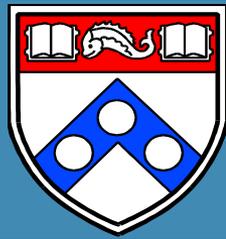


Listeria monocytogenes
Developing A Perfect Vector for
Cancer Immunotherapy

Dr. Yvonne Paterson, Ph.D.

Professor of Microbiology, Perelman School of Medicine &
Associate Dean for Research and Professor at the
University of Pennsylvania School of Nursing

Four Essential Elements of Cancer Immunotherapy



1



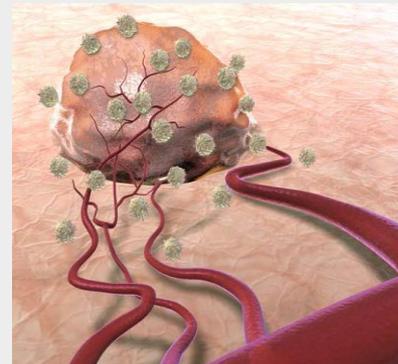
Access Antigen Presenting Cells (APC) to direct and target the immune response

2



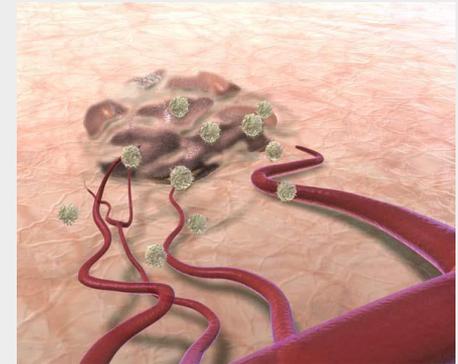
Generate Specific cytotoxic T cell response against tumor antigens

3



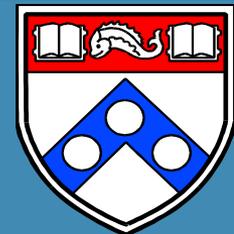
Get past checkpoint inhibitors and negative regulators of cellular immunity

4

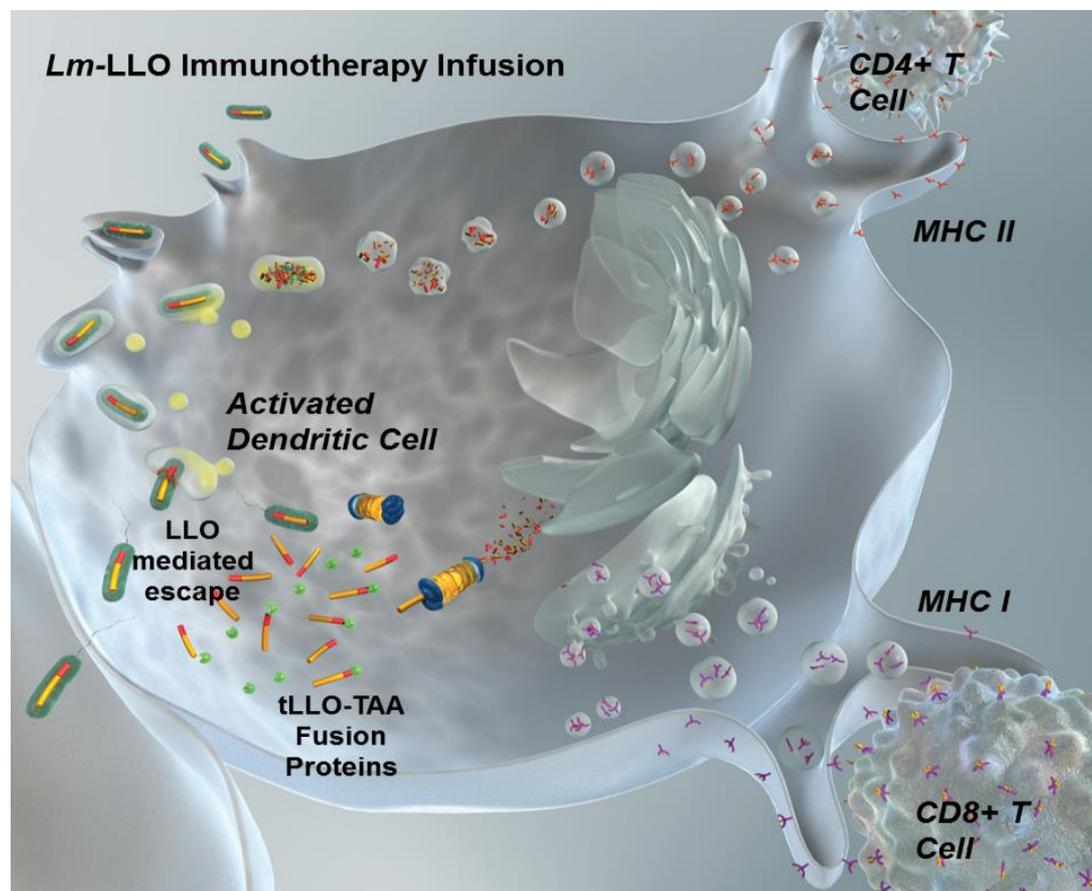


Change the tumor microenvironment (TME) to disarm immune tolerance, neutralize Tregs and myeloid-derived suppressor cells (MDSC)

* Data published in *Journal for Immunotherapy of Cancer* 2013, 1(Suppl 1):O18 doi:10.1186/2051-1426-1-S1-O18

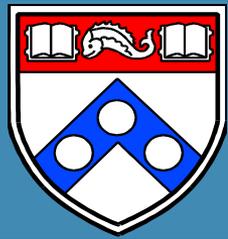


Unique Life Cycle of *Lm* in APC



- Live Vector Accesses APC
- TAA-Fusion Peptide Secreted
- Triggers Innate and Adaptive Pathogen Immune Response
- Tumors Now “Seen” as Pathogen-Infected and Targeted By T-Cells

Why This Vector? Ideal System for a Cellular Immune Response



Powerful Innate Immunity

- **Live Vector serves as multiple adjuvants**
- Expresses multiple PAMPs
- Activates external and internal TLRs and NOD-like proteins
 - (TLRs 1, 2, 5, 6, NOD-1, NOD-2, CpG, LLO is a PAMP)
- Creates TH-1 “Immunotype”

Access to APCs (circulating and tissue-based)

- **Facilitated phagocytosis by dendritic cells and APCs**
- Escapes phagolysosome via LLO
- Replicates and secretes gene products within cytoplasm of APC
- Bridges innate and adaptive immunity

Advaxis Constructs Secrete Fusion Protein: tLLO-TAA within APC

- **“Programs” APCs *in situ* within each patient**

Can be administered repeatedly; no neutralizing antibodies

Adaptive Immunity

- **Cross presents to MHC I and II pathways**
- Matures and activates dendritic cells
- Drives CTL-focused immune response in context of “perceived” listeriosis.
- Reveals “hidden” CTL epitopes
- Induces PD-1 / PD-L1 expression

Changes Tumor Microenvironment

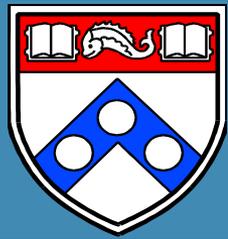
- **Specifically breaks tolerance within tumors**
- Chemokines facilitate infiltration of CD4+ and CD8+ T cells and MDSCs
- Reduces and disables Tregs, reduces and disables MDSCs
- Antigen spreading observed

Directly invades tumors

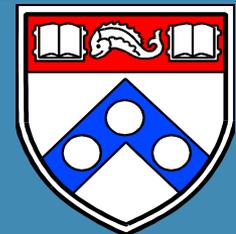
- Redirects *Listeria* specific killing
- Directly kills tumor cells by apoptosis

Vector can be cleared with antibiotics

Developing the Perfect *Lm* Vector from Over Twenty Years of Experience



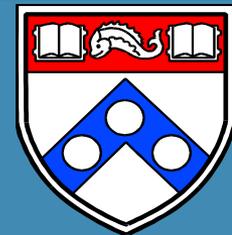
- The first paper that showed that *Lm* could be used in cancer immunotherapy was published in 1995
 - Pan, Z-K, Ikonomidis, G., Lazenby, A., Pardoll, D., and **Y. Paterson**. A recombinant *Listeria monocytogenes* vaccine expressing a model tumour antigen protects mice against lethal tumour challenge and causes regression of established tumours. *Nature Medicine* 1:471-477. 1995
- The first patent was filed November 8, 1994, protecting the use of *Lm* for cancer immunotherapy
 - United States Patent Number: 7,135,188. Inventor; Yvonne Paterson “Methods and compositions for immunotherapy of cancer”
 - Advaxis intellectual property portfolio now includes over 50 patents filed and 70 patents pending



Developing the Perfect *Lm* Vector: Step 1 – Choice of Host Strain

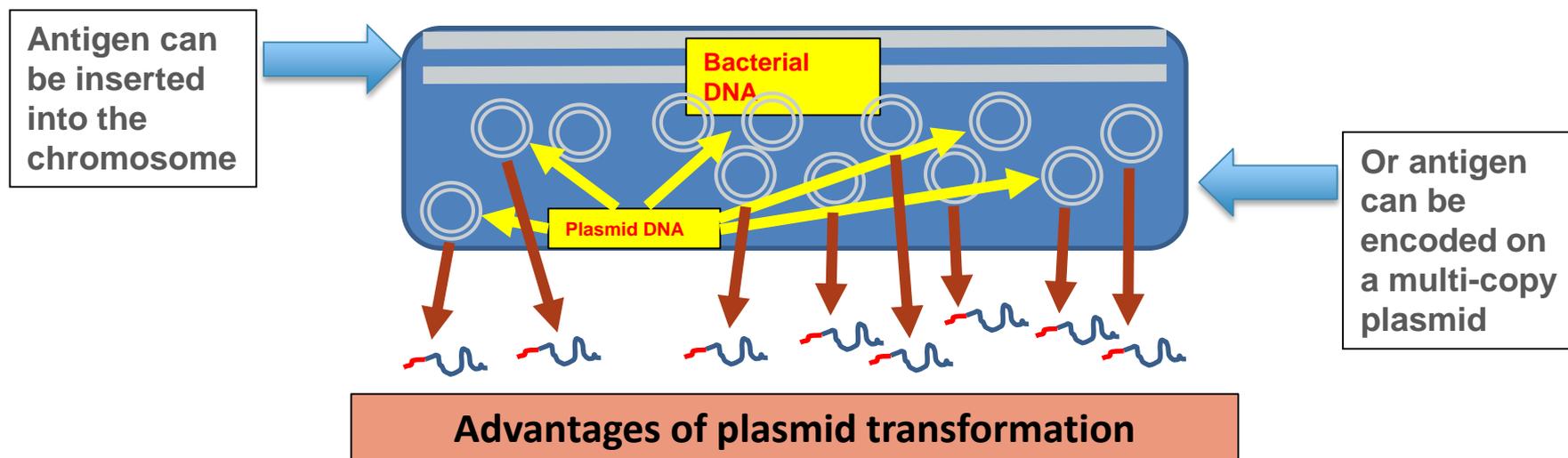
Attenuate the wild-type strain:

- Natural attenuation due to metabolic load placed on the bacterium. This is much greater for multi-copy plasmid expression systems compared to single-gene expressing strains. Metabolic load reduces virulence by one or two logs.
- To design for added safety delete a virulence factor such as ActA and/or InIB. Disadvantage of deleting InIB is that *Lm* cannot invade non-phagocytic cells such as tumor cells (no direct killing). ActA deletion further attenuates by two logs.
- Advaxis vectors have an LD₅₀ of 1×10^8 - 1×10^9 cfu. LD₅₀ of ActA⁻InIB⁻ vectors is 1×10^7 cfu. Higher LD₅₀ allows shorter infusion times, lower AE grades and side effects.
- Mean tolerated dose of Advaxis vector ADXS-HPV is 3.3×10^9 cfu. Compared to 1×10^9 cfu for ActA⁻InIB⁻ vectors that have been used clinically.



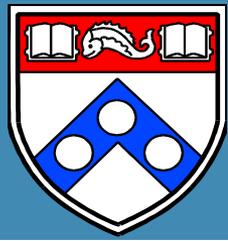
Developing the Perfect *Lm* Vector: Step 2 – Method of Transformation

Lm functions as a vector system that delivers and expresses tumor antigens



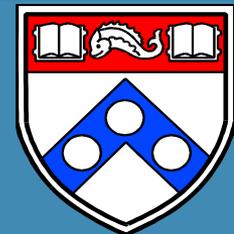
- Multiple plasmid copies per live attenuated *Lm*, therefore more tumor antigen presented
- More LLO PAMP reconditions the tumor microenvironment (lower levels of Tregs and MDSCs). Thus does not require prior deletion with cyclophosphamide.
- Using plasmid complementation, the newest Advaxis platform is antibiotic resistance free

Developing the Perfect *Lm* Vector: Step 3 – Choice of Promoter to Drive Antigen Expression



Promoter usage: Both the ActA promoter and the LLO promoter have been used in *Lm* vectors.

- The disadvantage of the ActA promoter is that it is only active in the cytosol of the cell thus does not turn on protein production until escape from the cytosol.
- Advaxis vectors use the LLO promoter, which has two advantages.
 - First, it has a perfect PrfA binding site and binds the pluripotential transcription factor PrfA more tightly with enhanced transcription
 - Secondly, it is expressed in the phagolysosome
 - Thus when *Lm* escapes it also releases antigen made in that compartment and allows antigen presentation to occur earlier in infection



Developing the Perfect *Lm* Vector: Step 4 – Choice of Fusion Protein

Both LLO and ActA are used as fusion partners in *Lm*

- In our preclinical research, both LLO and ActA truncated to about 400 residues, have been equally effective
- Shorter versions of LLO have been less effective but better than no fusion protein
- We found that the first 200 residues of ActA were required for good cytotoxic T lymphocyte (CTL) induction

Fusing LLO to E7 improves efficacy when delivered by other vectors, in addition to *Lm*

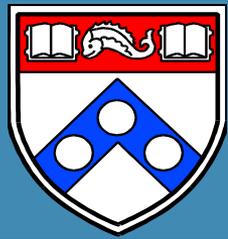
VACCINE	% MICE TUMOR FREE
Lm-LLO-E7	70%-100%
Lm-E7	0%
Vac-LLO-E7	50%
Vac-E7	0%
LLO-E7-DNA*	50%
E7-DNA*	12%

* Immunization started on day 3

LLO selected as our fusion protein

Developing the Perfect *Lm* Vector

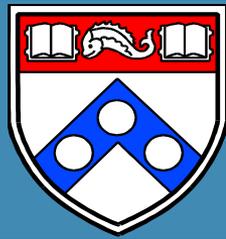
Step 5 – Choice of Tumor Antigen



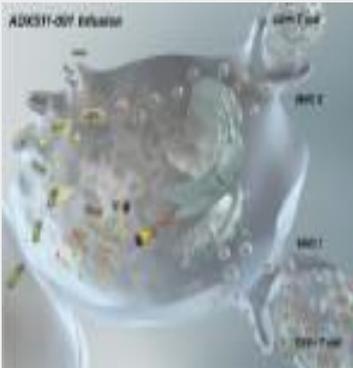
The ideal tumor antigen should have the following properties:

- Over expressed or better still uniquely expressed on tumors versus normal tissue, e.g. HPV-16 E7
- No homology to self proteins - this is only going to be the case for viral oncogenes – or little homology
- Contributes to the tumor phenotype such that the tumor cannot lose expression of that antigen under immune pressure. (Which it may, for example melanomas become amelanotic in response to immunotherapy that targets the melanin synthesis pathway). HER-2/neu and HPV-16 E7 both conform to this requirement.

Four Essential Elements of Cancer Immunotherapy



1



Access Antigen Presenting Cells (APC) to direct and target the immune response

Advaxis immunotherapies preferentially infect the APCs and escape into the cytoplasm to secrete antigens for the targeted tumor

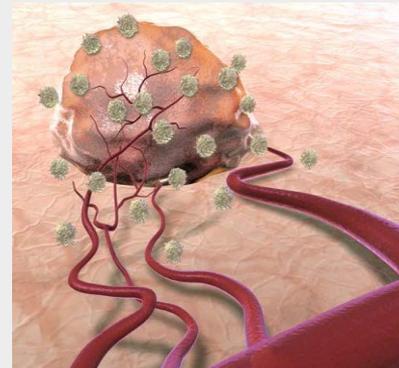
2



Generate Specific cytotoxic T cell response against tumor antigens

Advaxis immunotherapies generate a strong T cell response to clear Listeria that is redirected to the tumor via the secreted antigens

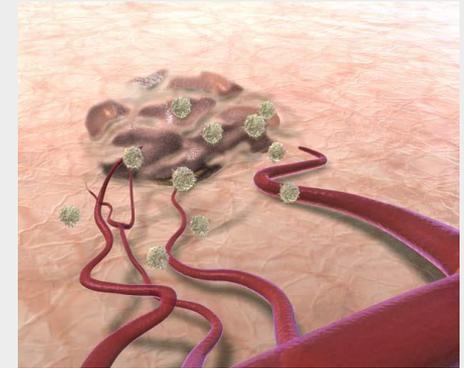
3



Get past checkpoint inhibitors and negative regulators of cellular immunity

Acute “perceived” listeriosis stimulates a maximum immune response that bypasses immune checkpoints

4

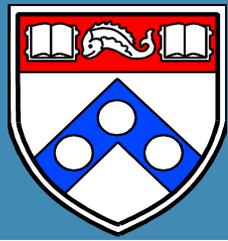


Change the TME to disarm immune tolerance, neutralize Tregs and MDSC*

Advaxis immunotherapies generate TAA-specific T cells and decrease the number and function of Tregs and MDSCs in the tumors, enabling TAA-specific T cells to infiltrate and kill tumors

* Data published in *Journal for Immunotherapy of Cancer* 2013, 1(Suppl 1):O18 doi:10.1186/2051-1426-1-S1-O18

Advaxis *Lm*-LLO Immunotherapies: Moving From the Benchtop into the Clinic...



- *Lm*-LLO immunotherapies are attenuated and non-pathogenic
- *Lm*-LLO immunotherapies act as their own adjuvant; no additional adjuvants required for activity
- *Lm*-LLO vectors reduce immunosuppression mediated by Tregs and MDSCs within the tumor microenvironment
- *Lm*-LLO immunotherapy has single agent activity in clinical trials
- *Lm*-LLO immunotherapy has the potential for prolonged survival and objective tumor responses, including complete responses
- *Lm*-LLO immunotherapy has the potential to be combined with other active treatments like checkpoint modulators