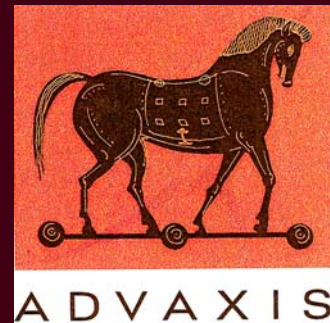


LM-LLO-E7, a Novel Vaccine for the Treatment of Cervical Cancer

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Our Team

- Yvonne Paterson, PhD, Scientific Founder; Professor of Microbiology at University of Pennsylvania
- Thorsten Verch, PhD, Senior Scientist; University of Pennsylvania
- Bennett Lorber, MD, Scientific Advisor; Professor of Medicine and Chief, Section of Infectious Diseases, Temple University

Today's Presentation

- Description of our technology
- Data in animal models
- Summary of our proposed clinical trial

Technology-Concept

1. Clinical cancer is a failure of the immune system to recognize tumor-associated antigens and eliminate neoplastic cells.
2. Cell-mediated immunity is the key to the elimination of solid tumors.
3. *Listeria monocytogenes* is an excellent vector to deliver a specific tumor antigen in a way that will stimulate a cell-mediated response.

Why *Listeria monocytogenes*?

Why is *Listeria monocytogenes* a food safety concern?

Why is *Listeria monocytogenes* a public health concern?

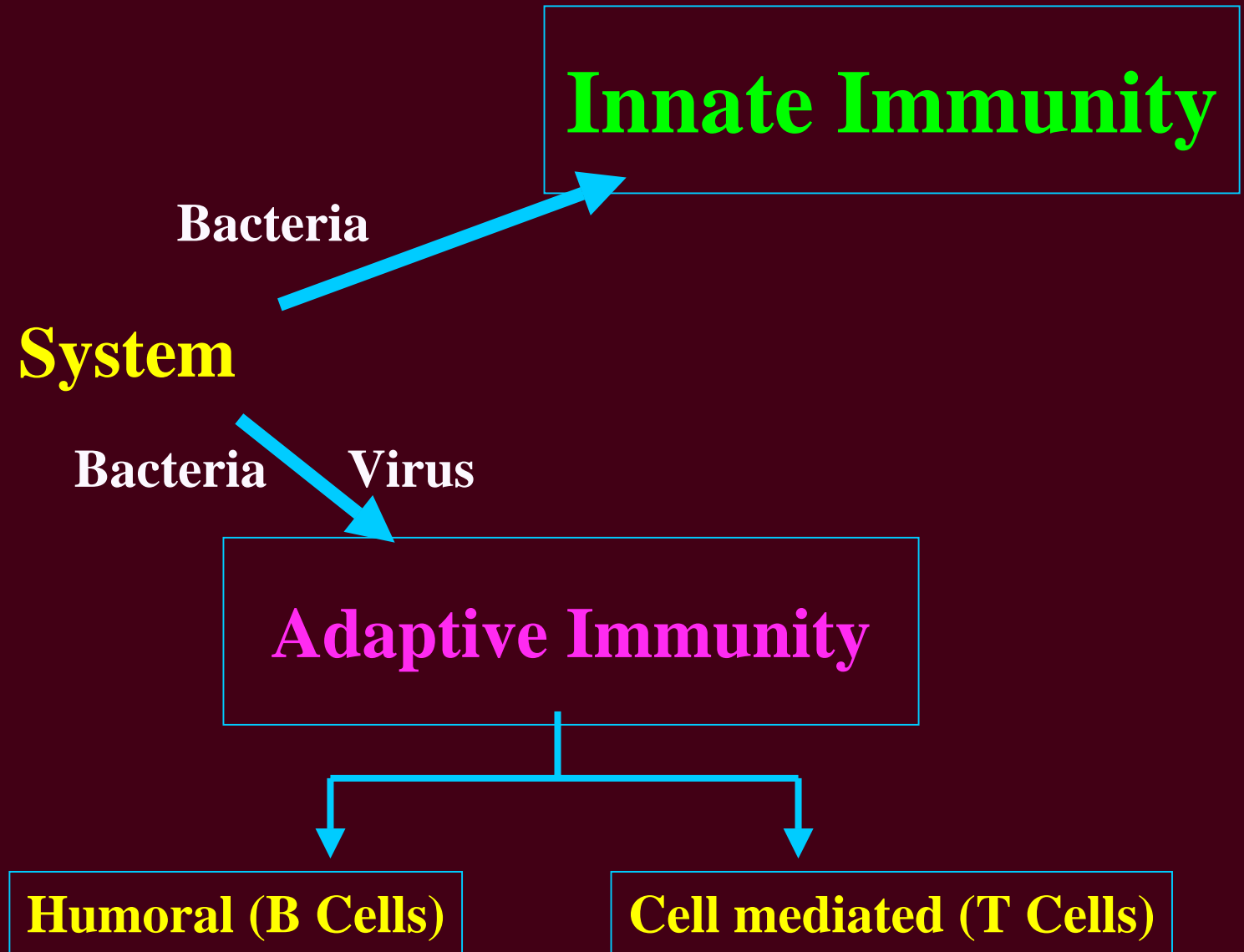
Why is *Listeria monocytogenes* a food safety concern?

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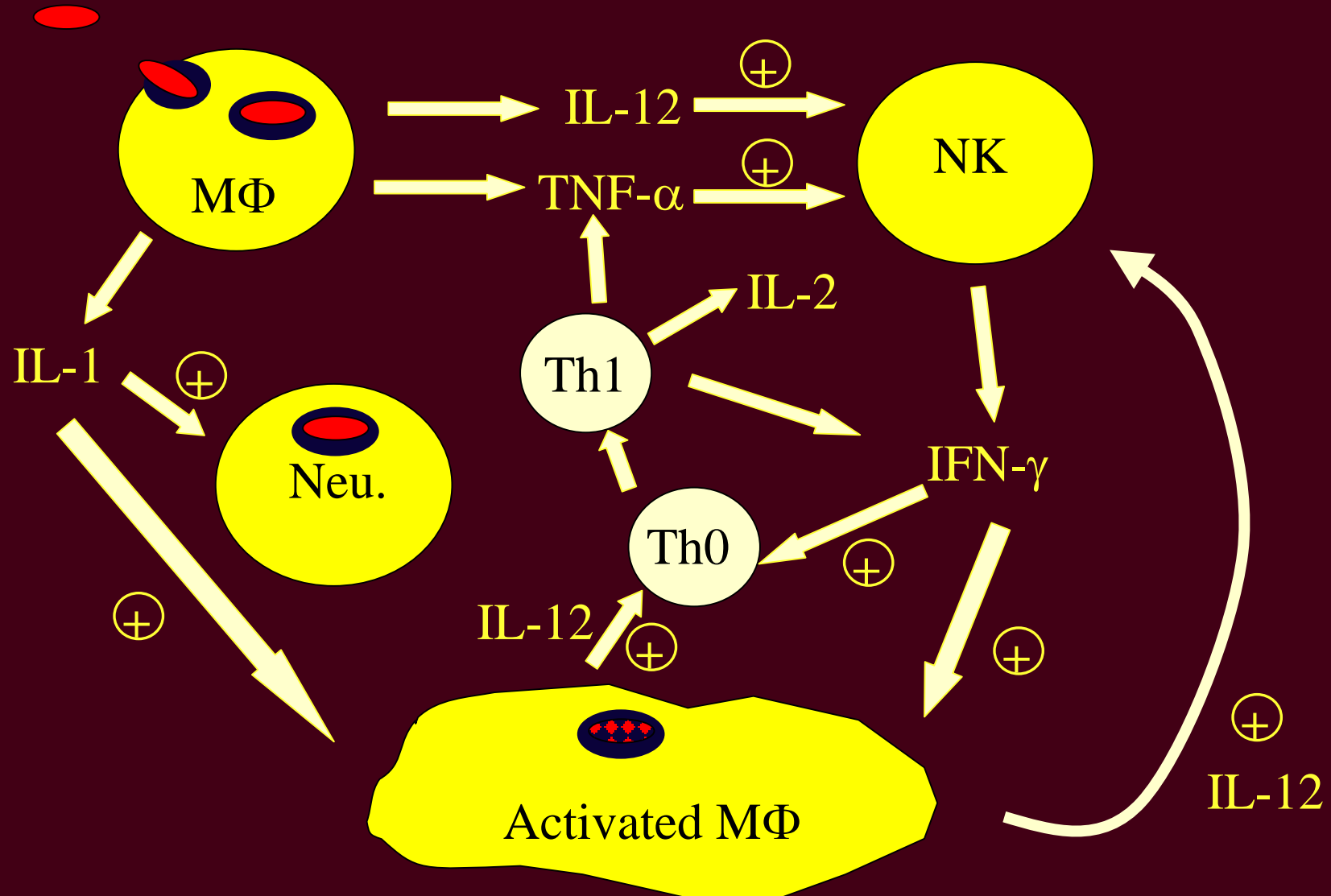
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Why is *Listeria monocytogenes* a public health concern?

Immune System



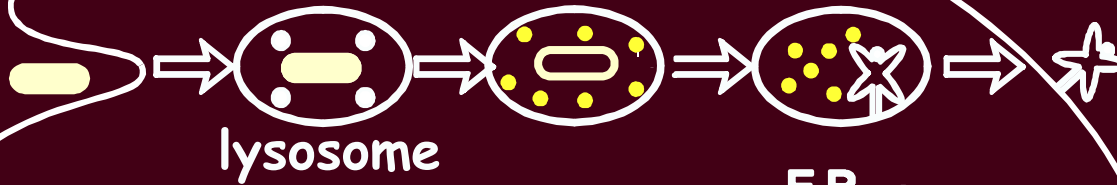
Bacteria induce an inflammatory cytokine cascade early after infection



Life Cycle of *Listeria monocytogenes*

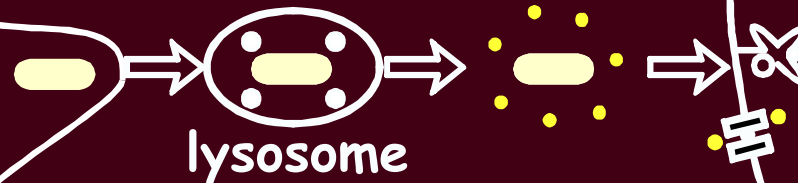
Antigen Processing Cell

Listeria,
Salmonella,
BCG etc



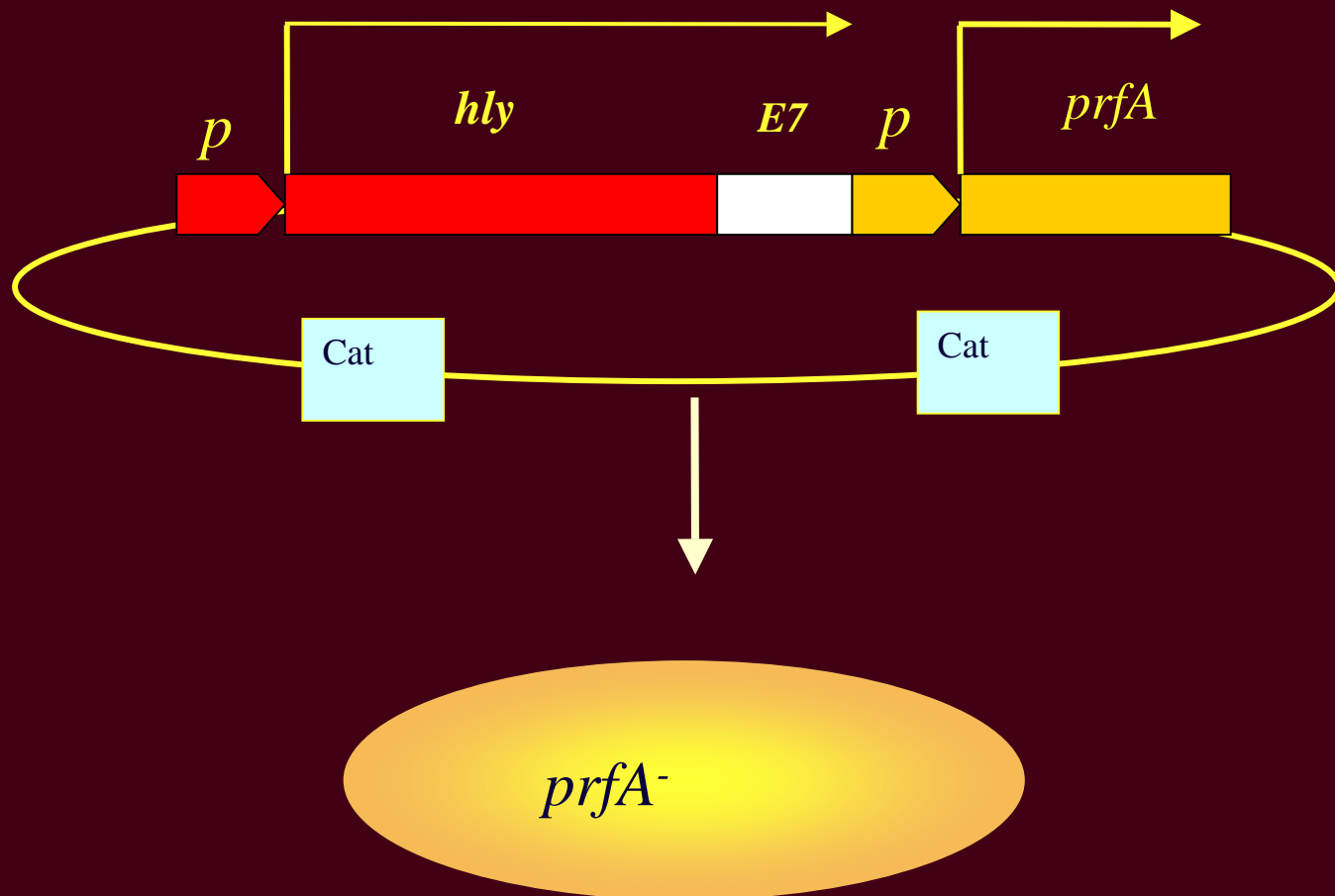
CD4
T-cell

Listeria



CD8
T-cell

LM-LLO-E7 Expression System



Listeria monocytogenes as a Delivery Vector

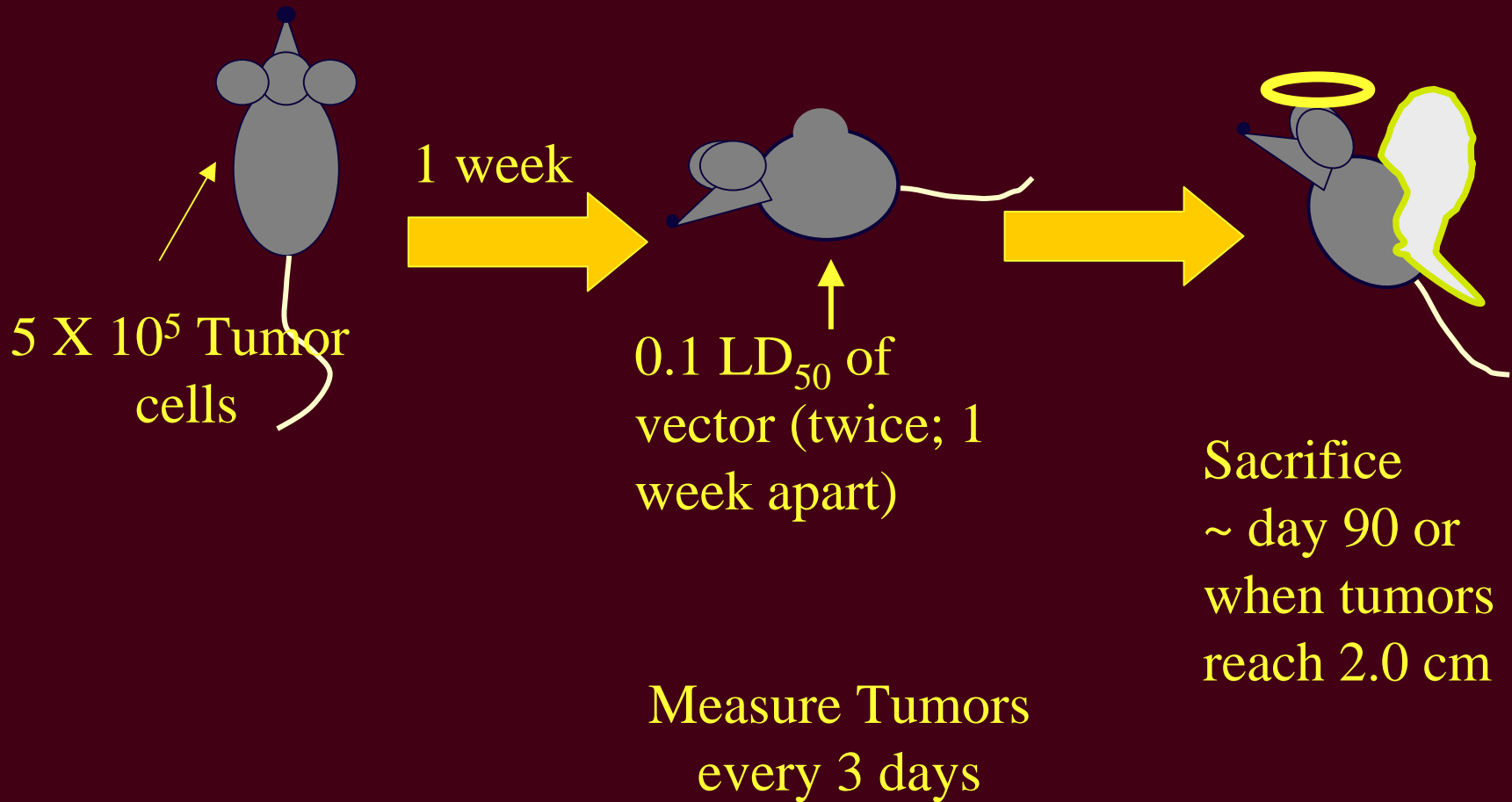
- Turns on innate immunity
- Generates powerful cell-mediated immunity
- Readily engineered to produce antigens
- No gram-negative endotoxins
- Sensitive to antibiotics
- Stable vaccine that may be stored frozen
- Can be grown on completely synthetic media

A Platform Technology

- LM-LLO-E7 is our first application
- This technology can be adapted to other antigens
- Potential value in other cancers and infectious disease

Mouse Data

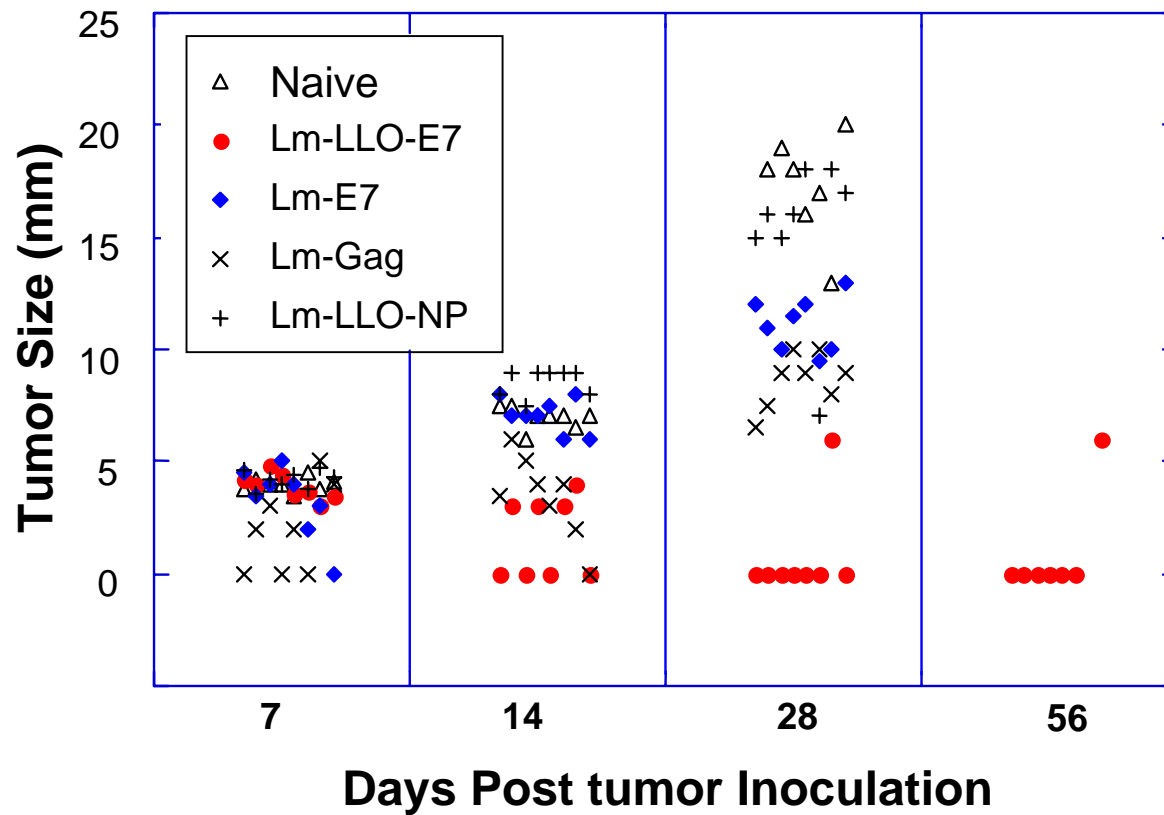
Tumor Regression Study



TC-1 tumor

- C57Bl/6 derived epithelial tumor
- Immortalized with HPV-16 E6 and E7
- Transformed with oncogenic *ras*
- Expresses E6 and E7 constitutively at low levels similar to human cervical tumors
- Grows rapidly in syngeneic mice compared other HPV-immortalized tumors
- Used extensively to test therapeutics for HPV transformed tumors.

LM-LLO-E7 induces regression of established TC-1 tumors



Clinical Protocol

- We have a vector that generates significant anti-tumor activity in animal models of cervical cancer.
- Next step is human clinical testing.

Human Papillomavirus and Cancer

- > 95% of cervical cancers are associated with HPV
- 2nd leading cause of cancer death for women world-wide
- About 5,000 deaths p.a. of cervical cancer in the USA
- HPV strains 16 and 18 most commonly linked to cancer
- HPV E6 and E7 gene products immortalize cells
- E6 and E7 are constitutively expressed in HPV-associated tumors
- 25% head & neck cancers express HPV antigens; may also be expressed in lung, prostate and other tumors.

Protocol-Outline

- Give an attenuated vector to patients with advanced cervical cancer under inpatient hospital evaluation.
- Monitor for adverse events, treat promptly as indicated.
- Incrementally increase dose as tolerated.
- Purpose is to determine the safety and immunogenicity of the vector in a patient population.

Patient Population

- Adult women with stage III or stage IV disease who have failed standard therapy
- Must be HPV-16 positive
- Must be 4 weeks or more out from last chemotherapy or radiation
- Exclusions: steroid use, active brain mets, valvular disease, bone marrow or renal insufficiency, anergy, pregnancy, severe debilitation, HIV

Clinical Protocol Summary

- 20 patients in 4 dose cohorts
- Dose escalation based on tolerance in lower cohort
- Day 1: admit to hospital, vaccination
- Day 5: 1 dose IV ampicillin and discharge on 10 days of oral ampicillin
- Return on Day 21 for second dose, repeat above

Clinical Protocol Endpoints

- Safety
- Immunogenicity
- Clinical response

Is it safe to give *Listeria* to Cancer Patients?

- Exclusion of many high risk people
- Initial dose is equal to the lowest dose used in the rhesus study
- Attenuation: engineered bacteria has LD₅₀ in mice about 10^3 times wild-type
- Patients will be monitored in hospital
- The bacteria is sensitive to ampicillin

Last Slide

1. Plasmid Transfer

Plasmids are small, circular, self-replicating DNA molecules that can be transferred between bacterial cells.

Plasmids can carry genes that confer antibiotic resistance, virulence, or other traits that can be passed on to other cells.

Plasmid transfer can occur through direct contact between cells (conjugation) or through the release of plasmids into the environment (transformation).

Plasmid transfer is a key mechanism for the spread of antibiotic resistance and other traits among bacterial populations.

Understanding plasmid transfer is important for developing strategies to control the spread of antibiotic resistance and other harmful traits.

Plasmid transfer is a complex process that involves many factors, including the presence of specific proteins and the state of the recipient cell.

Research on plasmid transfer is ongoing, and new insights are being gained into the mechanisms of this important process.

Plasmid transfer is a key area of research in microbiology and has important implications for public health and the environment.

Methods

- Mix LM-LLO-E7 with E. coli in vitro
- Grow in a high concentration chloramphenicol media
- Every two days, sample the mixture and culture on media specific for E. coli with chloramphenicol resistance

Results

Day	Results
1	no growth
3	no growth
5	no growth
7	no growth
9	no growth
11	E. coli

Conclusions

- Unrealistically high concentrations of bacteria (iv route)
- Full chloramphenicol pressure
- Still took over 9 days to push plasmid into E. coli (antibiotics at day 5)

2. Route of Administration

• The route of administration is the way a drug is given to a patient.

• The route of administration affects the drug's absorption, distribution, and elimination.

• The route of administration also affects the drug's toxicity.

• The route of administration is chosen based on the drug's properties and the patient's condition.

• The route of administration is also chosen based on the drug's cost and availability.

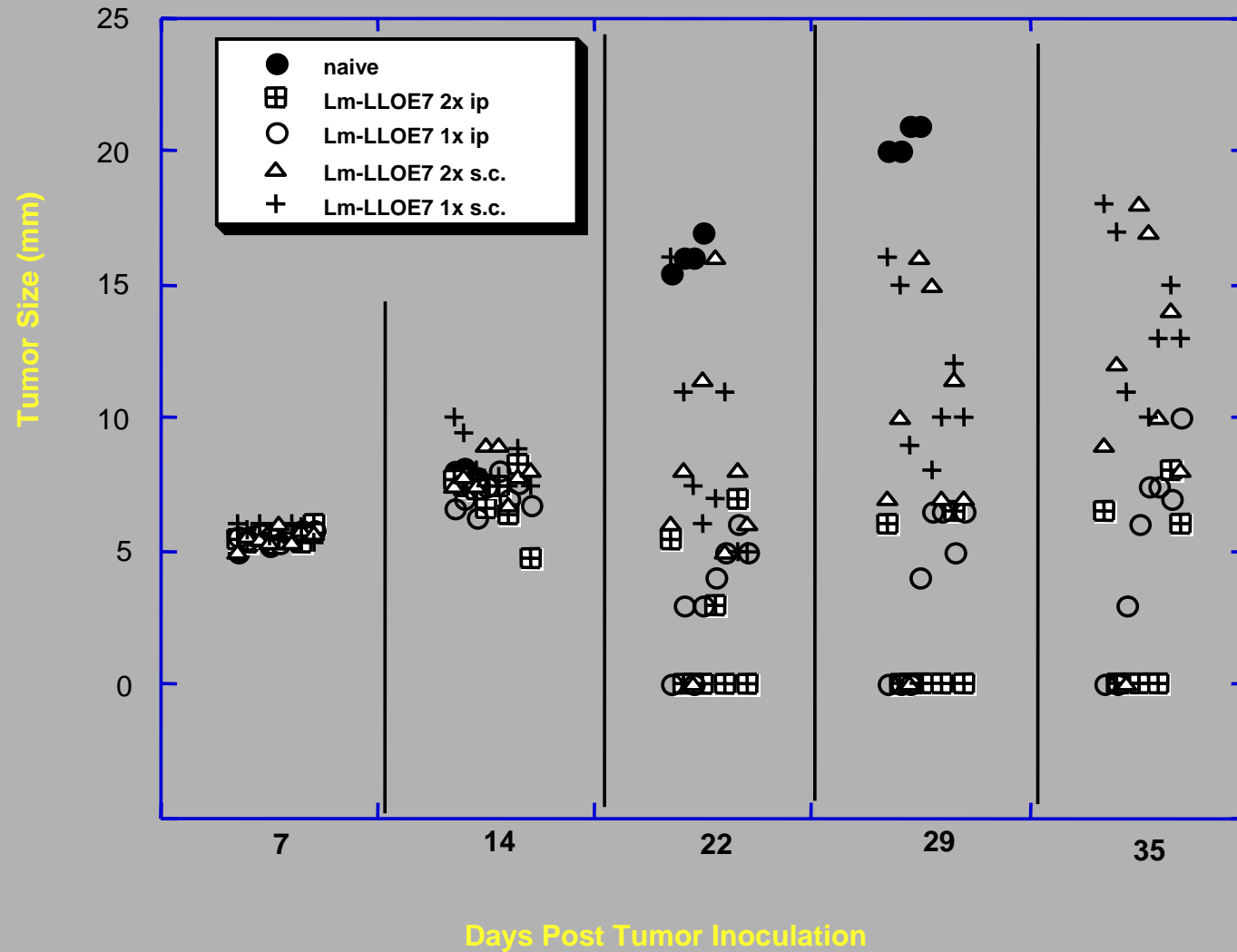
• The route of administration is also chosen based on the patient's preferences.

• The route of administration is also chosen based on the drug's stability.

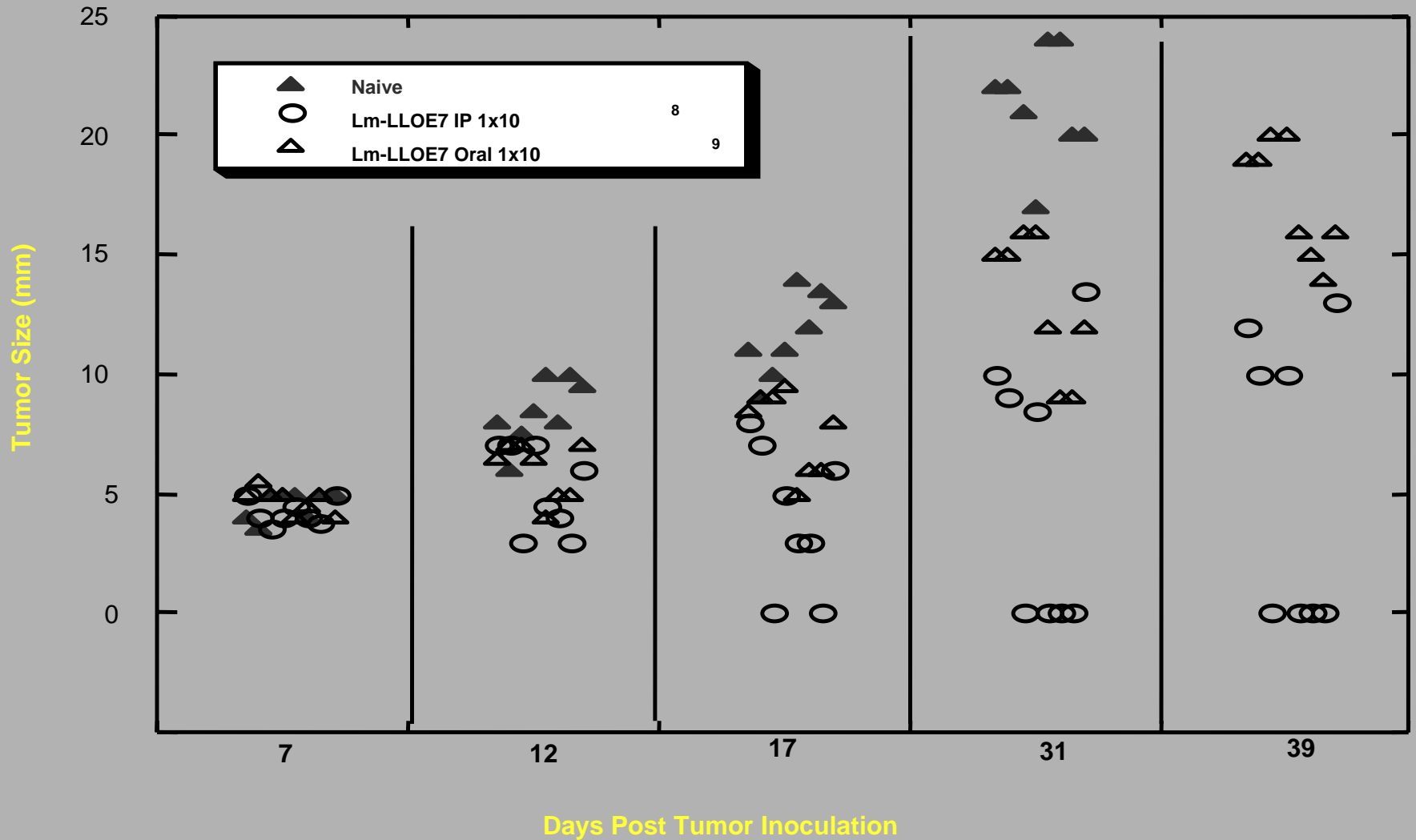
• The route of administration is also chosen based on the drug's shelf life.

• The route of administration is also chosen based on the drug's packaging.

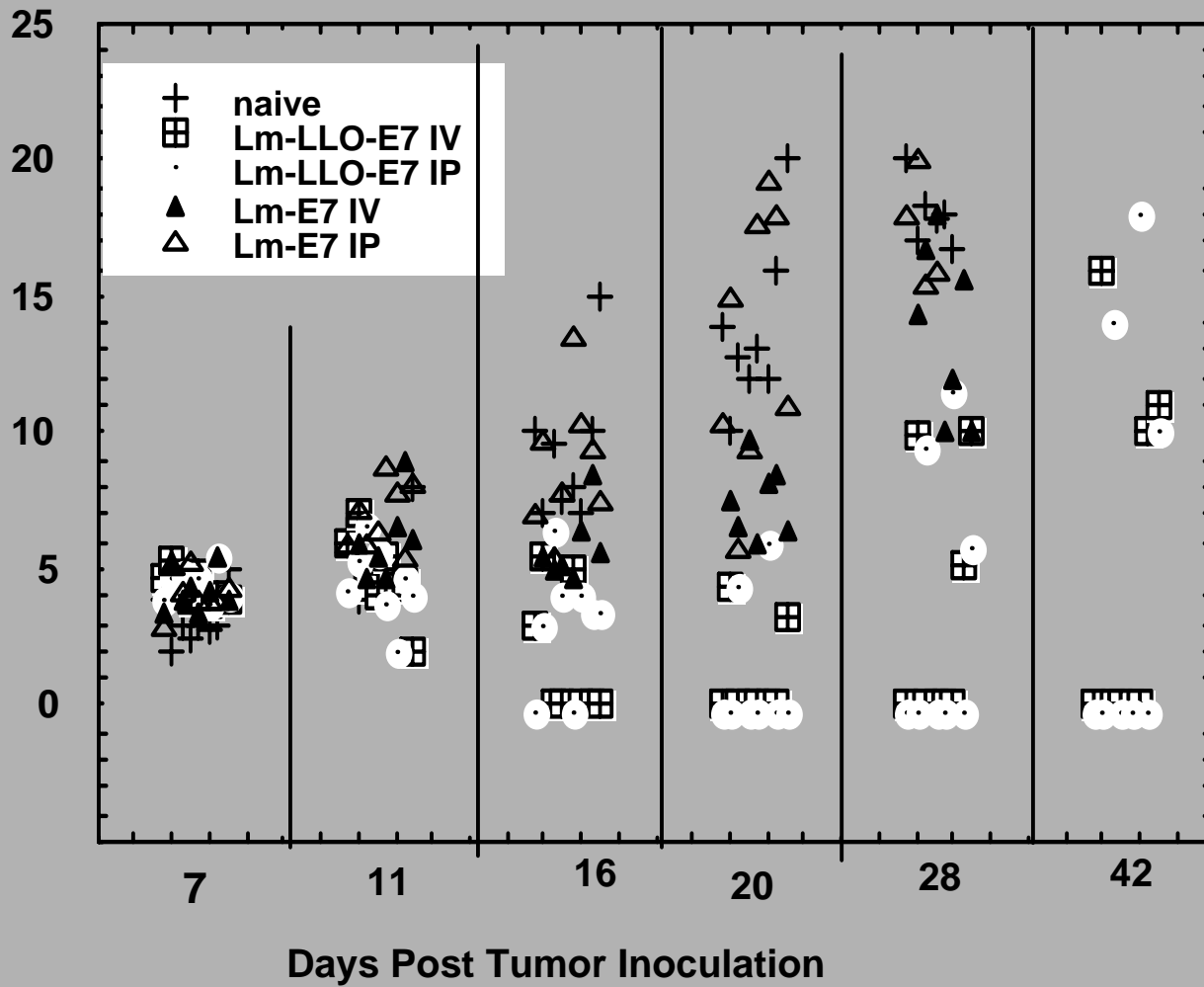
IP compared to SC administration



IP compared to oral administration

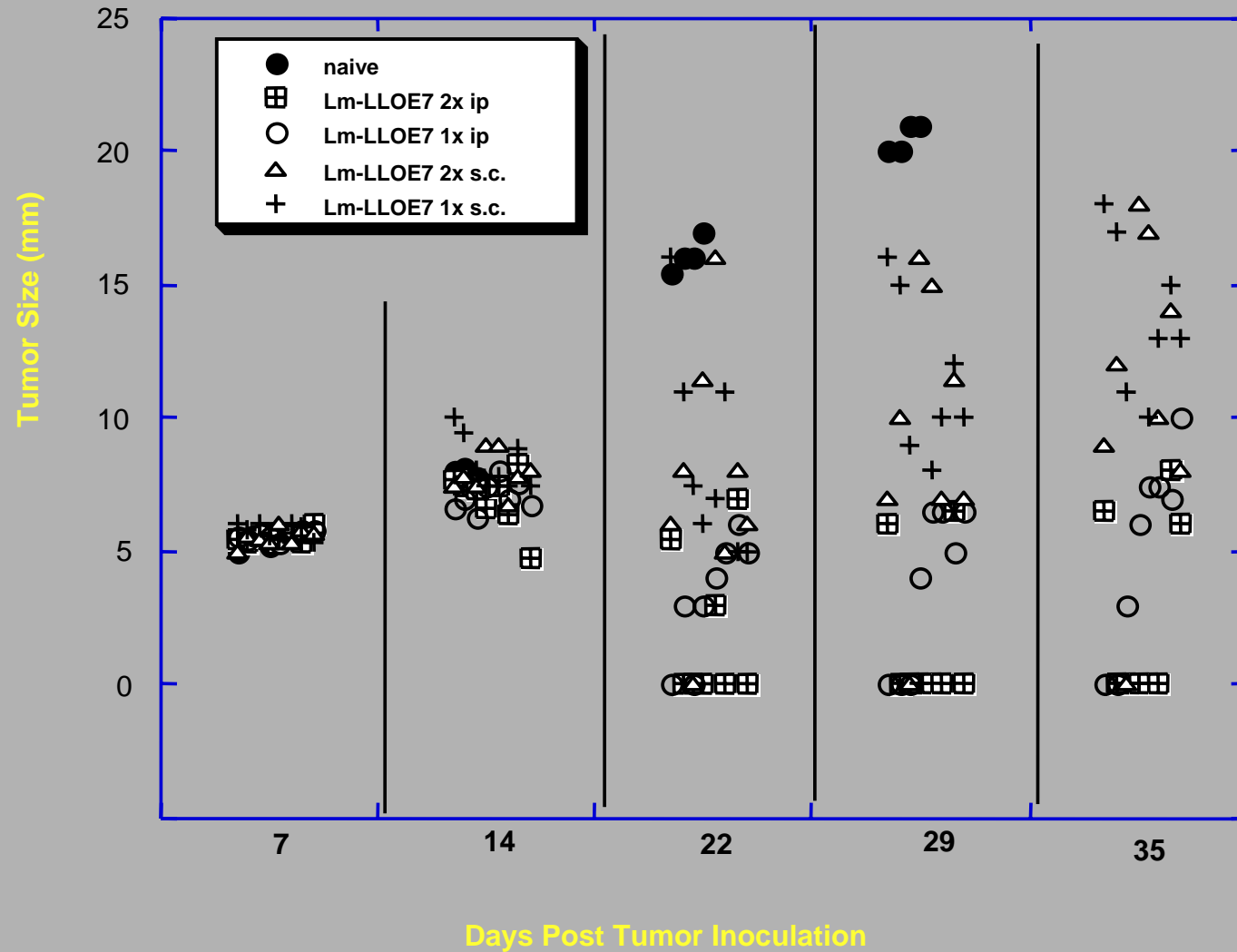


Comparison of I.V. versus I.P. immunization of Listeria based E7 vaccines



3. Dose Schedule

One vs Two doses of LM-LLO-E7



4. Planned Studies

• **Prevalence** of disease in a community

• **Incidence** of disease in a community

• **Prognosis** of disease in a community

• **Etiology** of disease in a community

• **Control** of disease in a community

• **Health status** of a community

• **Health care** in a community

• **Health policy** in a community

• **Health economics** in a community

• **Health law** in a community

• **Health ethics** in a community

Biodistribution in Mice

- Dose route: iv and sc
- Check a cohort at days 2, 10, 20, 90
- Check blood, liver, spleen, brain, nodes, kidney, intestine, ovaries, injection site, bone marrow
- Use PCR to look for plasmid DNA

Acute Dose Toxicity

- Determine MTD in wild-type *Listeria* and LM-LLO-E7
- Compare iv and sc

Longer Term Toxicity in Mice

- 3 dose levels each for iv and sc
- Dose on days 1, 8, 15, and 22
- Monitor response and sacrifice on day 23 with full necropsy

Monkey dosing study

- Cynomolgus monkeys will receive LM-LL0-E7 intravenously
- Dose up to 10^{12} cfu iv
- Follow clinically and with blood tests

5. Dose Levels

• Dose levels are the amount of a drug or treatment administered to a patient.

• Dose levels are typically expressed in milligrams (mg) or grams (g).

• Dose levels are determined by the patient's weight, age, and medical condition.

• Dose levels are typically determined by a healthcare provider.

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Dose

- Starting dose 10^9 cfu i.v. with LM-LO-E7
- 10^{10} LM-SIV i.v. tolerated in rhesus macaques with vector 2 logs more virulent
- Human study: 10^9 tolerated p.o. with vector 1.5 logs more virulent
- Further studies planned

6. Misc

Karnofsky Scale

- 100 Normal, no complaints, no evidence of disease
- 90 Able to carry on normal activity: minor symptoms of disease
- 80 Normal activity with effort: some symptoms of disease
- 70 Cares for self: unable to carry on normal activity or active work
- 60 Requires occasional assistance but is able to care for needs
- 50 Requires considerable assistance and frequent medical care
- 40 Disabled: requires special care and assistance
- 30 Severely disabled: hospitalization is indicated, death not imminent
- 20 Very sick, hospitalization necessary: active treatment necessary
- 10 Moribund, fatal processes progressing rapidly