ADXS-HPV: A therapeutic *Listeria* vaccination targeting cervical cancers expressing the HPV E7 antigen

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Abbreviations: HPV, human papillomavirus; FDA, Food and Drug Administration; VLP, virus like protein; *Lm*, *Listeria monocytogenes*; LLO, listeriolysin O; PLC, phospholipase C; MHC, major histocompatibility complex; GOG, Gynecologic Oncology Group; OPSCC, oropharyngeal cancer

Listeria monocytogenes is a bacterium that can be genetically modified to express fusion proteins with antigens specific to certain cancer models. This technology has been harnessed to develop ADXS11-001, a vaccine that aims to elicit an immune response against human papillomavirus (HPV) oncoprotein E7. Pre-clinical studies assessing the efficacy of recombinant *Listeria* vaccination targeting this same oncoprotein have consistently demonstrated successful reduction of in vivo tumor burden among animal cancer models. Several clinical trials are underway to assess the efficacy of ADXS11-001 in eliciting both immune and clinical responses against HPV-related human cervical, oropharyngeal and anal cancers.

The causal relationship between human papillomavirus (HPV) infection and cervical cancer is well established. Human papillomavirus is a double-stranded DNA virus that maintains oncogenic potential primarily through the actions of its oncoproteins E6 and E7. Cellular dysregulation by HPV's oncogenes E6 and E7 is also implicated in the pathogenesis of anorectal and head and neck cancers.^{6,7} Presence of the E6 oncoprotein leads to degradation of the p53 tumor suppressor gene. Oncoprotein E7 contributes to the oncogenic potential of HPV through multiple mechanisms, including inactivation of the retinoblastoma tumor suppressor gene and disruption of the function of cyclin dependent kinases and their inhibitors.⁷

Introduction

Representing the third most common gynecologic malignancy in the United States, cervical cancer poses therapeutic challenges to both national and global health care sectors.¹ In the United States alone, cervical cancer was estimated to represent more than 12,000 new cancer diagnoses and 4,000 cancer related deaths in 2013.² Worldwide, however, cervical cancer represents a larger health care problem with about 528,000 new women diagnosed in 2012. Disease burden and disease-related mortality in under-developed countries account for approximately 85% and 87% of worldwide cases, respectively.^{3,4} The disproportionate disease burden faced by developing nations poses complicated challenges, as lack of infrastructure and financial resources make development of sustainable programs for the implementation of cervical cancer screening and/or HPV vaccination difficult.⁵ Given these challenges, there may be a role for therapeutic cervical cancer vaccination until sustainable preventative programs are successfully implemented in resource poor areas.

HPV Vaccination

Of the more than 120 strains of HPV identified to date, only 40 are thought to be acquired through sexual transmission. HPV strains can further be classified into high or low risk regarding their oncogenic potential. Human acquisition of 13 strains of HPV has been linked to the subsequent development of cervical cancer.⁸ Despite the numerous identified strains of HPV, it is estimated that strains 16 and 18 account for 70% of cervical malignancies.⁹

Two prophylactic vaccines targeting high risk HPV strains have been developed in response to the causal relationship between high risk HPV infection and the subsequent development of cervical cancer. Gardasil, a quadrivalent vaccine providing immunologic protection against HPV strains 6, 11, 16 and 18, was licensed by the Food and Drug Administration (FDA) in 2006.¹⁰ When administered prior to exposure, the vaccine is 98% effective at preventing HPV 16/18 related CIN 2+.⁹ Cervarix, a bivalent vaccine targeting high-risk HPV strains 16 and 18, subsequently obtained FDA approval in 2009. This bivalent vaccine appears to be slightly less efficacious at preventing HPV 16/18 related CIN 2+ disease with a reported efficacy of more than 92%.¹⁰ Both vaccines utilize HPV L1 virus like particles (VLPs) to elicit an immunological response.^{10,11} Preclinical

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animal studies suggest that exposure of naïve animals to HPV L1 VLPs leads to production of antibodies protective against HPV infection.¹¹ In December of 2013, preliminary reports of a phase III study evaluating an investigational 9-valent HPV vaccine, V503, in comparison to the Gardasil showed that the new agent prevented approximately 97% of precancerous lesions of the cervix, vulva, and vagina caused by HPV 31, 33, 45, 52, and 58. V503 also elicited immune responses to HPV types 6, 11, 16, and 18 that were the same or better than the FDA-approved Gardasil vaccine.¹²

Despite the demonstrated efficacy of various preventative HPV vaccines, a 2012 CDC report of the National Immunization Survey – Teen (NIS-Teen) reported that 53.8% and 33.4% of females aged 13 to 17 had received ≥ 1 dose and ≥ 3 doses of the HPV vaccine, respectively.¹³ While such data emphasize the need for ongoing efforts to improve uptake of HPV preventative strategies, they also highlight the need to explore new therapeutic interventions in the treatment of cervical malignancies. One such promising strategy is the use of ADXS11-001 as an immunotherapy based on live attenuated *Listeria monocytogenes* to target HPV-associated tumors.

***Listeria monocytogenes* as a mediator of immune response**

Prophylactic HPV vaccines target HPV VLP L1 as a means to prevent primary infection with specific HPV strains.^{10,11} However, L1 is not expressed in HPV related neoplasms that develop subsequent to primary infection and, as such, is not an effective target for therapeutic vaccines. Development of an effective therapeutic vaccine necessitates that the vaccine can elicit a potent immune response against an antigen that is consistently expressed on target cells.¹⁴

Listeria monocytogenes (*Lm*) is a facultative gram-positive intracellular bacterium. It accounts for approximately 2,500 infections annually and most commonly causes clinically significant illness (es) in neonates, pregnant women and immunocompromised hosts.¹⁵ Following interaction with surface proteins on a host cell, *L. monocytogenes* is phagocytosed. Unlike other intracellular bacteria, *Listeria* then utilizes listeriolysin O (LLO) and phospholipase C (PLC) to enable their escape from the host cell phagosome into the cytoplasm of the host cell.¹⁶⁻¹⁸ Bacteria released into the cytosol are then able to utilize actin to promote their own motility and movement between host cells via expression of the bacterial surface protein ActA.^{16,19}

Once the bacterium successfully infects host cells, it maintains the ability to activate the adaptive immune response through 2 different major histocompatibility complex (MHC) pathways. Those bacteria that do not escape the host cell phagosome illicit an immune response through the MHC II pathway with subsequent activation of CD4+ T cells. For those bacteria that successfully escape from the host cell phagosome, peptides derived from bacterial antigens via the MHC I pathway are presented to the host cell surface where they activate CD8+ T cells.¹⁹ Activation of CD8+ T cells has been well studied and explored as a mechanism to direct evolving vaccine technology against tumors.

Listeria also maintains the ability to activate the innate immune system. Activation of this arm of the immune system

leads to recruitment of phagocytic cell types, including macrophages and neutrophils, which work to control infection through various mechanisms (engulfment, production of free radicals).²⁰ In addition to recruitment of phagocytic cells, a variety of inflammatory cytokines and chemokines are produced.^{19,20} For example, interferons are produced in response to *Listeria* infection. While interferon gamma plays a protective role against infection, production of interferon α and β may further support bacterial infection. Additional cytokines are released when the bacterium and its antigens are recognized by toll like receptors (TLR) and dendritic cells.²⁰ Once *Listeria* antigens are presented on the cell surface, TLR2 and 5 play a role in antigen recognition. Myeloid differentiation primary-response protein 88 (MyD88) then plays a role in translating signals originating from TLRs to recruit the innate immune system.²¹

Because *Listeria* is capable of activating both MHC pathways secondary to its presence within the phagosome as well as the cytosol, it is able to elicit a potent immune response, and was identified as a potential vector for therapeutic vaccinations.¹⁸ However, in order to fully develop a recombinant therapeutic *Listeria* vaccination, an antigenic target that is consistently expressed on target cells needed to be identified. In the case of therapeutic HPV vaccination, HPV oncoprotein E7 was identified as a possible antigenic target given its reliable expression in HPV 16 related neoplasms and its ability to elicit an immune response through MHC class I pathway.^{22,23}

Early targeted uses of *Listeria Monocytogenes*

Use of *L. monocytogenes* to direct an immune response at a specific tumor burden dates back to 1995 when a study demonstrated that use of recombinant *Listeria* could lead to tumor regression. Pan et al.²⁴ provided evidence at that time that recombinant *Listeria* engineered to secrete influenza virus nucleoprotein could decrease an injected tumor load, as well decrease established tumor, expressing the influenza virus nucleoprotein in colon and renal cancer models. This study also established a critical role for adaptive immunity, more specifically CD4+ and CD8+ T cells, in directed immune responses elicited from recombinant *Listeria*. Depletion of CD4+ T cells led to inability to reject antigen expressing tumor cells, though tumor growth was still slowed in these models. However, depletion of CD8+ T cells rendered the animal model incapable of mounting an effective immune response against tumor cells in response to recombinant *Listeria*.²⁴

Since that initial study in 1995, several additional experiments utilizing recombinant *Listeria* in multiple animal cancer models have been completed (Table 1). Work to date has evaluated the use of recombinant *Listeria* as a delivery vector for tumor specific antigens investigating several cancer models, including breast, cervical, colon, skin and renal cancers.¹⁶ In 2005, Singh et al.²⁵ demonstrated the efficacy of HER-2/neu targeted recombinant *Listeria* vaccines in a rat breast cancer model. Five strains of recombinant *L. monocytogenes*, each secreting a unique fusion protein representing fragments of HER-2/neu in combination with LLO, were developed. Following establishment of tumors bearing the targeted antigen, mice were injected with one of the

Table 1. *Listeria* as anti-cancer therapy

Author	Year	Cancer Model	Investigational Agent	Outcomes
Pan et al. ²⁴	1995	Colon, renal	<i>Lm</i> -NP ¹	Protection against tumor load; regression of established tumors
Gunn et al. ³³	2000	Cervical	<i>Lm</i> -LLO-E7	Recombinant <i>Listeria</i> vaccine secreting LLO-E7 fusion protein leads to tumor regression, while one secreting E7 does not; different immune responses detected between the 2 recombinant vaccines
Lin et al. ²³	2002	Cervical	<i>rLm</i> -E7	Oral vaccination leads to immune response with vaccine efficacy
Sewell et al. ⁵⁰	2004	Head and neck	<i>Lm</i> -ActA-E7	Tumor regression with T cell response
Singh et al. ²⁵	2005	Breast	<i>Lm</i> -LLO-EC1 <i>Lm</i> -LLO-EC2 <i>Lm</i> -LLO-EC3 <i>Lm</i> -LLO-IC1 <i>Lm</i> -LLO-IC2	Efficacious anti-tumor agents, impact of agents abolished by absence of T cells
Kim et al. ²⁶	2008	Breast	<i>Lm</i> -LLO-Mage-b	Reduction in metastatic disease
Sewell et al. ⁵¹	2008	Cervical	<i>Lm</i> -LLO-E7	Successful reduction in autochthonous tumor following vaccination
Seavey et al. ²⁹	2009	Breast	<i>Lm</i> -hHer2-neu	Tumor regression; limited metastatic disease
Seavey et al. ²⁷	2009	Breast	<i>Lm</i> -LLO-O-fetal liver kinase-1	Anti-angiogenesis vaccine effective without negatively impacting wound healing
Shahabi et al. ²⁸	2011	Breast	ADXS-164	Highly attenuated recombinant <i>Listeria</i> vaccine without antibiotic selection markers remains efficacious
Wood et al. ³¹	2011	Breast	<i>Lm</i> -LLO-CD105A <i>Lm</i> -LLO-CD105B	Elicited anti-angiogenesis immune response effective against primary and metastatic cancer
Wood et al. ³²	2012	Breast	<i>Lm</i> -LLO-ISG15	Reduction of tumor burden

¹ Influenza nucleoprotein

5 recombinant strains and monitored for changes in tumor size. Results ranged from stasis in tumor development to complete tumor regression, with results varying somewhat based on the strain of recombinant *L. monocytogenes* injected.²⁵ Similar to the results reported by Pan et al. in 1995²⁴, CD8+ T cell depleted models demonstrated nullification of anti-tumor effects previously demonstrated by the vaccine.²⁵

Several subsequent studies have produced similar results regarding the efficacy of recombinant *Listeria* vaccination in the treatment of breast cancer in animal models.²⁶⁻²⁸ Kim et al.²⁶ investigated the role of recombinant *Listeria* vaccination in improving the efficacy of Mage-b vaccination in the treatment of metastatic breast cancer tumors in animal models. Results showed improved efficacy of the Mage-b vaccination in the treatment of metastatic tumors when *Listeria* was used as the delivery vector.²⁶ Seavey et al.²⁷ explored vaccination with recombinant *Listeria* secreting a vascular endothelial growth factor receptor-LLO fusion protein in the treatment of animal breast cancer models expressing HER-2/neu. Results demonstrated that some of the recombinant *Listeria* vaccinations targeting angiogenesis led to resolution of some tumors and, in those subset of resolved tumors, protection against a subsequent tumor reload.²⁷

With ongoing investigation, use of recombinant *Listeria* vaccinations in the treatment of breast cancer evolved to focus on human models.^{28,29} Seavey et al.²⁹ demonstrated that a *L. monocytogenes* hHer-2/neu chimera vaccine was able to inhibit or lead to regression of tumor formation, as well as limit metastatic disease, in murine models.²⁹ Shahabi et al.²⁸ subsequently published a study that showed a highly attenuated vaccine utilizing a new *Listeria* vector without antibiotic selection markers (ADXS31-164) maintained anti-tumor properties in a mouse

model with Her2/neu expressing cancer with potential for use in human malignancies.²⁸ Immunotherapy targeting Her2 remains in pre-clinical stages.³⁰ *Listeria* vaccination has also been used to explore other novel targets in breast cancer models.^{31,32}

The role of *Listeria monocytogenes* in cervical cancer therapy

Similar to the evolution of *Listeria* vaccination in breast cancer models, its role in cervical cancer has been thoroughly explored. In 2001, Gunn et al.³³ published a study assessing 2 recombinant *L. monocytogenes* strains as an immunotherapy targeting tumors bearing the HPV-16 E7 antigen. A comparison of a strain secreting *Lm*-E7 to a strain secreting *Lm*-LLO-E7 fusion protein demonstrated that only the strain secreting the fusion protein reduced tumor burden.³³ Lin et al.²³ then demonstrated that oral administration of recombinant *Listeria* secreting HPV 16 E7 can successfully slow tumor growth and reduce tumor burden in a mouse model.²³ Similar to the work completed in breast cancer models, Verch et al.³⁴ demonstrated that a strain of *Listeria* not encoding for an antibiotic resistance gene could maintain efficaciousness in tumor regression.³⁴ Given success in pre-clinical studies, interest emerged in investigating an intervention applicable to human cancer models.

ADXS as an immunotherapy for cervical cancer

Following pre-clinical outcomes, Advaxis, Inc. developed ADXS11-001 (ADXS-HPV).³⁵ ADXS11-001 is an immunotherapy based on live attenuated *Listeria monocytogenes* that secretes fusion protein *Lm*-LLO-E7 targeting HPV-associated tumors.³⁶

Table 2. Investigations of ADXS11-001 as anti-cancer therapy

Investigator	Cancer Model	Phase	Dosing Regimen	Enrollment (n)	Results
Maciag et al. ³⁸	Cervical	I	Dose escalation scheme (1×10^9 , 3.3×10^9 , 1×10^{10} CFU)	15	Dose limiting toxicity reached at 1×10^{10} CFU
GOG ³⁹	Cervical	II	1×10^9 CFU	67	Pending
Petit et al. ⁴⁵	Cervical	II	1×10^9 CFU	100	36% 12 month survival, 11% response rate (preliminary)
Jones et al. ^{46,47}	Oropharyngeal	I	Dose escalation scheme (3.3×10^8 , 1×10^9 , 3.3×10^9 CFU)	36	Pending
Safran et al. ^{48,49}	Anal	I/II	1×10^9 CFU	25	Pending

Genetic engineering was utilized to achieve an attenuated form of the bacterium that is deficient in PrfA, a transcription factor essential for the expression of the bacterium's virulence factors. Without functional PrfA, the bacterium is unable to function as an intracellular parasite.³⁷⁻³⁹ Advaxis, Inc., currently has clinical trials in various stages underway, including a Phase I trial for head and neck cancer in the United Kingdom, a Phase I/II trial for anal cancer in the United States and Phase II trials for cervical cancer in India and the United States (Table 2).³⁵

In 2009, Maciag et al.³⁸ published a Phase I clinical trial assessing the safety of ADXS11-001 in 15 subjects with advanced cervical cancer. Women ≥ 18 years old with advanced cervical cancer following failure of traditional therapies and meeting additional inclusion criteria were eligible for the study. Women with a history of listeriosis were excluded from the study. Pathologic confirmation of HPV 16 infection was only required for the first 6 subjects and was not used thereafter as an inclusion criteria.³⁸ Interestingly, previous studies have demonstrated that HPV 16 E7 has cross-reactivity with other HPV types and maintains the ability to trigger an immune response against varying HPV strains.⁴⁰⁻⁴² As such, it may not be entirely necessary to screen for HPV type prior to therapeutic vaccination.

A dose escalation scheme was then employed, administering escalating doses to subjects in groups of 5 on an every 21 day schedule for a total of 2 intravenous doses (1×10^9 CFU, 3.3×10^9 CFU, 1×10^{10} CFU). Dose limiting toxicity was reached at 1×10^{10} CFU with 3 of 5 subjects experiencing hemodynamic instability necessitating medical interventions. Given establishment of dose limiting toxicity, the dose escalation scheme was stopped prior to administration of 3.3×10^{10} CFU. Subjects were monitored as inpatients for 5 days following therapy, received prophylactic antibiotics and were followed as an outpatient after completion of their second dose as per the published protocol. Both safety and efficacy were monitored.³⁸

Adverse events were classified by the Common Terminology Criteria for Adverse Events version 3.0 without report of any grade 4 events associated with ADXS11-001.⁴³ All subjects enrolled in this Phase I clinical trial reported at least one adverse event (Table 3). Pyrexia was the most common reported event with some subjects experiencing associated changes in hemodynamics. This and other common side effects comprising a flu-like illness (Table 3) were felt to be related to the release of

chemokines and cytokines as a part of the innate immune response triggered in response to vaccination. Of the reported grade 3 events in 9 subjects, 6 were associated with administration of ADXS11-001 (pyrexia, fatigue and elevation in gamma-glutamyltransferase levels). Laboratory assessments revealed alterations in liver enzyme and complete blood count panels, but these alterations were felt to lack clinical significance or to lack association with administration of ADXS11-001. Microbial surveillance of blood, urine and feces yielded only one subject (1×10^9 cohort) with blood cultures transiently positive for *Lm*-LLO-E7. Two subjects died for reasons characterized as unrelated to the investigational agent within the study follow up period. One death was attributed to disease progression (study day 87), while the other occurred in the setting of renal failure complicated by metabolic acidosis and cardiac arrest (study day 36). An additional 9 deaths occurred outside of the initial study period with a median survival of 347 days for all 11 deceased subjects.³⁸

While this Phase I clinical trial was implemented to assess vaccine safety, its authors also make preliminary comments on efficacy, as assessed through this study protocol. Mixed responses were observed in 13 of the 15 subjects included in this portion of the analysis. One subject was reported to have demonstrated a possible partial response, but no confirmatory imaging study was performed following her potential response. Of the remaining 12 subjects, stable disease was noted in 7 while 5 were found to have progression of disease.³⁸

Given the acceptable safety profile of *Lm*-LLO-E7 at 1×10^9 CFU, a Phase II clinical trial is currently being conducted by the Gynecologic Oncology Group (GOG) for patients with advanced, metastatic, or recurrent cervical cancer that has failed prior systemic chemotherapy. The primary endpoints of this single-arm study include safety and tolerability of the agent, as well as 12 month survival. Secondary endpoints include progression-free and overall survival, as well as objective response rate. Under this study protocol, 3 doses of ADXS11-001 1×10^9 CFU will be administered every 28 days. Non-steroidal anti-inflammatory agents will be given peri-administration as prophylaxis against the common adverse events reported in the Phase I trial (fever, chills, headache, myalgia). A total enrollment of 67 subjects is expected.^{39,44} Additional investigations of ADXS11-001 are ongoing, including a Phase II trial in India involving randomization of 110

Table 3. Most frequent adverse events in Phase I trial assessing safety of ADXS11-011³⁸

Adverse Event (AE)	Reported percentage (%)	Adverse Event (AE)	Reported percentage (%)
Pyrexia	100	Chills	53.3 each
Vomiting	60	Headache	
Nausea	46.7 each	Anemia	
Tachycardia		MS* pain	56.7

* Musculoskeletal

subjects with recurrent cervical cancer to use of AXS11-001 with or without cisplatin.^{19,45}

ADXS for other HPV-related cancers

In addition to the work in cervical cancer, investigation of ADXS11-001 in other HPV related cancers is ongoing. A Phase I study investigating the use of ADXS11-001 in the treatment of oropharyngeal cancer (OPSCC) is currently underway. This study, with an anticipated conclusion in October 2014, aims to assess the safety of ADXS11-001 in eliciting an immune response to OPSCC associated with HPV infection.^{46,47} Initiated in April 2013, there is also an ongoing Phase I/II clinical trial exploring the potential role of ADXS11-001 as an adjunct to chemoradiation in the treatment of anal cancer. Goal enrollment for this study is 25 subjects with 2 treatment schedules built into the protocol to carefully monitor for the use of this immunotherapeutic in subjects receiving potential myelosuppressive therapy.^{48,49}

Conclusion

Cervical cancer is a malignancy with a well-known causal relationship to infection with HPV. Prophylactic HPV

immunizations are a promising mechanism by which to prevent infection, but uptake of these vaccines remains suboptimal.¹³ *Listeria monocytogenes* serves as an ideal model for delivery of fusion proteins to patients given the robust immune response it elicits¹⁹ and has been well studied in this capacity. Engineered to deliver a fusion protein eliciting an immune response against the HPV 16 E7 protein commonly found in cervical carcinomas, ADXS11-001 serves as a potential therapeutic for advanced cervical cancer, as well as other HPV-related cancer models. History of listeriosis was an exclusion criterion in the Phase I study investigating ADXS11-001 in cervical cancer, but is not currently being used as an exclusion criterion for the other ongoing Phase I and II clinical studies.^{38,46-49} It is possible that prior *Listeria* infection may impact the efficacy of this therapeutic vaccine, but data analysis and outcomes from these ongoing studies will need to be explored before making additional conclusions.¹⁴ This potential therapeutic requires ongoing investigation with outcomes of a Phase II clinical trial by GOG pending.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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