**Window of opportunity trial of HPV E7 antigen-expressing Listeria-based therapeutic vaccination prior to robotic surgery for HPV-positive oropharyngeal cancer**

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**Introduction**

*In the USA, the prevalence of human papillomavirus (HPV)-associated oropharyngeal cancer (HPVOPC) is increasing [225% from 1988 to 2004].1,2*

- Patients tend to be younger and have a favorable prognosis, with a 69% reduction in the risk of death compared with HPV-negative patients.1,2
- Most HPVOPC patients present with advanced stage, and standard chemoradiation regimens can be associated with significant toxicity.3
- It is a paradox of treatment that patients who have a good prognosis are at risk of therapy-related long-term poor quality-of-life outcomes.

**Immunotherapy has the potential to reduce toxicity through de-escalation of chemoradiation regimens, and potentially enhance long-term disease control.**

- *Listeria monocytogenes* (Listeria]tio-synthetic O [LLO] immunotherapies have been shown to generate antigen-specific T-cell responses and neutralize Tregulatory (Treg) and myeloid-derived suppressor cells (MDSCs) that protect the tumor microenvironment against immunologic attack (Figure 1).4
- **ADXS11-001** is an attenuated, genetically modified Lm vector that secretes an HPV-E7 tumor antigen as LLO-E7 fusion protein; LLO refers to the truncated form of non-hemolytic LLO protein.5
- **ADXS11-001** can be combined with different treatment modalities, and data in cervical cancer support potential clinical benefit.6-7

We hypothesize that **ADXS11-001** neoadjuvant immunotherapy will induce a robust HPV-specific cytotoxic T-lymphocyte (CTL) response in the blood and tumor of HPVOPC patients who are vaccinated prior to surgery.

**Methods**

**Window of opportunity, non-randomized, single-arm phase 2 trial of neoadjuvant ADXS11-001 treatment before standard of care transoral robotic surgery (TORS) in patients with stage II-IV HPVOPC (NCT02002182; Figure 2).**

- Patients in the ADXS11-001 treatment arm (study arm) will be enrolled according to a Simon's two-stage design.
- **Initial cohort of 9 patients** enrolled before preliminary analysis, and a subsequent cohort of 13 patients enrolled if statistical criteria are met.
- **ADXS11-001** will be administered as an intravenous infusion at a dose of 1x107 colony forming units (CFUs) at Days 1 and 15.
- Ibuprofen, diphenhydramine, and an antiemetic will be given before infusion, with ibuprofen also administered after infusion; a course of amoxicillin (or alternative antibiotic) will be administered 72 hours after each ADXS11-001 dosing.
- An observational arm of up to 10 patients, who will undergo TORS without previous treatment with ADXS11-001, will also be enrolled.
- Standard of care TORS will be performed in all patients.
- Adjuvant radiation/chemoradiation will be as per standard of care (4-6 weeks after TORS).
- Blood, tumor specimens, and tumor-infiltrating lymphocytes will be collected at different time points from study patients (Figure 2), and processed and stored prior to analysis.

**Figure 1. Step by step Lm-LLO immunomodulation**

1. APCs are genetically altered so they do not have the body
2. The bacilli are further modified to develop a vector targeting the specific tumor of interest
3. The lmx1-measured can vector the LLO-E7 fusion protein
4. The iSOI vector contains the tumor-specific immune signature
5. The TME contains in vivo the tumor-invasive signature
6. The TME contains the tumor-invasive signature
7. The programmed CTLs will enter into the tumor and destroy the tumor cells

**Objectives**

To determine the immunogenicity of **ADXS11-001** treatment in patients with stage II-IV HPV-positive squamous cell carcinoma of the oropharynx.

- Primary endpoint: change from baseline in HPV-specific CD8+ CTL responses in peripheral blood at the time of surgery.
- Secondary endpoint: change in HPV-specific CD8+ CTL responses in peripheral blood at various time points after surgery.
- Exploratory endpoint: changes in the profile of tumor-infiltrating effector (natural killer [NK] cells, CD4+ and CD8+ T cells) and suppressor (Treg and MDSCs) immunocytes.

To evaluate the tolerability, safety, and nature and degree of **ADXS11-001** toxicity in patients with HPVOPC.

**Key inclusion criteria**

- Adult patients (≥18 years) with newly diagnosed, biopsy proven, stage II-IV HPVOPC.
- Eligible to undergo TORS with or without neck dissection.
- Eastern Cooperative Oncology Group performance status ≤ 2.
- Able to understand and give informed consent.

**Key exclusion criteria**

- Active cancer at another site, or history of cancer in the past 3 years.
- Prior systemic chemotherapy or radiotherapy.
- Immunosuppressive condition, or taking immunosuppressive medication.
- Liver disease or other medical contraindication to study medications.

**Blood and tumor assessments**

- Blood and tumor analyses include immunophenotyping and characterization of HPV-specific T-cell responses in blood, seroreactivity to HPV and cancer-testant antigens in blood, and immunophenotyping and molecular profiling of tumor tissue (summarized in Table 1).
- Tissue-based changes will be correlated with comprehensive analysis of immune changes in peripheral blood.

**Table 1. Laboratory studies**

<table>
<thead>
<tr>
<th><strong>Assay</strong></th>
<th><strong>Questions to be answered</strong></th>
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<tr>
<td>ELISPOT for HPV-negative T-cells in peripheral blood</td>
<td>Does <strong>ADXS11-001</strong> induce robust systemic antigen-specific immunity?</td>
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<tr>
<td>IHC/IF for tumor-infiltrating CD8+ T-cells and other immunocytes</td>
<td>Do <strong>ADXS11-001</strong> induced T-cells penetrate the tumor? Is the overall balance of suppressor and effector immune cells in the TME improved after treatment?</td>
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<tr>
<td>Immunophenotyping of suppressor and effector immune cell subsets in blood by flow cytometry</td>
<td>Does <strong>ADXS11-001</strong> improve the systemic balance of suppressor and effector immunocytes?</td>
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<tr>
<td>Seroreactivity to HPV antigens and HNSCCA-associated cancer-testant antigens in blood</td>
<td>Does targeting a foreign viral antigen (E7) lead to epitope spreading and induction of a broad-based response to self-derived tumor antigens?</td>
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<tr>
<td>Immune gene expression signatures in TME by Nanostring</td>
<td>Is <strong>ADXS11-001</strong> associated with an “immune response signature” of altered gene expression? Can we identify potential molecular targets for combination therapy?</td>
</tr>
<tr>
<td>Multiplex serum cytokine and soluble immunomodulator levels by Luminex analysis</td>
<td>Does <strong>ADXS11-001</strong> induce a durable inflammatory/cytokine signature?</td>
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<tr>
<td>Tcell receptor diversity profiling by Immunoseq</td>
<td>How does <strong>ADXS11-001</strong> treatment affect the depth and breadth of the tumor-infiltrating T-cell repertoire?</td>
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**Statistical considerations**

- The trial is designed to conclude that **ADXS11-001** treatment is highly immunogenic and worth further investigation if post-treatment T-cell responses in peripheral blood at least two-fold greater than pretreatment baseline response are observed in ≥ 75% of patients.

**Trial status**

- This phase 2 study is open and actively enrolling at Icahn School of Medicine at Mount Sinai, NY, USA [Site PI Brett Miles]. The IND for this study is held by the Baylor College of Medicine (FDA IND#15688, PI Andrew Sikora).
- Eight of a maximum of 22 **ADXS11-001** treated patients and 2 of a maximum of 10 observational patients have been enrolled to date.

**References**


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