1).

Toxicities will be recorded by NCI common toxicity version 4. Toxicities will be monitored continuously. Unacceptable toxicities will be defined as grade 3 toxicities lasting > 72 hours or grade 4 toxicities of any duration, related to ADXS11-001. For toxicity assessment, the target probability of unacceptable toxicities is set at 20% for this study (pT0). A rate of grade 3 or grade 4 toxicities related to ADXS11-001 of >20% is considered a reason to terminate the study and accept the null hypothesis.

An interim analysis for toxicity will be carried out after accruing the first 16 patients. If 3 or more patients develop unacceptable toxicities, the study will be stopped early for excess toxicity. If <3 patients have unacceptable toxicities, the study will continue until 25 patients are accrued. If 5 or more patients developed unacceptable toxicities at this stage, the null hypothesis will be accepted and no further investigation of the drug is warranted.

Total sample size = 25 treated patients.

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APPENDIX A
Suggested INFORMED CONSENT
Agreement to Participate in a Research Study
And Authorization for Use and Disclosure of Information

**A Phase I/II Evaluation of ADXS11-001, Mitomycin,
5-fluorouracil (5-FU) and IMRT for Anal Cancer BrUOG 276**

You are being asked to take part in a research study. All research studies carried out at _____ institutions are covered by rules of the Federal government as well as rules of the State and _____ institutions. Under these rules, the researcher will first explain the study, and then he or she will ask you to participate. You will be asked to sign this agreement that states that the study has been explained, that your questions have been answered, and that you agree to participate.

The researcher will explain the purpose of the study. He or she will explain how the study will be carried out and what you will be expected to do. The researcher will also explain the possible risks and possible benefits of being in the study. You should ask the researcher any questions you have about any of these things before you decide whether you wish to take part in the study. This process is called informed consent.

This form also explains the research study. Please read the form and talk to the researcher about any questions you may have. Then, if you decide to be in the study, please sign and date this form in front of the person who explained the study to you. You will be given a copy of this form to keep.

Nature and Purpose of the Study

Your doctors are participating in this research study sponsored by the Principal Investigator, Dr. Howard Safran, in collaboration with Brown University Oncology Research Group (BrUOG), which will serve as the central coordinating office for the study. This study is supported by Advaxis (the makers of ADXS11-001).

You have been diagnosed with anal cancer. The standard treatment for anal cancer is to use a combination of chemotherapy and radiation treatments over approximately 6 weeks. This study will test the addition of an experimental drug called ADXS11-001 to standard chemotherapy and radiation for the treatment of anal cancer.

The main purpose of this study is to evaluate the safety and effectiveness of ADXS11-001 when combined with standard chemotherapy and radiation treatment for anal cancer. ADXS11-001 is an investigational agent that is not approved by the FDA to treat anal cancer or any other cancer.

About 25 patients will take part in this study in about 5-7 cancer centers across the United States.

How Does ADXS11-001 Work?

One of the most important risk factors for the development of anal cancer is the presence of the Human Papilloma Virus (HPV). A part of a protein made by HPV called HPV E7 can be found inside almost all anal cancer cells. ADXS11-001 is an investigational drug that tries to stimulate your body's immune system to attack cancer cells that contain HPV E7. As of September 2015, approximately 675 doses of ADXS11-001 have been given to 263 patients with HPV associated cancers.

ADXS11-001 is a modified and weakened version of a bacteria called *Listeria monocytogenes*.

The experimental product ADXS11-001 uses a live strain of the *Listeria monocytogenes* (*Lm*) bacteria that has been genetically modified such that the risk of getting an infection is significantly reduced. Inside the *Listeria* bacteria has been placed part of the molecule for HPV E7. This modified *Listeria* bacteria (ADXS11-001), is injected into a patient's bloodstream. ADXS11-001 then goes inside a patient's lymphocytes which are blood cells of the immune system. This stimulates other immune system cells to attack cells that contain HPV E7 including anal cancer cells.

Explanation of Procedures

What will happen if I take part in this research study?

Before you begin the study, you will need to have the following exams, tests or procedures to find out if you can be in the study. These exams, tests, or procedures are part of regular cancer care and may be done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. This will be up to the study doctor.

- A physical examination and an examination of your anus and groin
- Examination and measurement of the cancer using a tube with a light on it (a "scope") in order to see the cancer better; sometimes you will be given a short general anesthetic for this examination.
- A specimen of the cancer (biopsy) will be removed to examine. If the study doctor suspects cancer in the lymph nodes in the groin, a biopsy may be taken from there too.
- An x-ray or a CT scan (a computerized x-ray that gives your doctor clearer pictures of the inside of your body) of your chest to make sure your lungs are clear.
- A CT scan or an MRI (a test that uses magnets to take pictures of the inside of your body) of your abdomen and pelvis to check for any spread of the cancer.
- Blood tests to check your bone marrow, kidneys, and liver.
- For women able to have children, a pregnancy test because the treatments in this study could harm an unborn baby.
- An HIV test. If you are HIV positive you will not be eligible for this study because of increased chance of side effects. If your HIV test comes back as positive, you will be referred to a specialist in HIV treatment. You will still be able to get standard treatment for anal cancer but you will not be able to be part of the study and you will not be able to receive ADXS11-001.
- If you have a previous history of heart disease, your heart strength will be evaluated by an ultrasound of your heart called a cardiac echo to see if your heart function is adequate to participate in this study.
- If you have a previous history of lung disease, your lung function will be evaluated by breathing into a machine that measures your lung strength (PFTs) to see if your lung function is adequate to participate in this study.
- You will be asked about your current health, medical history, surgical history, and the medicines that you have taken within 30 days of study entry or are currently taking. The study doctor may ask you specifically about medicines called Phosphoinositide 3-kinase inhibitors (PI3K, such as idelalisib (Zydelig)) and Tumor Necrosis Factor (TNF, such as humira (adalimumab), enbrel (etanercept)) alpha inhibitors. These medicines might interact with ADXS011-001 to cause adverse effects. You cannot be on one of these medicines to enter the study, take one during your participation in the study or after your participation in the study is completed.
- You will be asked if you have received any live vaccines within the past 30 days as this may affect your study participation. Examples of live vaccines include, but are not limited to: measles, mumps, rubella, chicken pox, yellow fever, rabies, 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 not allowed within 30 days. Please speak with your study doctor about any vaccines you have recently received.

Treatments:

If you are determined to be eligible for the study, and you consent to be part of the study, you will receive standard radiation and chemotherapy for anal cancer. You will also receive ADXS11-001.

Standard Radiation Therapy and Chemotherapy (Mitomycin and 5-FU) For Anal Cancer:

Radiation:

You will receive radiation once a day, five days a week, for 30 treatments (6 weeks) depending on your cancer.

Sometimes you may need a break (usually a week but it may be longer depending on your case) in the radiation treatment due to the side effects from the radiation, such as vomiting, diarrhea, skin rash, or lowered blood counts. The study doctor will be checking your side effects throughout treatment.

Chemotherapy:

You will receive two drugs: Mitomycin and 5-fluorouracil (5-FU) in two treatments. The first chemotherapy treatment starts on the same day as your first radiation treatment (day 1). The second treatment is given at the start of the fifth week of radiation (approximately day 29). The mitomycin is given through your vein over about 15 minutes. The 5-FU also is given through your vein, but it has to be given continuously over 96 hours (starting on day 1 and approximately again on day 29). Typically, if you start radiation on a Monday, your 5-FU will be started that day and be stopped on Friday. This means that although the 5-FU is given for 96 hours, you will receive some 5-FU on each of 5 days (Monday to Friday).

Treatment with ADXS11-001

You will receive 4 treatments with ADXS11-001 as a 60-minute infusion through the vein. Your first treatment will be 6-14 days *before* your first radiation. The second treatment of ADXS11-001 will be given approximately 10 days - 28 days after completion of radiation. The third treatment will be given approximately 28 days after the second treatment and the fourth treatment will be approximately 28 days later.

- Administration of drug: You will have a new infusion line place for each infusion of ADXS11-001. Central venous catheters or infusion ports that you currently have or that you recently had placed are not allowed to be used for ADXS11-001 administration.
- Patients with central venous catheters or infusion ports: If you currently have or will have a central venous catheter or infusion port placed, it is important you understand that the catheters or port **must not be used** for 72 hours following the completion of the ADXS11-001 infusion **and** not until after you take your first dose of antibiotics. This is required with each of the 4 infusions of ADXS11-001.
- **Medications prior to ADXS11-001:** You will be asked to take an oral medication to control any possible nausea or vomiting related to the infusion, such as promethazine, ondansetron, or prochlorperazine. You will also receive an acid reducer (medicines used to reduce amount of acid in stomach such as famotidine), and an antihistamine (medicines used in treatment of allergies such as diphenhydramine) approximately 30 minutes before you receive the study drug.
- **Naproxen to prevent fever and body aches from ADXS11-001:** One day prior and on the day of your infusion with ADXS11-001 you will take naproxen, which is a non-steroidal anti-inflammatory drug that is available over the counter. Naproxen is being used to try to help prevent fever, aches and pains that you may get from taking the ADXS11-001. On the day of your infusion, you will be asked to take your Naproxen dose prior to arrival to the study site

and the evening dose at home (to be taken 8-12 hours after morning dose). For further information on naproxen please speak with your study doctor.

- **Clinic Observation after ADXS11-001:** You will be observed at the study site for approximately 4 hours to make sure there are no unanticipated side effects from the infusion. You will need to come back to clinic 24 hours after your infusion and again 72 hours after the infusion so that the study team can document your vital signs.
- **Antibiotics to prevent infection from ADXS11-001:** Three days after your infusion of ADXS11-001 you will be given either a seven (7) day course of an oral antibiotic called Bactrim® (Trimethoprim and Sulfamethoxazole). Depending on the strength of the antibiotic your doctor prescribes you will either take the antibiotic once a day for 7 days (daily) or you will take it three (3) times over the 7 days. Your doctor or the research team will explain how often you are required to take the antibiotics. . If you know you are allergic to Bactrim®, you will be given ampicillin in its place, which you will take four times a day for seven days. Please ensure that you complete the entire course of antibiotics as prescribed by your physician. The reason for the antibiotic being given is as a precaution to try to ensure that all of the Listeria has been eliminated from your body. If you develop persistent fever within the 72-hour window between your receiving ADXS11-001 and starting the antibiotic, you will be given the 7-day course of antibiotic by vein instead of by mouth. Your doctor may decide to reduce the dose of antibiotic or change to a different antibiotic based on your medical condition.
- **Drug Interactions with ADXS11-001:** While you are on this study you should not take any immunosuppressive steroids, such as prednisone and prednisolone. Your doctor will review all of the medications and supplements you are currently taking before starting you on this drug. You may need to change one or more of your regular medications under the direction of your doctor if it is safe to do so in order to participate in this study. You should not take any new medications or dietary supplements without discussing it with your study doctor first. It is important that you know you cannot receive any live vaccines within 30 days prior to the first dose of ADXS11-001 and while on receiving ADXS11-001. This includes but is not limited to: measles, mumps, rubella, chicken pox, yellow fever, rabies, intranasal influenza (eg: Flu-mist). The seasonal influenza vaccine for injection is generally a non-live virus vaccine and this is allowed.
- **Devices or surgery:** If during the study you need to undergo any surgery during which you will have a medical device (such as a prosthetic joint, artificial heart valve, pacemaker, orthopedic screw, bone graft, or other implant) implanted you will be need to be taken off the study secondary to the small, but potential risk of delayed listeria. 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.
- **Additional Precautions after treatment with ADXS11-001:** There may be a live bacterium in your body for a few days after your treatment. The ampicillin is given after 3 days to try to eliminate the bacteria. The bacteria can, under certain circumstances, be contained in your bodily fluid. Healthy people around you are not at risk of getting sick from this bacteria from you. However, it could be transferred to a baby if you are pregnant or through breast milk if you are nursing. This is why women who are pregnant or nursing cannot participate in the trial. As a precaution, you should not exchange bodily fluids with someone who has a weakened immune system (for example, persons with leukemia, lymphoma, AIDS, or a positive blood test for HIV) during the time between when you receive the infusion until 3 days after you start the antibiotics. In clinical studies, the bacteria are cleared from the bloodstream quickly with no evidence of the bacteria being present in the urine or feces of any patient. ADXS11-001 has been administered over 500 times to patients. There have been no instances of person-to-person transmission.

- ***Lm* Surveillance Monitoring:** After study treatment is completed, you will be monitored for 3 years for a possible delayed listeria infection. During the first 6 months of your participation in this period you will receive a daily dose of oral Bactrim which is an antibiotic used to treat an infection from listeria. The goal of receiving this treatment is that the Bactrim will kill any listeria that might still be in your body from the ADXS11-001 treatment. In addition, you will have routine blood tests and cultures which will require about 5 teaspoons of blood. These will be obtained approximately every 4 months for 3 years. The first blood test and culture will be obtained approximately 4 months after you receive your first dose of Bactrim. If you are allergic to or cannot tolerate Bactrim or develop a hypersensitivity to it during your participation in the study you may receive Ampicillin. This is another antibiotic which is used to treat an infection from listeria and kill it in the body. The study doctor will be able to help you decide which antibiotic is the one you will receive.
- **Other treatments:** You will be able to participate in other clinical trials only following the completion of the 6 month oral antibiotic treatment,.

Tests You Will Need Weekly For 6 Weeks While You Are Receiving Chemotherapy and Radiation:

During the study treatment you will need the following tests and treatments. They are part of regular cancer care:

- Weekly physical examination to check for side effects
- Weekly examination of your anus and groin
- Weekly blood tests to check for side effects

Follow up:

You will be seen 1 to 2 weeks after the completion of radiation, then 2-6 weeks after completing treatment with ADXS11-001. Following the completion of your study treatment, there will be a 3-year follow-up and surveillance period which will include a study visit approximately every 4 months. After 3 years you will be followed once a year for 2 years (year 4 and year 5). Your participation in this study could be a maximum of 6years. In follow-up, you will need the following tests and examinations:

- A physical examination
- An examination of your anus and groin
- Blood tests
- A CT scan or an MRI of your abdomen and pelvis
- A chest x-ray
- An examination of the area of the cancer using a tube with a light on it (a “scope”)

You will be monitored for 5 years after your study treatment is completed. You will be given an oral antibiotic for 6 months at the start of this monitoring period. You will have blood tests approximately every 4 months during this period for 3 years. The reason for this monitoring is to check to see if any *Lm* bacteria can be found in your blood after receiving treatment with ADXS11-001 which uses *Lm* bacteria. This is called a delayed listeria infection. After 3 years, you will be seen once a year for 2 years (year 4 and year 5).

Biopsy of the Anal Cancer:

Approximately 6 months after you started treatment with radiation and chemotherapy you will have a “scope” of your anus and you may have a biopsy. This “scope” is part of standard treatment for anal cancer. Your doctor may recommend additional evaluations and biopsies as part of standard follow-up if your doctor thinks this is helpful.

If your cancer is still present at the completion of treatment or recurs in the anus, your doctor may recommend surgery to remove the anus and rectum.

How long will I be in the study?

The period from the first treatment of ADXS11-001 which is given 6-14 days prior to starting radiation and chemotherapy to the final treatment of ADXS11-001 (which is given after completion of chemotherapy and radiation), to the post-treatment scope and biopsy (biopsy only if applicable) is approximately 6 months. Afterwards you will be followed every 6 months for 1 year and every year for an additional 4 years, to make sure that there are no delayed side effects and that any side effects that did happen during the study have improved.

It is important that we know what happens to you over a long-term period of time, so you will be followed for 5 years after your study treatment. Also, it is very important that any time you have medical problems, medical procedures or surgery that you inform your study doctor.

Can I stop being in the study?

Yes. You can decide to stop at any time. Tell the study doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely. If you decide to stop immediately after receiving treatment with ADXS11-001, or before completion of the following course of antibiotics, it is critically important that you complete the entire course of antibiotics to make sure none of the ADXS11-001 bacteria remain.

It is important to tell the study doctor if you are thinking about stopping so any risks from the discontinuation of radiation, or chemotherapy can be evaluated by your doctor. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest; if you do not follow the study rules; or if the study is stopped.

Costs for participating in this study

The investigational drug, ADXS11-001, will be provided at no cost to you by Advaxis.

Other services you will receive during this research study are considered "routine clinical services" that you would have received even if you were not in the research study. These "routine clinical services" include the administration of ADXS11-001, pre-medications, all study doctor visits, chemotherapy, blood tests, CT scans, PFTs, echocardiogram, HIV test, pregnancy test, the scope at 6 and 12 months after last treatment with study drug and then yearly, the post-treatment biopsy at approximately 6 months, radiation treatments and MRI scans. These services will be billed to your health insurance company, but you will be responsible for paying any deductibles, co-payments, or co-insurance that are a normal part of your health insurance plan. If you do not have health insurance, you will be responsible for those costs.

Contact Information: If you have any questions regarding this study, you may contact your sites' Principal Investigator, <INSERT NAME>, MD at <INSERT PHONE NUMBER>.

Discomforts and Risks

You may have side effects while on this study. We will monitor everyone in the study for any side effects. Contact your study doctor if you experience a side effect or have any questions about possible side effects.

Side effects may be mild or serious. We may give you medicines to help lessen side effects. Some side effects will go away as soon as you stop taking the drug. In some cases, side effects can be serious, long-

lasting, or may never go away.

Risks and side effects related to the **Radiation Therapy** include those which are:

Likely (more than 10%)

- Redness and skin irritation in the treatment area that may result in bleeding and/or infection, which may require hospitalization
- Loss of pubic hair in the treated area, usually temporary
- Tiredness
- Nausea and/or vomiting
- Sterility (inability to bear children) in fertile women
- Sterility (inability to produce children) in men

Less Likely (3-9%)

- Diarrhea
- Sores and bleeding from the bowel (these side effects may occur well after treatment and be serious enough to require surgery)
- Narrowing and dryness of the vagina (birth canal) and genital area with painful or difficult intercourse and possibly bleeding
- Development of extra tissue (fibrosis) in the anal canal, which may result in decreased function
- Long-term dryness of the skin
- Inability to have or keep an erection (impotency)
- Hip fracture
- Build up of fluid in ankles, feet, and/or legs

Rare, but serious (less than 2%)

- Narrowing or blockage of the bowel (these side effects may occur well after treatment and be serious enough to require surgery)
- Blockage of the urinary tubes
- Development of an abnormal path or connection between organs (fistulae)
- Skin damage (tissue death), which may result in surgery
- Narrowing of or persistent bleeding in the vagina (birth canal), which may result in surgery

Risks and side effects related to the **Chemotherapy** (5-FU and Mitomycin) include those which are:

Likely (more than 10%)

- Diarrhea with cramping or bleeding
- Nausea and or vomiting
- Change in taste, particularly a metallic taste
- Loss of appetite
- Dry skin with rash, cracking, and/or peeling
- Mouth sores and sore throat
- Temporary thinning or loss of hair
- Low white blood cell count, which may increase the risk of infection
- Low red blood cell count, which may result in anemia, tiredness, and/or shortness of breath
- Low platelet count, which may result in increased bruising and bleeding

Less Likely (3-9%)

- Eye irritation, watery eyes, and/or runny nose
- A blocked tear duct, which may require treatment
- Blurred vision
- Darkening and thinning of the skin
- Darkening, dryness, and marking of the nails
- Increased sensitivity to sunlight
- Headaches, which may continue

- Light-headedness
- Fever
- Puffiness of the hands and feet
- For women, missed menstrual periods
- Redness, tenderness, peeling, and/or tingling of the palms and soles of the feet
-

Rare, but serious (less than 2%)

- Confusion
- Unsteadiness, loss of coordination
- Temporary loss of consciousness
- Slurred speech
- Dry cough and shortness of breath
- Tissue damage from leakage of mitomycin from a vein, which may require surgery
- Vomiting blood from the digestive tract
- Serious infection, which may be life threatening
- Irritation of a vein due to a blood clot, which may result in tenderness over the vein and pain in the part of the body affected and which may require treatment
- Allergic reactions, which can involve flushing, difficulty breathing, and low blood pressure and which can be life threatening
- Change in heart rhythm
- Damage to the heart or spasm of the heart's blood vessels that can cause chest pain
- Heart attack
- Kidney damage
- Inflammation of the liver, which may result in yellowing of the skin and eyes, tiredness, and/or pain on the upper right of the stomach area

Risks and side effects related to the **ADXS11-001** include those which are:

Wild-type *Listeria monocytogenes* (*Listeria*) is a naturally occurring bacteria that is present in the environment and is known to cause illness in some people when they eat foods contaminated with *Listeria* (such as deli meats and hot dogs, unpasteurized [raw] milk and soft cheeses, uncooked vegetables [such as lettuce or celery] and fruits [such as cantaloupe]). ADXS11-001 is a *Listeria* that has been genetically altered in the laboratory so that its ability to cause sickness and infection is reduced.

Wild type *Listeria* does not usually cause disease, and most people who encounter it clear the infection without even knowing that they have been exposed. However in some instances *Listeria* exposure can result in an infection with fever, chills or muscle aches or sickness to your gastrointestinal tract (such as nausea or diarrhea) or nervous system. Both wild type *Listeria* and ADXS11-001 are sensitive to antibiotics and are killed after treatment with antibiotic therapy. However in rare instances *Listeria* and ADXS11-001 can remain in the body even after antibiotic treatment.

So far, as of September 2015, over 675 doses of ADXS11-001 have been given to over 263 subjects with HPV associated cancers in 9 research studies with ADXS11-001 alone or in combination with chemotherapy.

From the clinical experience, the most common side effects associated with ADXS11-001 treatment were mainly individual flu-like symptoms and/or cytokine release symptoms. The symptoms usually start within 2-4 hours after the completion of an ADXS11-001 dose, are often mild to moderate in nature, short lived, and respond quickly to symptomatic treatment. In rare instances severe or life-threatening/disabling side effects that last up to 24 hours have been observed, such as moderate to severe low blood pressure.

Flu-like and/or cytokine release symptoms: a group of symptoms resulting from a substance (cytokines) secreted by cells of the immune system which causes an increase in the number of a specific immune blood cells, called T-cells.

Symptoms related to cytokine release may include symptoms that affect the whole body such as fever, chills, shaking, feeling physically and mentally exhausted, headache, nausea, vomiting, rash, rapid heartbeat, low blood pressure, and shortness of breath which usually occur within 2-4 hours after the completion of an ADXS11-001 dose and may last up to 24 hours. The symptoms are caused by an increase in cytokines which have been shown to occur after ADXS11-001 administration, resulting from the body's immune response to the therapy.

On rare occasions, these side effects may be severe enough to require hospitalization and/or urgent treatment. In these cases, your study doctor may choose to treat these side effects by giving you additional fluids, high dose vasopressors (to increase your blood pressure), tocilizumab, corticosteroids, or other treatments not listed here. The study doctor will determine the best treatment needed based on the severity of your symptoms.

Risk of Delayed/Late Listeria Infection:

Listeremia is the appearance of the strain of Listeria bacteria used in the investigational product ADXS11-001 in the blood. It is detected by taking a sample of your blood and testing it. In rare circumstances, Listeria bacteria may appear in the blood many weeks to years after receiving a dose of ADXS11-001. This is called delayed or late listeria infection. There was one recently reported case of delayed listeria infection from a subject with metastatic cervical cancer who participated in a clinical research study. It occurred approximately 2.5 years after the subject received a dose of ADXS11-001. In this case, the subject had an artificial body part and received an investigational treatment (PI3K inhibitor) for her cancer after completing the study. It is believed that people who have an implanted medical device, an artificial body part or bone graft may have a higher risk for getting a delayed Listeria blood infection. In most cases of delayed listeria infection, common symptoms such as a fever, chills, nausea and/or diarrhea may be present. A fever was the only symptom associated with a Listeria infection that this subject showed. Treatment with an antibiotic may be able to get rid of Listeria symptoms and also kill it in the blood. This subject received treatment with an antibiotic in the hospital which took care of her fever and she was able to go home. The subject died about 2 weeks after going home from progression of her cervical cancer according to her study doctor. However, it was not known if there was still listeria bacteria in her body.

Therefore to help ensure your safety you will receive oral antibiotics for 7 days after each dose of ADXS11-001 you receive during the treatment phase, a 6-month oral antibiotic course after your study treatment with ADXS11-001 is over and your blood will be monitored every 4 months for 3 years after your study treatment is over to see if any Listeria bacteria can be found. If it is found, you will start taking antibiotics intravenously (i.e. the medicine is given to you through a tube in your arm). This treatment can

usually kill the bacteria. You will also be asked to see a doctor who specializes in treating infectious diseases like Listeria bacteria. Also, if at any time during your participation in this clinical study, you develop any of the symptoms that are seen with an infection, please immediately let the research staff or study doctor know so that they may test your blood for a Listeria blood infection. If at any time after your participation in this clinical study, you develop any of these symptoms, you should immediately contact your primary care doctor and inform him/her that you have participated in a clinical study with and have received a dose of ADXS11-001 so that he/she may test your blood for a possible delayed Listeria blood infection.

Likely (more than 15%)

- 'Flu like' Symptoms including chills, headache, muscle aches and fever
- Chills or rigors
- Nausea
- Fever
- Headache
- Vomiting
- Fatigue
- Anemia
- Abdominal pain

Less Likely (5-15%)

- Hypotension (i.e. abnormal lowering of blood pressure)
- Pain (general). May include muscle, back, bone, lymph node, chest, neck, extremity, jaw, mouth.
- High heart rate
- Constipation
- Diarrhea
- Swelling of extremities
- Decreased appetite
- Short term changes in liver function or liver enzymesChanges in blood cell counts including kidney function tests (creatinine)
- Changes in blood chemistry and blood counts
- Dizziness
- Shortness of breath at rest or with activity

Rare (less than 5%)

- Cytokine release syndrome (a disorder characterized by nausea, headache, tachycardia, hypotension, rash, and shortness of breath caused from the release of cytokines from the cells.)This is similar to a flu-like illness. Symptoms related to cytokine release may include symptoms that affect the whole body such as fever, chills, shaking, feeling physically and mentally exhausted, headache, nausea, vomiting, rash, rapid heartbeat, low blood pressure, and shortness of breath which usually occur within 2-4 hours after the completion of an ADXS11-001 dose and may last up to 24 hours. The symptoms are caused by an increase in cytokines, which have been shown to occur after ADXS11-001 administration, resulting from the body's immune response to the therapy.
- Muscle aches
- Short term increase in enzyme released during tissue damage
- Short term increase in inflammatory response protein
- Short term increase in pancreatic enzymes (lipase),
- Shaking
- Abdominal bloating

- Infection such as: fungal, lung, nerve, upper respiratory tract, candidiasis
- Tingling in hands and feet or weakness of the nerve
- Depression
- Sweaty palms or excessive sweating
- Weight loss or gain
- Migraine
- Sleepiness or insomnia (difficulty falling or staying asleep)
- Anxiety
- Acute kidney failure
- Cough
- Itching, which may be severe
- Rash
- Sensitivity to light
- Skin ulcer
- Blushing or flushing
- Muscular weakness
- High blood pressure
- Gastroesophageal reflux disease (GERD)
- Lack of energy
- Uneasiness or discomfort (general)
- Inflammation of the lining of the mouth
- Infusion reaction
- Feeling of happiness
- Not able to empty bladder
- Delayed/late Listeria infection

Serious/Life Threatening

- Lung infection
- Sepsis, which can be life threatening and lead to septic shock and even death.

On rare occasions, these side effects may be severe enough to require hospitalization and/or urgent treatment. In these cases, your study doctor may choose to treat these side effects by giving you additional fluids, high dose vasopressors (to increase your blood pressure), tocilizumab, corticosteroids, or other treatments not listed here. The study doctor will determine the best treatment needed based on the severity of your symptoms

Side Effects Related to Antibiotic use:

The most common possible side effects seen with antibiotic use may include:

- Gastrointestinal disturbances (diarrhea, nausea, vomiting, and anorexia [eating disorder with excessive weight loss]),
- allergic skin reactions (rash, urticaria [swollen, pale red bumps or patches], and itching),
- vaginal itching or discharge,
- white patches on tongue,
- allergic reaction (shortness of breath, hives, swelling of lips, face or tongue, and fainting).

In addition, specific antibiotics may have unique side effects and these side effects should be discussed with your study doctor.

Side effects associated with Bactrim®:

Common Bactrim side effects may include:

- nausea, vomiting, loss of appetite; or
- mild itching or rash.

Less common Bactrim side effects may include:

- diarrhea that is watery or bloody;
- pale skin, feeling light-headed or short of breath, rapid heart rate, trouble concentrating;
- sudden weakness or ill feeling, fever, chills, sore throat, new or worsening cough;
- cold or flu symptoms, swollen gums, painful mouth sores, pain when swallowing, skin sores;
- low levels of sodium in the body--headache, confusion, slurred speech, severe weakness, vomiting, loss of coordination, feeling unsteady;
- liver problems--upper stomach pain, tired feeling, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes); or
- severe skin reaction--fever, sore throat, swelling in your face or tongue, burning in your eyes, skin pain, followed by a red or purple skin rash that spreads (especially in the face or upper body) and causes blistering and peeling.

Get emergency medical help if you have any of these **signs of an allergic reaction to Bactrim**: hives; difficult breathing; swelling of your face, lips, tongue, or throat.

Side effects associated with the use of nonsteroidal anti-inflammatory products (NSAIDs), such as naproxen:

- heart attack,
- stroke,
- high blood pressure,
- heart failure from body swelling from fluid retention,
- kidney problems including kidney failure,
- bleeding and ulcers in the stomach and intestine,
- low red blood cells,
- life-threatening skin reactions,
- life threatening allergic reactions,
- liver problems including liver failure,
- asthma attacks in people who have asthma.
- Other possible side effects can include stomach pain, constipation, diarrhea, gas, heartburn, nausea, vomiting, dizziness, and ringing in the ears.

Side effects associated with Tocilizumab:

Tocilizumab is a drug approved by the FDA for the treatment of rheumatoid arthritis (RA). However, a dose of tocilizumab has been shown to be beneficial in the case of severe cytokine release related low blood pressure. **In the event you experience severe low blood pressure, your doctor may choose to give you a dose of tocilizumab.** Because tocilizumab is continually used for RA, the known risks are based on continuous use. The risks for a single dose of tocilizumab are not known.

The most common side effects (more than 10%) reported with continuous use of tocilizumab are listed below:

- Headache
- Common cold
- Increased blood pressure
- Infections
- Changes in laboratory results

Risks and side effects related to **biopsies** include infection, bleeding, and/or pain.

Reproductive risks:

You should not become pregnant or father a baby while on this study because the drugs and the radiation in this study can affect an unborn baby. Women should not breast feed a baby while on this study. It is important you understand that you need to use birth control while on this study. Check with the study doctor about what kind of birth control methods to use and how long to use them. Some methods might not be approved for use in this study. Both men and women of childbearing age are required to use 2 effective methods of birth control (such as a condom and spermicide) during the study and for at least 90 days after the last dose of study drug.

Bactrim: Some research studies suggest that taking Bactrim during pregnancy may be associated with an increased risk of birth defects. Therefore, if you become pregnant while taking this drug, you should immediately stop taking it and contact the study physician. He/she will be able to advise you on what to do

Radiation therapy to the pelvis will cause fertile women to lose the ability to bear children since radiation causes loss of ovary function and will cause men to lose the ability to produce children. Women may need hormones to relieve symptoms such as hot flashes or vaginal dryness caused by the loss of ovary function.

Cancer treatments often have side effects. The treatment used in this program may cause all, some, or none of the side effects listed. In addition, there is always the risk of very uncommon or previously unknown side effects occurring.

If you or your partner becomes pregnant, inform your study doctor immediately. If your partner becomes pregnant, your study doctor will ask her to sign a separate form to allow your study doctor to follow the pregnancy.

By signing this document you are acknowledging that you understand and agree to the information presented in this Reproductive risk section.

For more information about risks and side effects, ask the study doctor.

Antiemetics (anti-nausea medications): Various medications used to prevent nausea and vomiting may cause drowsiness, dry mouth, diarrhea, constipation, headache, restlessness, agitation, anxiety, dizziness, involuntary tremors, skin rash, and possible allergic reaction.

You will receive pre-medication to reduce the risk of infusion/injection reactions on your treatment days. Overall, the pre-medications you will be given are well tolerated.

Venipuncture (inserting a needle into a vein to obtain blood or give medication): May cause inflammation, pain, bruising, bleeding, or infection.

When you receive chemotherapy by vein, there is a slight risk that some of the drug may leak out around the needle at the injection site. A skin burn may result. Most skin burns are treatable and heal well.

In order to monitor the side effects, your physician will examine you frequently and obtain laboratory tests (blood tests, chest x-rays, or MRI scans as needed) to determine the effects of your treatment and alter the drug dosages if necessary.

Risk of CT imaging: CT imaging uses x-rays. The radiation dose associated with this procedure is estimated to be a small fraction of the annual permissible dose to an x-ray technologist.

Risk of MRI Imaging: Rarely the IV contrast used for MRIs, called gadolinium, has been associated with kidney damage and thickening of the skin.

Your doctors will be carefully monitoring your condition to minimize any possible risks to you. If your doctors feel that the side effects are too severe in your particular case, they will lower the dose of the medications or even stop them.

There may be other side effects that have not been reported. If you have any unusual symptoms, you should report them immediately to your doctor or nurse.

Benefits

Taking part in this study may or may not make your health better. While doctors hope that the addition of ADXS11-001 to standard chemotherapy and radiation will be more effective against anal cancer compared to standard chemotherapy and radiation alone, there is no proof of this yet. The possible benefits of ADXS11-001 are better control of your cancer, however, this is not guaranteed.

We do know that the information from this study will help doctors learn more about ADXS11-001 as a treatment for anal cancer. This information could help future cancer patients.

Alternative Therapies

What other choices do I have if I do not take part in this study?

Your other choices may include:

- Getting treatment or care for your cancer without being in a study, such as standard radiation and chemotherapy; radiation alone; or surgery in which the anus and rectum are removed and the bowel is attached to a permanent opening in the skin of the stomach (colostomy).
- Taking part in another study, if available
- Getting no treatment or comfort care, also called palliative care. In this case the cancer will likely continue to grow and spread to other parts of the body. This type of care may help reduce pain, tiredness, appetite problems and other problems caused by the cancer. It does not treat the cancer directly, but instead tries to improve how you feel. Comfort care tries to keep you as active and comfortable as possible.

Talk to your doctor about your choices before you decide if you will take part in this study.

Refusal/Withdrawal

You decide whether or not you want to be in the study. Participation is voluntary. If you decide now to participate, you can change your mind later and quit the study. If you decide not to participate, or if you quit the study, it will not affect the health care services that you normally receive. If the researcher or your doctor feels it is in your best interest, they may choose to take you out of the study at any time before you complete the study.

As soon as it becomes available, the researcher will give you new information about the study that may or may not affect your decision to stay in the research study.

If you decide to withdraw from this study (stop taking study medication) for any reason, you will be asked to sign a form indicating whether you give your permission for your doctor and the research staff to continue to collect and submit follow-up information on your health status from your physicians and medical record. After signing the form, you still have the right to change your mind, at any time, regarding follow-up after withdrawal.

Medical Treatment/Payment in Case of Injury

If you experience a research injury _____ (your institution) , or the study doctor, will arrange for medical treatment at no cost to you. The cost of your treatment will be paid for as described below. A research injury is any physical injury or illness caused by your participation in the study. If you are injured by a medical treatment or procedure that you would have received even if you were not in the study, that is not a research injury. To help avoid injury, it is very important to follow all study directions.

If you suffer a research injury and you are covered by insurance, it is possible that some or all of the costs of treating your condition could appropriately be billed to your insurance company. If such costs are not covered either by health insurance or the study sponsor, _____ (your institution) will pay for what it considers fair and proper treatment. <INSERT HOSPITAL> has no policy to cover payment for such things as lost wages, expenses other than medical care, or pain and suffering. Neither Dr. Howard Safran, the Principal Investigator, nor BrUOG, the coordinating center, have money set aside to reimburse you for medical bills from treatment of a research related injury or otherwise compensate you in the event of a study-related injury.

Signing this form does not lessen or take away any of your lawful rights. For more facts, please contact _____ name in the Office of Research Administration at _____ phone.

Rights and Complaints

If you have any complaints about your taking part in this study, or would like more facts about the rules for research studies, or the rights of people who take part in research studies, you may contact _____ name, in the _____ institutions

Confidentiality

The section at the end of this document called “Research Authorization for Use and Disclosure of Information” provides detailed information about how the information learned about you during this study will be used and shared. More generally, all of your records from this study will be treated as private health care records. The records will be protected according to the rules of _____ institutions. The _____ institutions privacy practices and policies are based on the rules about protection of private health care information contained in <INSERT STATE> law and in the Federal Health Insurance Portability and Accountability Act of 1996 and its regulations (“HIPAA”). The privacy practices of _____ institutions and of the people who provide services at or with <INSERT HOSPITAL> are explained in more detail in the _____ institutions Joint Privacy Notice (the “Privacy Notice”) that will be given to you.

You should also know that there are times when the law might require or permit _____ institutions to release your health information without your permission. The Privacy Notice explains when this might happen. To give you some examples, State law requires health care workers to report abuse or neglect of children to the Department of Children, Youth and Families (DCYF). State law also requires health care workers to report abuse or neglect of people age 60 and older to the Department of Elderly Affairs.

If you are found to have HIV/AIDS, <INSERT STATE> State law requires that the names of individuals diagnosed with HIV or AIDS be reported to the Health Department.

Research authorization for use and disclosure of information.

The purpose of this section of the document is to provide you with some more information about how the information learned about you during the study will be used and shared.

We understand that your medical information is very personal and we will work hard to keep it private. **If you sign this form you consent to participate in this research study and are giving us permission to use and share your personal health information in the ways described in this form.**

Understandings and notifications

The main purpose of permitting the use and release of your information is to allow the research project to be conducted and to ensure that the information relating to that research is available to all parties who may need it for research purposes. Your information may also be used as necessary for your research-related treatment, to collect payment for your research-related treatment (when applicable), and to run the business operations of the hospital.

All health care providers are required to protect the privacy of your information. However, most persons or entities (i.e., businesses, organizations) that are not health care providers are not bound by law to protect the privacy of your information. You understand that if the person or entity that receives your information is not a health care provider bound to protect your privacy, such person or entity might re-release your health information.

You have the right to refuse to sign this form. If you do not sign this form, none of your health care outside the study, or the payment for your health care, or your health care benefits will be affected. However, if you do not sign this form, you will not be able to enroll in the research study described in this form, and you will not receive treatment as a study participant.

If you sign this consent form, you may withdraw from the study at any time. However, if you do not want the researchers to use or disclose any further information in this study you must cancel permission in writing and may do so at any time. If you cancel your permission, you will stop taking part in the study and no new information will be collected about you. However, if you cancel your permission, it will not apply to actions already taken or information already collected about you by the hospital or the researchers before you canceled your permission. This information or action may be needed to complete analysis and reports of this research. This permission will never expire unless you cancel it. To cancel this permission, please write to <ENETER CONTACT INFORMATION FOR SITE PRINCIPAL INVESTIGATOR INCLUDING NAME, MAILING ADDRESS AND PHONE NUMBER>.

If after you have signed this form you have any questions relating to your rights, please contact _____ name _____ institutions at _____ Phone.

Uses and releases covered by this authorization (permission)

Who will release, receive, and/or use your information? This form will allow the following person(s), class(es) of persons, and/or organization(s)* to release, use, and receive the information listed below in connection with this Study, or as required by law:

- ☒ Every research site for this study, including this hospital, and including each site's research staff and medical staff
- ☒ Health care providers who provide services to you in connection with this study
- ☒ Laboratories and other individuals and organizations that analyze your health information in connection with this study, in accordance with the study's protocol

- ☒ The following research sponsors and the people and companies that they use to oversee, administer, or conduct the research: BrUOG, the group coordinating the study, Advaxis, the makers of ADXS11-001
- ☒ The United States Food and Drug Administration, the Department of Health and Human Services, the Office of Inspector General, and the Office of Civil Rights
- ☒ The members and staff of the Institutional Review Board(s) or Ethics Committee(s) that approves this study
- ☒ Principal Investigator and other Investigators
- ☒ Study Coordinator
- ☒ Additional members of the Research Team
- ☐ The Patient Advocate or Research Volunteer Protector: _____
- ☒ Members of the hospital's administrative staff responsible for administering clinical trials and other research activities
- ☐ Contract Research Organization (A contract research organization is an independent organization that agrees to oversee and make possible, various aspects of the clinical research process for the research sponsor.)
- ☒ Data and Safety Monitoring Boards and others that monitor the conduct of the Study, for example a Clinical Events Committee
- ☐ The members and staff of the hospitals affiliated Privacy Board (if such a board is used)
- ☐ Others: _____
- * If, during the course of the research, one of the companies or institutions listed above merges with or is purchased by another company or institution, this permission to use or release protected health information in the research will extend to the new company or institution.
- ☒ The entire research record and any medical records held by the hospital may be used and released.
- ☐ The following information: _____

SIGNATURE

I have read this informed consent and authorization form. ALL OF MY QUESTIONS HAVE BEEN SATISFACTORILY ANSWERED, AND I WANT TO TAKE PART IN THIS RESEARCH STUDY.

By signing below, I give my permission to participate in this research study and for the described uses and releases of information. *I also confirm that I have been now or previously given a copy of the Lifespan Privacy notice*

Signature of study volunteer/authorized representative* Date and Time when signed

I was present during the consent PROCESS AND signing of this agreement above by the study volunteer or authorized representative

Signature of witness (required if consent is presented orally or at the request of the IRB) Date

I ASSURE THAT I HAVE FULLY EXPLAINED TO THE ABOVE STUDY VOLUNTEER/ AUTHORIZED REPRESENTATIVE, THE NATURE AND PURPOSE, PROCEDURES AND THE POSSIBLE RISK AND POTENTIAL BENEFITS OF THIS RESEARCH STUDY.

Signature of researcher or designate Date and Time when signed

* If signed by agent other than study volunteer, please explain below.

Documentation that a copy of this Informed Consent was given to the research participant is a Federal requirement. Prior to making a copy of the signed and dated Informed Consent please check appropriate box(es) as applicable to indicate copy provided to:

☐ Study Volunteer ☐ Medical Record ☐ Researcher ☐ Other (Specify)

Appendix B:

A Phase II Evaluation of ADXS11-001, Mitomycin, 5-fluorouracil (5-FU) and IMRT for Anal Cancer: BrUOG 276

Fill out all fields below to confirm patient eligibility. Once documented send to BrUOG for confirmation. Once confirmed patient is ready to begin treatment.

Conditions for Patient Eligibility:

Date of initial biopsy _____

Date patient scheduled to begin treatment if determined to be eligible _____

Conditions for Patient Eligibility

_____ (Y/N) Histologically-proven, invasive primary squamous, basaloid, or cloacogenic carcinoma of the anal canal; pathology to be sent

_____ (Y/N) AJCC 2009 TN Stage: T1N1-N3, T2(< 4cm)N1-N3, T2(≥ 4cm)N0-N3, T3N0-3, T4N0-3; based upon the following minimum diagnostic workup: stage to be sent to BrUOG

_____ (Y/N) History/physical examination within 14 days prior to registration; to be sent to BrUOG

_____ (Y/N) Within 42 days prior to registration, the patient must have an anal examination by any of the following: colonoscopy, sigmoidoscopy, or rigid proctoscopy with documentation of primary anal lesion size, distance from anal verge. Reports to be sent. If patient underwent anoscope this can be used for documentation. If patient is unable to tolerate any scope, reason must be documented and MRI can be used for disease measurements, distance and monitoring of disease response. See eligibility for details

_____ (Y/N) Groin examination (clinical) within 42 days prior to registration with documentation of any groin adenopathy and lymphadenopathy (location: right vs. left; medial vs. lateral; mobile vs. fixed; and size); Must be documented and sent to BrUOG

_____ (Y/N) X-ray (PA and lateral), CT scan, or PET/CT scan of the chest within 42 days prior to registration; Report to be sent

_____ (Y/N) CT scan, MRI, or PET/CT of the abdomen and pelvis within 42 days prior to registration; Report to be sent

_____ (Y/N) Zubrod Performance Status 0-1; to be documented

_____ (Y/N) Age ≥ 18;

_____ (Y/N) Laboratory data obtained ≤ 14 days prior to registration on study, with adequate bone marrow, hepatic and renal function defined as follows: Lab report to be sent to BrUOG

- Absolute neutrophil count (ANC) ≥ 1,500 cells/mm³; **Institution ULN**_____, **Date**_____
- Platelets ≥ 100,000 cells/mm³; **Institution ULN**_____, **Date**_____
- Hemoglobin ≥ 8.0 g/dl (Note: The use of transfusion or other intervention to achieve Hgb ≥ 8.0 g/dl is acceptable.); **Institution ULN**_____, **Date**_____
- Serum creatinine ≤ 1.5 mg/dl; **Institution ULN**_____, **Date**_____
- Bilirubin < 1.4mg/dl; **Institution ULN**_____, **Date**_____
- ALT/AST < 3 x ULN; **Institution ULN**_____, **Date**_____
- Negative serum pregnancy test for women of child-bearing potential; **Date**_____

_____ (Y/N) Women of childbearing potential and male participants must agree to use a medically effective means of birth control throughout their participation in the treatment phase of the study.

_____ (Y/N) Patients must sign a study-specific informed consent prior to study entry.

_____ (Y/N/NA) Cardiac echo with LVEF ≥ 30% if preexisting significant cardiac disease. Report to be sent

_____ (Y/N/NA) PFTS with DLCO ≥ 40% if history of significant COPD. Report to be sent

_____ (Y/N) Patients must be able to swallow pills.

Conditions for Patient Ineligibility

_____ (Y/N) Prior invasive malignancy (except non-melanomatous skin cancer), unless disease free for a minimum of 2 years;

_____ (Y/N) Prior systemic chemotherapy for anal cancer;

_____ (Y/N) Prior allergic reaction to the study drugs involved in this protocol.

_____ (Y/N) Prior radiotherapy to the pelvis that would result in overlap of radiation therapy fields; Prior therapies including radiation to be sent to BrUOG

_____ (Y/N) Severe, active co-morbidity, defined as follows:

_____ (Y/N) Unstable angina and/or congestive heart failure requiring hospitalization within the past 6 months;

_____ (Y/N) Transmural myocardial infarction within the last 6 months;

_____ (Y/N) Acute bacterial or fungal infection requiring intravenous antibiotics at the time of registration;

_____ (Y/N) Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of registration;

_____ (Y/N) Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects;

_____ (Y/N) Patients known to be seropositive for HIV and/or active hepatitis, even if liver function studies are in the eligible range.

_____ (Y/N) Other immunocompromised status (e.g., organ transplant or chronic glucocorticoid use). If patient has diagnosis of immunodeficiency, is dependent on or has received systemic steroids therapy or any form of immunosuppressive therapy within 7 days prior to the first dose of ADXS11-001 they are ineligible. Topical corticosteroid or occasional inhaled corticosteroids are allowed.

_____ (Y/N) Women who are pregnant or lactating are ineligible because the treatment involved in this study may be significantly teratogenic and there is the potential for transmission of listeria to the infant.

_____ (Y/N) Patients allergic to or with sensitivity to penicillin, ampicillin, trimethoprim-sulfa **and** quinolones (including history of rash or anaphylaxis).

_____ (Y/N) Patients allergic to naproxen.

_____ (Y/N) Patients currently receiving oral or IV antibiotics.

_____ (Y/N) Patients with a prior history of a splenectomy and/or sickle cell trait/disease.

_____ (Y/N) 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. Sponsor must be contacted prior to consenting any subject who has any other device and/or implant. Site is required to submit to BrUOG ALL surgical implants patient has ever had in their medical history and ALL surgeries regardless of link to this cancer diagnosis.

_____ (Y/N) Subjects who are receiving or may receive future treatment with PI3K or TNF α inhibitors.

_____ (Y/N) Has 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: if patient underwent surgery > 6 weeks from start of ADSX11-001, all toxicities and/or complications must have recovered to baseline or Grade 1 prior to the initiation of ADXS11-001 study therapy and this must be documented for confirmation to BrUOG prior to registration., please confirm if this is applicable: _____ (y/n)

_____ (y/n)Patient not being willing to have new infusion line placed for each infusion of ADXS11-001 as existing or newly placed central venous catheter or infusion ports are not allowed to be used for ADXS11-001 administration.

_____(Y/N) Patient not being willing to comply with requirement 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.

_____(Y/N) 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.

_____(y/n) Patients with active infection requiring systemic therapy (oral or IV) or those currently receiving antibiotics that cannot discontinue prior to dosing are ineligible. (Subjects who discontinue an antibiotic prior to dosing must wait at least 5 half-lives after the last dose of antibiotic before receiving any ADXS11-001 infusion- documentation required to be sent to BrUOG to confirm eligibility)

_____(y/n) Patient has a history of listeriosis or prior ADXS11-001 therapy.

To be filled out by BrUOG Administrator:

Patient registered Yes _____ No _____

BrUOG ID Number _____ Randomization Assignment: _____

Signed informed consent: The patient must be aware of the neoplastic nature of his/her disease and must willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks, and discomforts.

The support documentation, per the requirements under the study parameters section of this study, as well as the consent form and this checklist, must be faxed to the BrUOG Central Office at the time of registration. Please check if "Enclosed", state reason when "Not Enclosed," or check if "Not Applicable."

Registration:

1) Eligibility Form with	Enclosed	Not Enclosed	Not Applicable
2) Heme/Onc initial note	Enclosed	Not Enclosed	Not Applicable
3) Pathology Report(s)	Enclosed	Not Enclosed	Not Applicable
4) Radiation Oncology note	Enclosed	Not Enclosed	Not Applicable
5) MRI Report(s)	Enclosed	Not Enclosed	Not Applicable
6) Lab reports	Enclosed	Not Enclosed	Not Applicable

****Please remember to send source and confirmation of all inclusion/exclusion and schedule of evaluations testing as this checklist does not count as source documentation.****

Other _____

IRB approval date of protocol: _____

Hospital where patient will be treated with Oncologist: _____

Hospital where patient will be treated with Radiation Oncologist: _____

Date patient will begin treatment: _____ Primary Physician: _____

Your signature: _____

APPENDIX C:

NCI CTC Version 4.0

Steps to determine if an adverse event is to be reported in an expedited manner:

Step 1: Identify the type of event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be downloaded from the CTEP home page (<http://ctep.cancer.gov>).

Additionally, if assistance is needed, the NCI has an Index to the CTCAE that provides help for classifying and locating terms. All appropriate treatment locations should have access to a copy of the CTCAE.

Step 2: Grade the event using the NCI CTCAE.

Step 3: Determine whether the adverse event is related to the protocol therapy (investigational or commercial). Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Step 4: Determine the prior experience of the adverse event. Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is NOT listed in:

APPENDIX D
ECOG / Zubrod Performance Status

- 0** Fully active, able to carry on all pre-disease performance without restriction
- 1** Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
- 2** Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
- 3** Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
- 4** Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
- 5** Death

**APPENDIX E
CASE REPORT FORMS**

Appendix F: Adverse Event form

Please note that the Protocol Event Type Form (OBA) below is REQUIRED to be submitted to BrUOG for all SAEs. A MedWatch 3500A is not required for reporting, only the OBA form is required.

<u>PROTOCOL AND EVENT TYPE</u>	
NIH/OBA (RAC) Protocol Number	1207-1172
FDA IND number	15057
Date this report completed:	
Seriousness of the AE (choose one)	Death Life-threatening Initial or prolonged hospitalization Disability Congenital anomaly Required intervention to prevent permanent impairment/damage Other medically important condition Non-serious
Severity of Event	Minimal Moderate Severe Life- Threatening Fatal
Was this event expected in terms of its severity?	Yes No
Was this event expected in terms of its specificity?	Yes No
Relationship of Event to gene transfer product	Unrelated Unlikely Possible Probable Definite
Attribution of AE Attribution of AE, continued	Concomitant medication Product Intervention Underlying disease Route of administration

	Other suspected cause (describe)
Type of report	Initial Follow-up
<u>DEMOGRAPHICS</u>	
PI Name	
Name of Clinical Trial Site/Organization	
PI Telephone Number	
PI E-mail Address	
Reporter name	
Reporter Telephone number	
Reporter E-mail address	
Research Participant's study identification number	
Research Participant's gender	
Research Participant's date of birth	
Research Participant's date of death	
Research Participant's weight in kgs	
Research Participant's height in cms	
Which Arm/Cohort/treatment group was the subject assigned to?	
Was subject dosed?	Yes No Information Not Available
What study agent was received:	IND agent Placebo Blinded Study Agent
Were there any Protocol Deviations/Violations/Exceptions for this participant?	Yes: _____ _____ _____

	No
<u>DETAILED ADVERSE EVENT INFORMATION</u>	
Adverse Event Date	
Description of Event	
Relevant tests (e.g. x-rays) and results	
Treatment (s) of Adverse Event (Include medications used to treat this event.)	
Name of Concomitant Medications (Do not include medications used to treat this event.)	
Pre-existing conditions/ relevant clinical history (if this is an oncology trial, please designate primary disease, e.g. ovarian cancer)	
Date(s) of treatment(s) of the adverse event	

Was autopsy performed?	Yes No
Date of autopsy	_____ or Not Applicable _____
Outcome of the event	Recovered/resolved Recovering/resolving Not recovered/not resolved Recovered/resolved with sequelae Fatal Unknown
Documentation accompanying the report (e.g., H& P, Progress Notes, Discharge Summary, Lab or Autopsy Reports, Other, etc.)	
Description of any “other” documentation	
<u>PRODUCT AND DOSING INFORMATION</u>	
Name of gene transfer product	ADXS11-001
Vector type (e.g. adenovirus)	Bacteria- Listera
Vector sub-type (e.g. type 5, also include relevant deletions)	Listeria Monocytogenes (ADXS11-001)
Lot number	
Was the agent manufactured at an NGVL?	No
Route of administration	IV injection
Site of administration	
Did subject receive the dose specified in the protocol?	
If not, what dose was given?	

Date of first exposure to study agent?	
Date of most recent exposure to study agent?	
Total dose received prior to this event?	
Total dose quantity administered to subject to date	
Unit of measure for a single dose	1x10 ⁹ cfu
Dose quantity in a single administration	1
If courses used, how many were given prior to this event?	
How many doses on the last course were given?	
Was the administration of this product stopped because of this adverse event?	
Name of other treatment (s) (medications, radiation, surgery) received by research participant as required by the protocol	