

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

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Scientific Abstract

BACKGROUND:

This is a phase I/II study for patients with large anal cancers that are confined to the anus and surrounding tissues. The standard treatment is with radiation and the chemotherapy drugs mitomycin and 5-FU. The recurrence rate for these patients is high and novel treatments are needed. This study will investigate the addition of a vaccine called ADXS11-001 to standard treatment for anal cancer. The vaccine is made by the pharmaceutical company Advaxis. The vaccine uses a type of bacteria called listeria.

Almost all anal cancers are caused by the human papilloma virus. Infection by HPV puts the protein HPV E7 into cells of the anus. This can cause the normal cells of the anus to become malignant. In the Advaxis vaccine ADXS11-001, the HPV E7 protein is put into the listeria bacteria. The listeria vaccine is given by IV injection. The listeria vaccine is taken up by cells of the immune system called antigen presenting cells. The presence of the listeria bacteria carrying HPV E7 inside antigen presenting cells will stimulate other cells of the body's immune system to attack cells that contain HPV E7 including anal cancer cells.

The listeria bacteria used to make this vaccine has been weakened to reduce the chance that the vaccine will cause an infection. Beginning 3 days after each vaccination, patients also will receive oral antibiotics for 7 days against listeria to reduce infection risk.

A phase I study, evaluating the optimal dose of the ADXS11-001 vaccine has previously been completed in cervical cancer. (Most cases of cervical cancer are also caused by HPV). A phase II study of ADXS11-001 is being performed in cervical cancer by the United States cooperative group, the Gynecological Oncology Group. A study of ADXS11-001 is also being performed in the United States for patients with "cervical intraepithelial neoplasia", a pre-cancerous abnormality of the cervix. A randomized phase II study of ADXS11-001 as a single agent and in combination with chemotherapy for women with cervical cancer is underway in India. Clinical tumor reduction was seen in patients with several different strains of HPV including HPV 16, 18, 31, 35 and 45. This is the first trial in anal cancer.

OBJECTIVES:

Primary Objectives

1. To evaluate the safety of the addition of ADXS11-001 to standard chemoradiation for patients with anal cancer.
2. To evaluate the 6-month clinical complete response rate for patients with anal cancer treated with ADXS11-001 mitomycin, 5-FU and IMRT.

Secondary Objectives:

1. To evaluate progression-free and overall survival for patients with anal cancer treated with ADXS11-001, mitomycin, 5-FU and IMRT.
2. To assess peripheral and histologic markers of immune response including measuring immunohistochemistry of biopsies for T cell infiltration: CD8+, CD4+ TAM (M1/M2) and T-regulatory cells and myeloid derived suppressor cells, following ADXS11-001 in patients with anal cancer.
3. To correlate HPV type with 6-month clinical complete response, and progression-free and overall survival, after treatment with ADXS11-001 and standard chemoradiation.

STUDY DESIGN:

All patients will receive standard radiation and chemotherapy for anal cancer. Radiation is given once a day, five days a week, for 28 or 30 treatments (5.5 to 6 weeks) depending on the stage of the anal cancer. All patients will receive standard chemotherapy with mitomycin and 5-fluorouracil on the first and fifth week of radiation.

The first part of this study is considered a phase I study since two treatment schedules of ADXS11-001 will be tested. Patients will be treated in groups of 3-6 patients to determine the safest treatment schedule of ADXS11-001 that can be given with radiation and chemotherapy for anal cancer.

Treatment Schedule #1

Patients will receive 4 treatments with ADXS11-001 as a 15-minute infusion through the vein. The first treatment will be 10-14 days *before* the first radiation treatment. The second treatment of ADXS11-001 will be given approximately 10 days after completion of radiation. The third treatment will be given approximately 28 days after the second treatment and the fourth treatment will be 28 days later.

Treatment Schedule #2

Only when Treatment Schedule #1 is found to be safe will Treatment Schedule #2 be evaluated. Patients on Treatment Schedule #2 will receive 4 treatments with ADXS11-001, approximately once every 28 days, as a 15-minute infusion through the vein. The first treatment will be 10-14 days *before* the first radiation treatment. The second treatment will be 21 days after starting radiation. (Thus patients will receive the second treatment of ADXS11-001 will be about half-way through completion of the 6 weeks of mitomycin, 5-FU and radiation.) The third treatment with ADXS11-001 will be approximately 10 days after completion of chemotherapy and radiation and the fourth treatment will be approximately 28 days later.

Depending on the safety of Treatment Schedule #1 and Treatment Schedule #2, a total of 25 patients will then receive treatment according to one of these schedules.

ACCRUAL:

Approximately 25-28 patients will take part in this study.

This study will be coordinated by the Brown University Oncology Research Group. An IND has been obtained to do this study. Other cancer centers, coordinated by the Brown University Oncology Group, will participate including The M.D. Anderson Cancer Center (Texas), Montefiore Medical Center (New York) and Boston Medical Center. Blood and tumor tissue will

be sent to the Radiation Therapy Oncology Group who will coordinate translation research studies.

DESCRIPTION OF ADXS11-001

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 (see protocol references 46-60 and please refer to Advaxis Inc IND#13712.)

Bulk drug substance consists of a recombinant strain of bacteria, *Listeria monocytogenes* (ADXS11-001), which secretes the HPV 16 E7 protein, and which has been attenuated by partial complementation of *prfA*, the transcriptional factor needed for expression of *Listeria* virulence genes. ADXS11-001 is generated by transforming the *prfA*-deficient strain XFL-7 with the multicopy plasmid pGG55, which contains a copy of *prfA* that partially restores the vector virulence ADXS11-001 uses this multicopy episomal expression system to secrete a 67-kDa fusion protein consisting of the first 417 amino acids of LLO, followed by the HPV 16 E7 protein.

BIOSAFETY OF ADXS11-001:

ADXS11-001 is a live attenuated strain of *Listeria monocytogenes*.

Listeria is gram-positive, non-spore-forming, facultative bacilli are hemolytic and catalase-positive. 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.

ADXS11-001 has been attenuated such that it is cleared by 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 *Listeria*. Phase 1 studies did not demonstrate significant bacterial shedding from patients treated with ADXS11-001. It has been administered intravenously over 480 times to over 180 patients (at the time of this writing) with only mild-moderate side effects associated with infusion. It is considered as a non-infectious BSL-1 agent for transport by the CDC.

Laboratory Hazards:

Wild-type *Listeria monocytogenes* is ubiquitous in the environment and may be found in feces, 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 *Listeria monocytogenes* infections in pregnant women occur most often in the third trimester and may precipitate labor. Transplacental transmission of *L. monocytogenes* poses a grave risk to the fetus and may result in disseminated abscesses contributing to a mortality rate of nearly 100%.

ADXS11-001 is highly attenuated from wild-type in such a way that it is cleared by SCID mice lacking cellular immunity and by gamma-interferon knock-out mice lacking adaptive cellular immunity but it is live bacteria and should be handled as such. Risks to laboratory workers and pregnant women from contact or ingestion exposure have not been established with ADXS11-001 and should be considerably less than those from a non-attenuated *Listeria* strain. Nevertheless, common safety practices for infectious materials should be followed in handling.

Recommended Precautions:

Both the CDC and the German government have confirmed that the strain of *Listeria* used in this study is non-pathogenic, however no mechanism currently exists to distinguish the biosafety level of an attenuated bacterium from the parent strain. Wild type *Listeria* is a BSL-2 pathogen, and so Bio-safety Level 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).

Because this agent is a live bacterium that will replicate upon thawing, it must be used to prepare a dose that is administered within 1 hour of thawing, or it is not to be administered.

Storage of ADXS11-001:

ADXS11-001 will be stored in -70 freezers within the pharmacies of participating hospitals. ADXS11-001 will not be constructed on-site at any of the participating hospitals.