

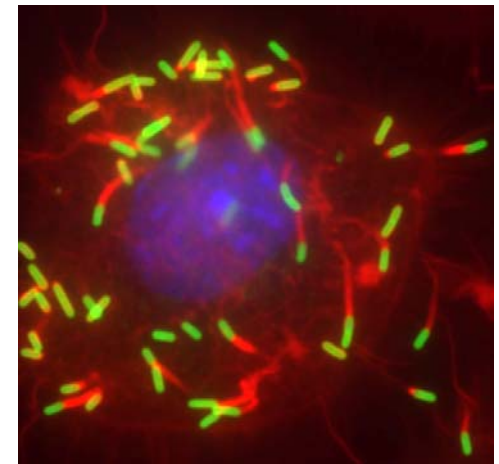
Harnessing (and Controlling) *L. monocytogenes* to Treat and Prevent Human Disease

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Presentation Overview

- Rationale for *L. monocytogenes* based vaccines
- Attenuation & potency of **Live-Attenuated** *Listeria* vaccine platform strain
 - Clinical trial status of live-attenuated vaccine platform
- **Killed But Metabolically Active (KBMA)** *Listeria* HPV vaccines
 - Rationale and potency
- Summary and CAVD activity



Clinical Translation of *L. monocytogenes* Vaccine Platforms

Wild-Type *L. monocytogenes*



Attenuation

- **Live**
 - PrfA-dependent genes
 - Separate immunogenicity & potency
- **KBMA**
 - Inactivate by DNA crosslinking DNA repair mutants
 - Similar mechanism to live attenuated

Potency

- **Antigen Expression & Secretion**
 - *prfA*-dependent promoter
 - Bacterial protein fusion partner
- **T cell function**
 - Role of phagosomal escape after LLO expression
 - Intracellular signaling



Toxicity and Potency in Mice and then Primates



Human Clinical Trials

Live Attenuated *Listeria* Platform

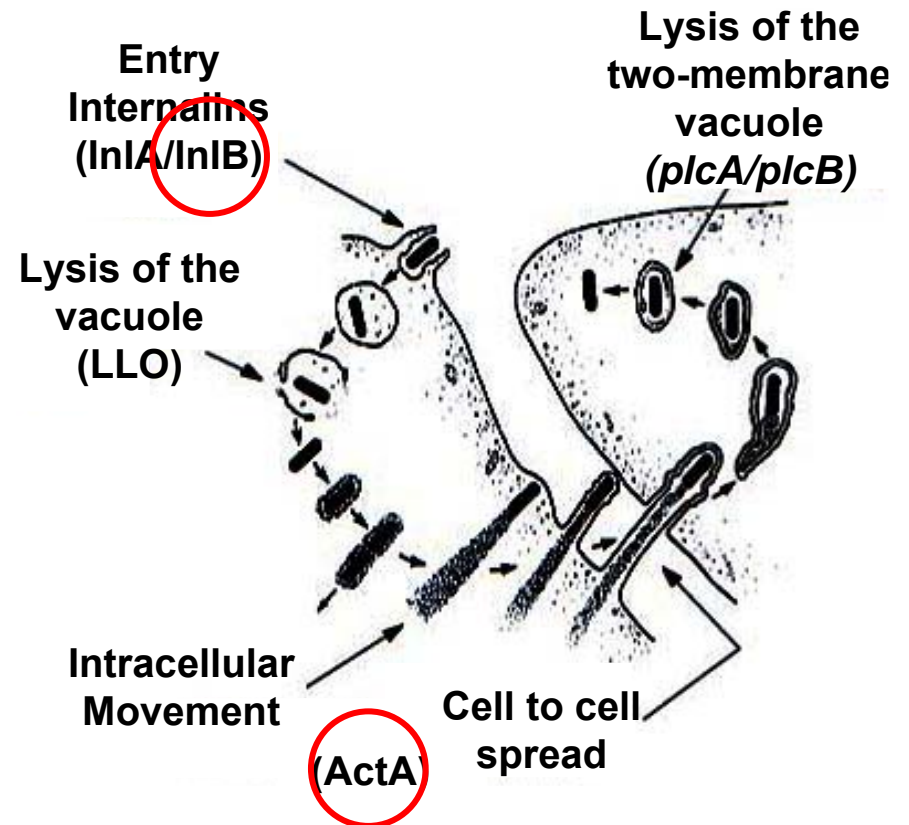


L. Monocytogenes Lifecycle and Attenuated Vaccine Platform Strain

Overview

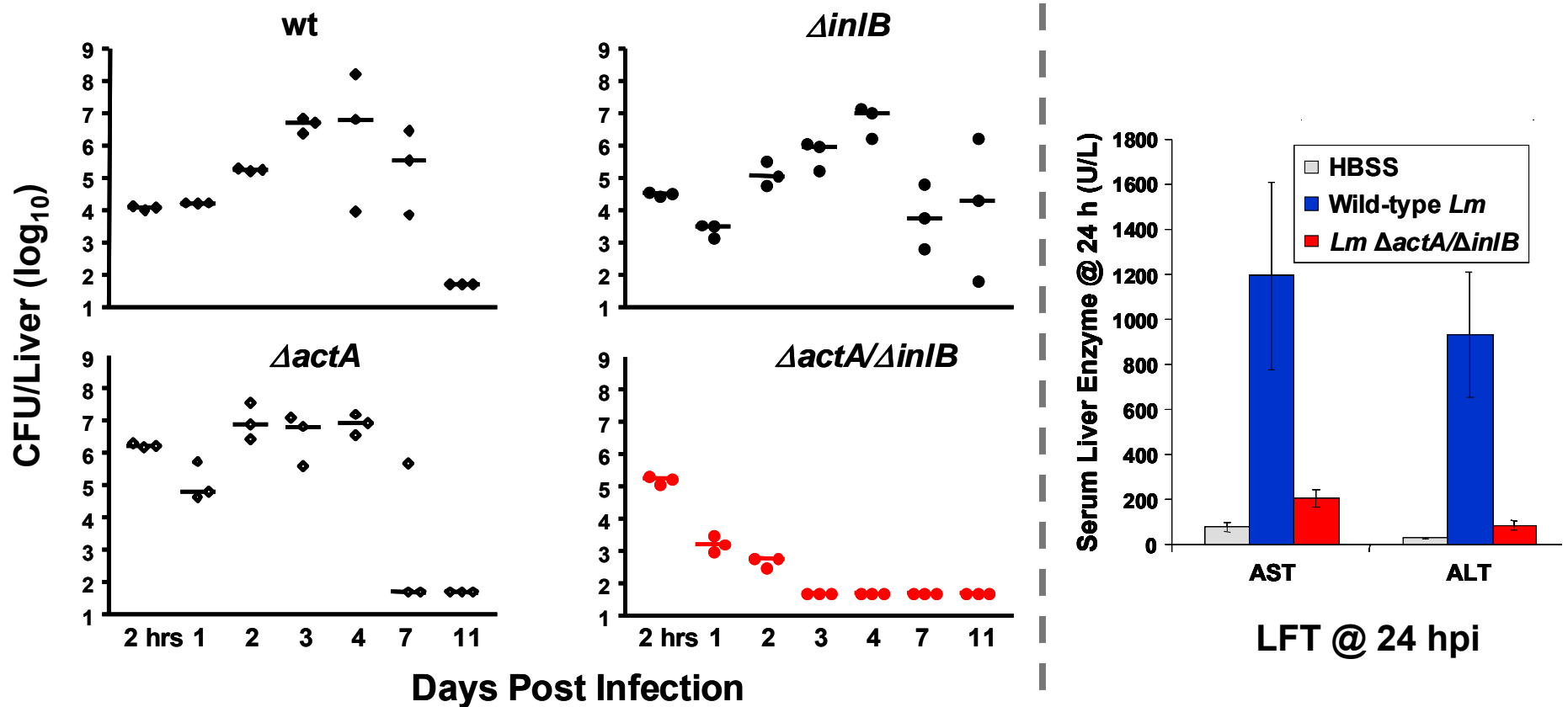
- Gram-positive bacterium that is ubiquitous in nature (food-borne)
- Facultative intracellular lifecycle
 - Taken up by and multiplies within phagocytic cells, including macrophages and dendritic cells
- Potent activator of innate immune effectors
- Model pathogen for the study of cell-mediated immunity
- Induces robust CD4⁺ and CD8⁺ T-cell immunity

Lifecycle of attenuated vaccine platform strain



Accelerated Clearance and Diminished Hepatotoxicity of *Lm* $\Delta actA\Delta inlB$

Pharmacodynamics of vaccine strain in C57Bl/6 mice following IV administration

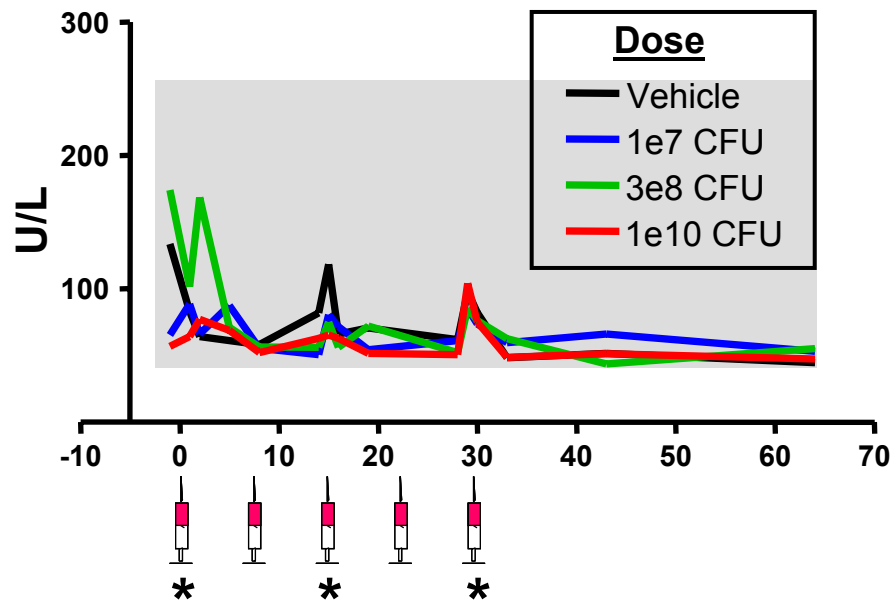


➔ The LD₅₀ value of *Lm* $\Delta actA\Delta inlB$ in mice is >1000-fold less than wild-type *Lm*

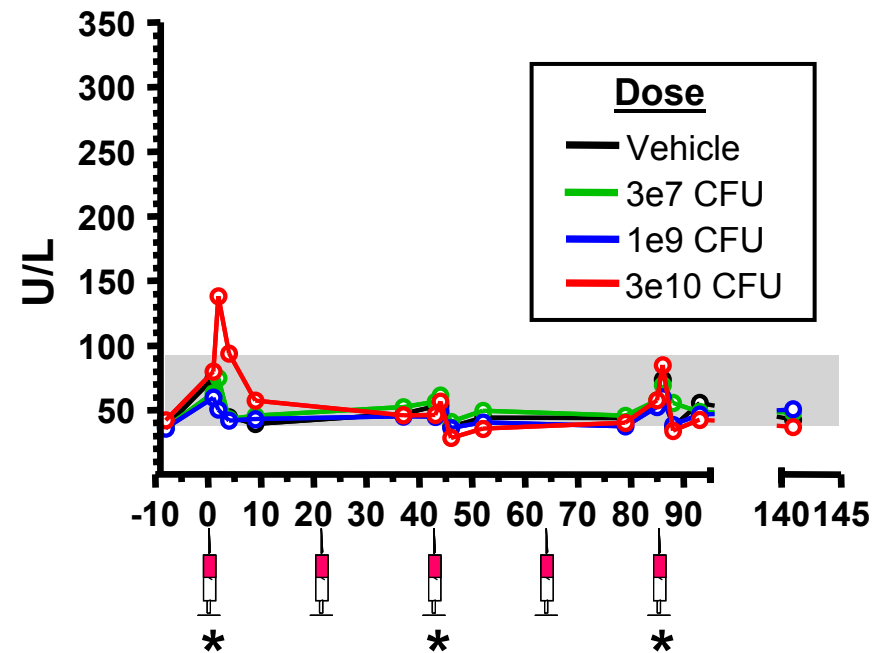
Minimal Change in Liver Function Tests with *Lm* $\Delta actA\Delta inlB$ Strains in Cynomolgus Monkeys

Serum ALT levels in female animals following IV administration of live-attenuated *L. monocytogenes*

CRS-100



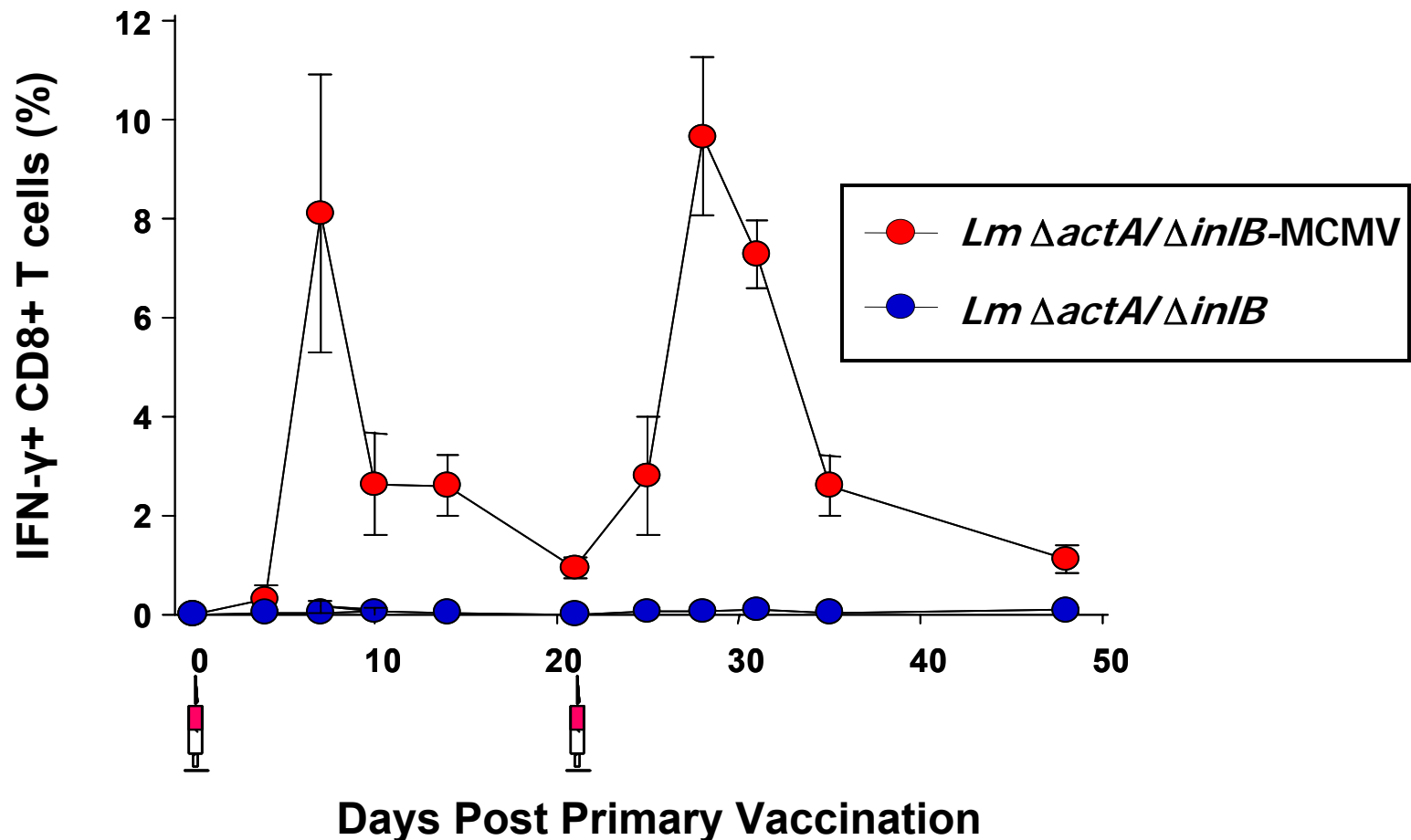
CRS-207



Study Day

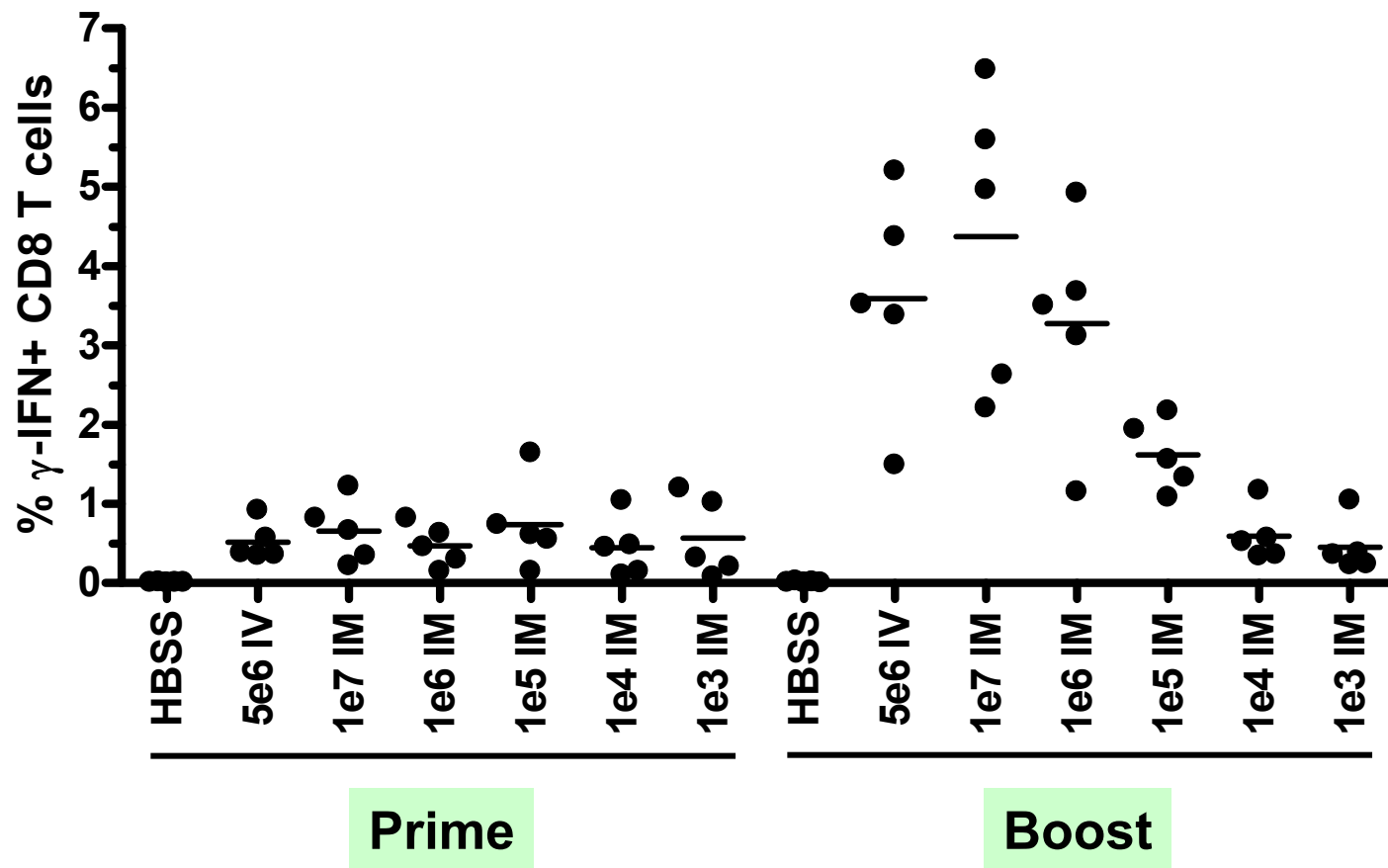
Lm $\Delta actA/\Delta inlB$ -MCMV Elicits Potent CD8+ T Cell Responses in C57BL/6 Mice

MCMV-Specific T Cell Kinetic Following Homologous Prime-Boost Vaccination



Intramuscular Immunization with *Lm* Vaccines Elicits Robust CD8+ T Cell Responses in Mice

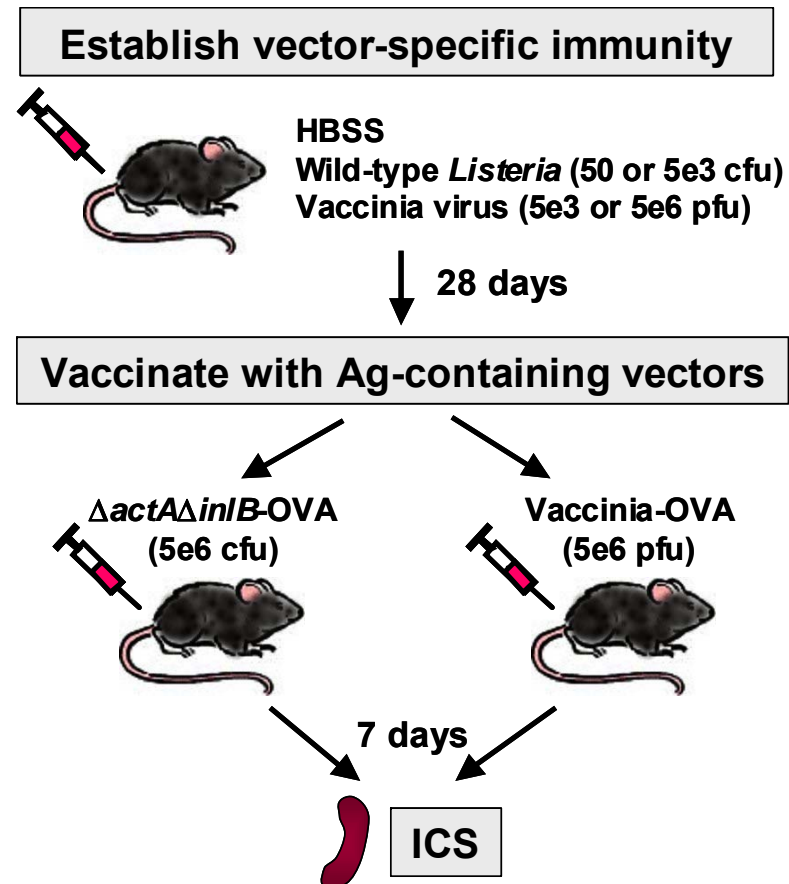
Primary and secondary HIV Gag (AMQMLKETI) CD8+ T cell responses



Immunization with *Listeria* in the Presence of Protective Immunity

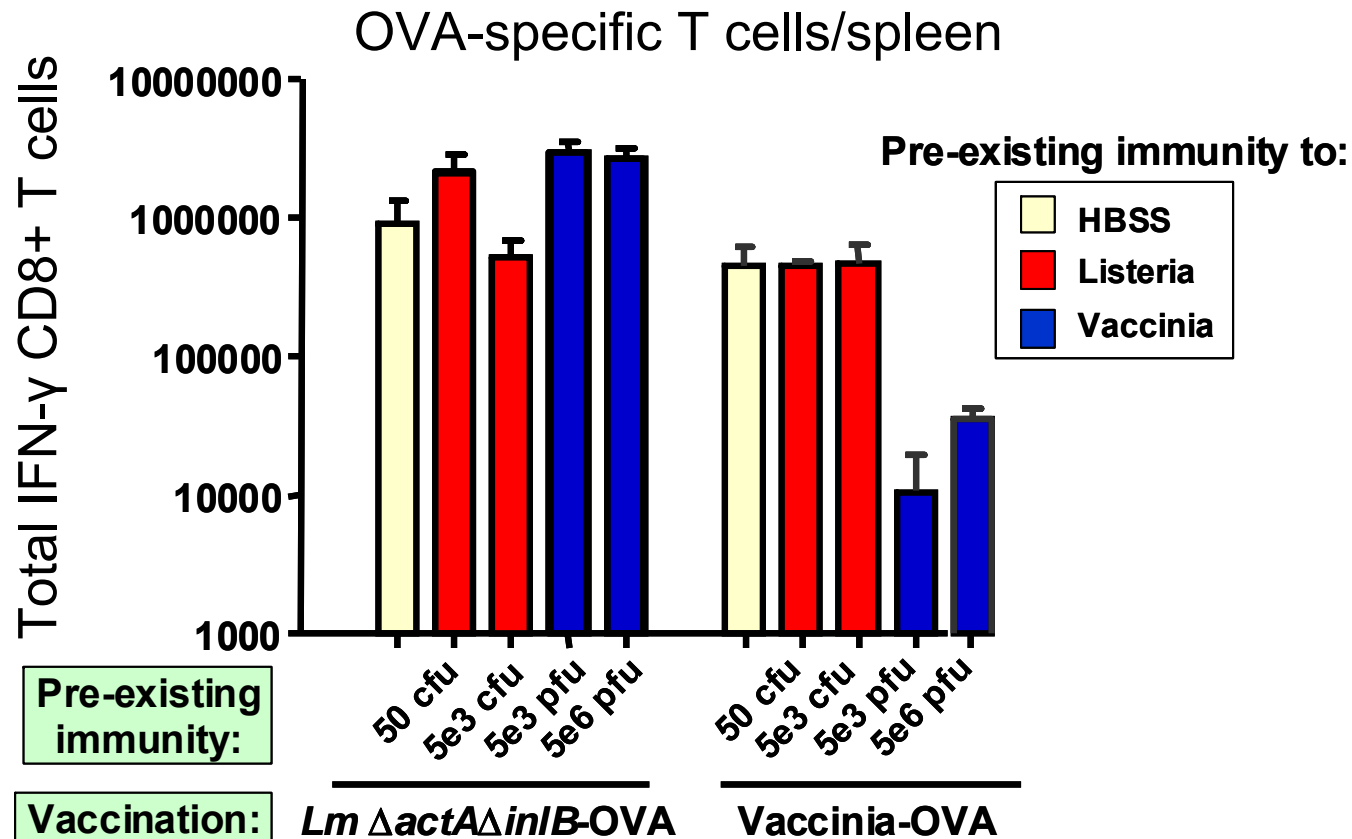
Comparison with Vaccinia Virus

Experimental Protocol



Immunization with *Listeria* in the Presence of Protective Immunity

Comparison with Vaccinia Virus

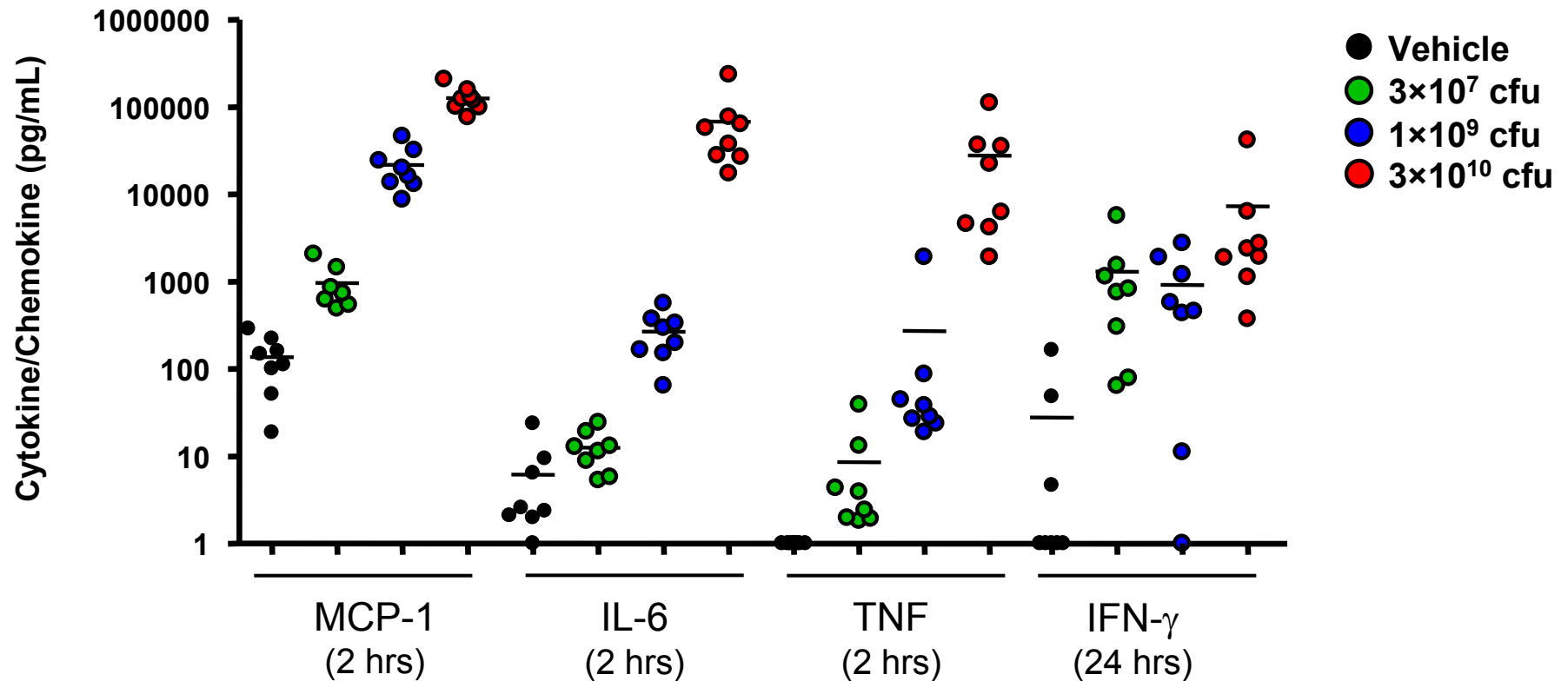


Mesothelin-Targeted Vaccine (CRS-207)
for Ovarian, Pancreatic and NSCL
Cancers

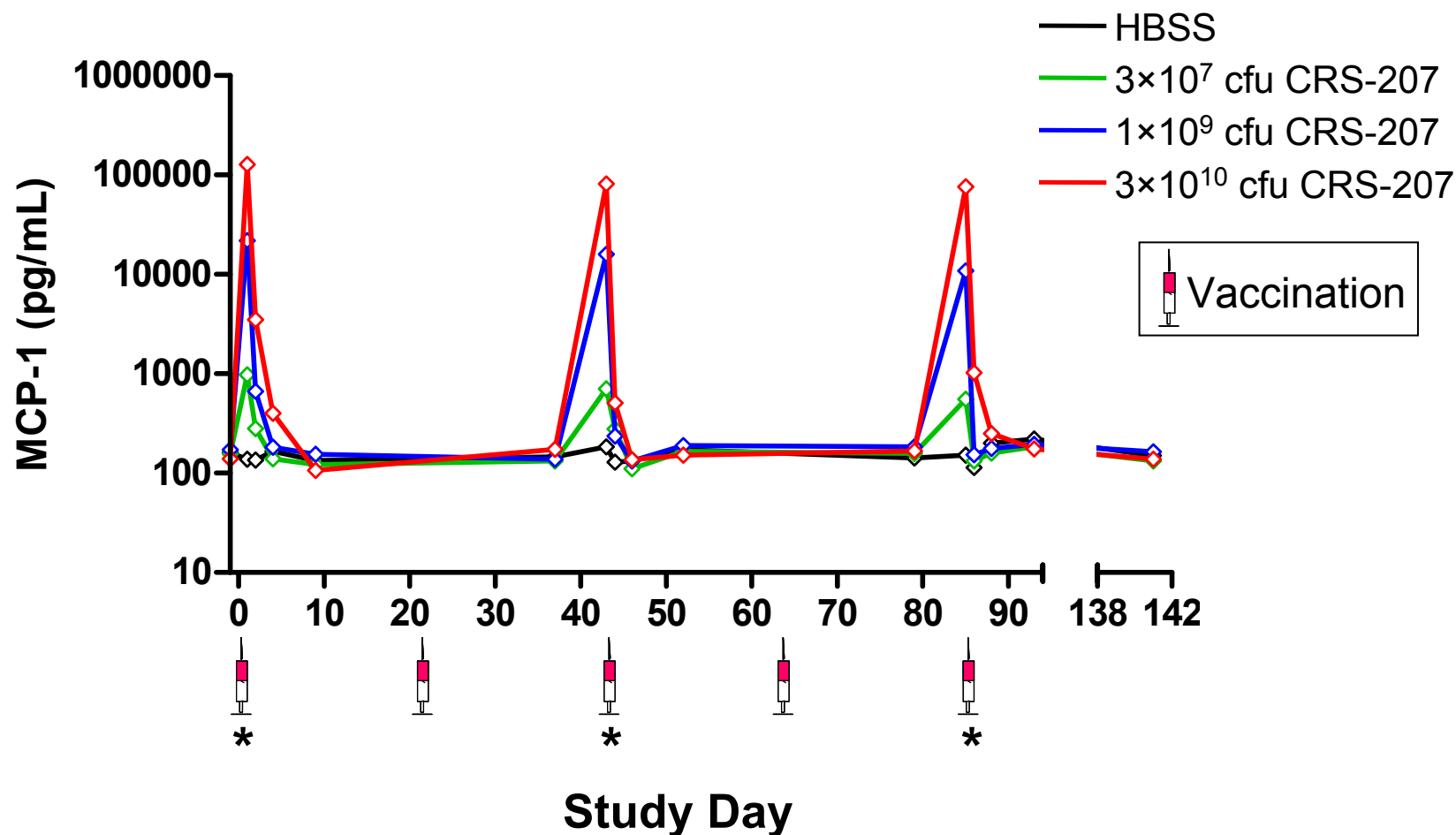


Induction of Cytokines and Chemokines by CRS-207 in Cynomolgus Monkeys

Peak Cytokine/Chemokine Levels Post First CRS-207 Dose



Induction of MCP-1 Following CRS-207 Administration in Cynomolgus Monkeys

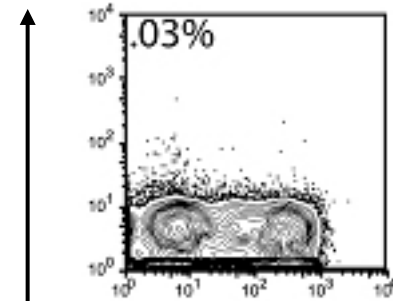


Breaking of T Cell Tolerance Against Mesothelin (a Human Tumor Antigen) in Monkeys

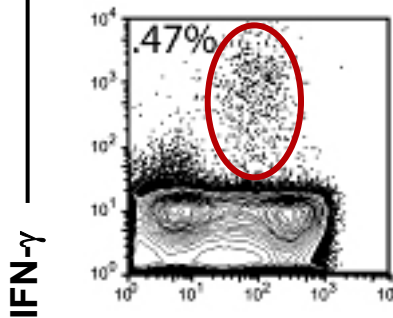
Intracellular Cytokine Staining

IFN- γ ELISPOT

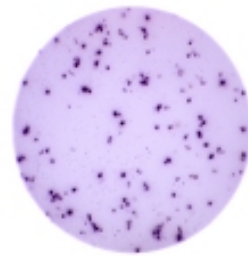
Unstimulated



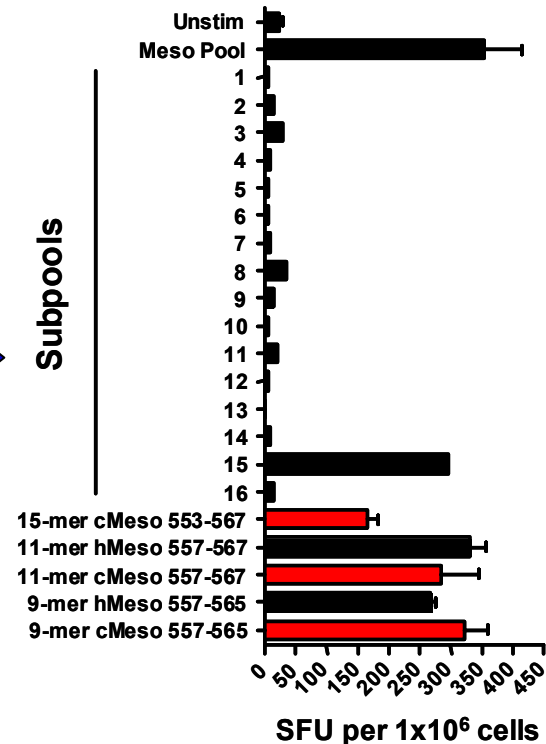
Mesothelin



CD8+



Subpools



Peptide Library

Individual Peptides

Clinical Trial Status of Live-Attenuated Vaccine Platform

- **CRS-100**

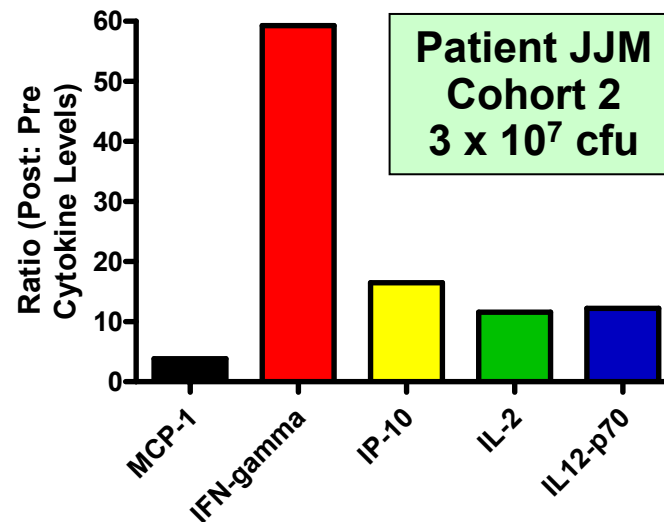
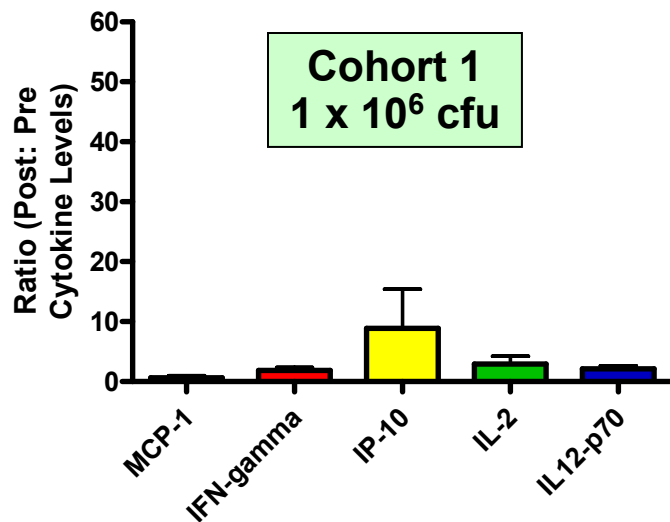
- *Lm ΔactAΔinlB* (non-Ag encoding strain)
- Ongoing dose escalation Phase 1 study in patients with carcinoma and hepatic metastases
- Completed first cohort: 10^6 cfu, given IV (N = 3)
- Treatment well-tolerated
- Initiated second cohort: 3×10^7 cfu

- **CRS-207**

- *Lm ΔactAΔinlB* encoding Mesothelin tumor Ag
- Mesothelin is over-expressed in pancreatic, ovarian, and non-small cell lung cancers and malignant Mesothelioma
- Demonstrated breaking of tolerance against endogenous Mesothelin in CRS-207 vaccinated cynomolgus monkeys
- IND filed in June 2007
- Establishing clinical sites

Early Results from CRS-100 Phase 1 Study

- No DLT reported for first 5 study patients
 - First cohort completed at 1×10^6 (N = 3)
 - No related DLT reported after single intravenous administration
 - CRS-100 not detected in blood, feces, urine or sputum
 - Second cohort enrolling at dose of 3×10^7 cfu
 - Increased cytokine response and cellular activation by first patient
- NK cell activation & cytokine response confirms pre-clinical models



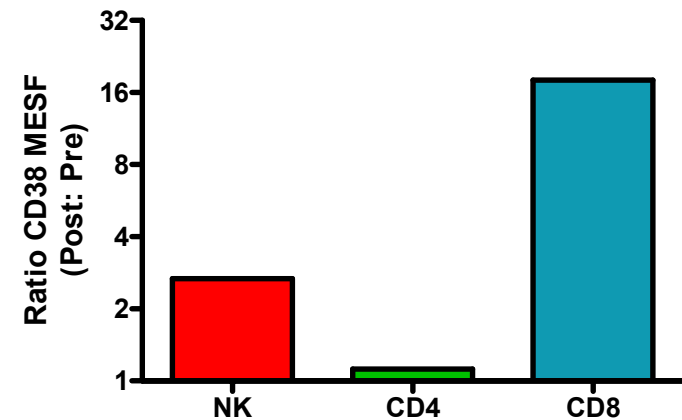
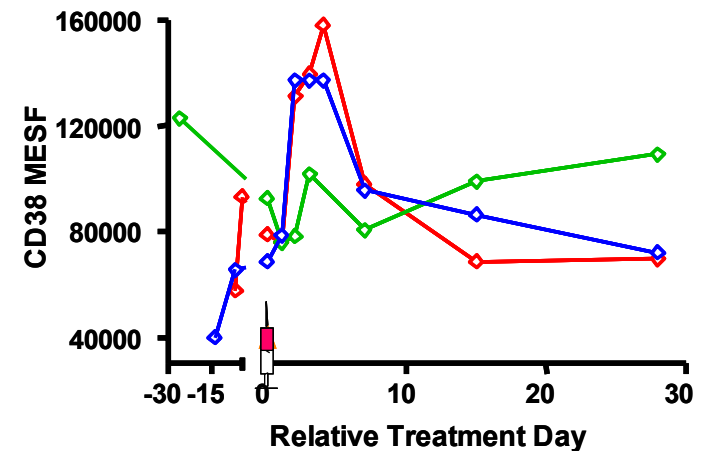
Preliminary Evidence of Biological Activity in Patients

- Recruitment and activation of NK cells and T cells from peripheral blood
- Stimulation of broad array of Th1 cytokines and chemokines
- Similar profiles observed in mouse and non-human primate

Cohort 1
 1×10^6 cfu

Patient JJM
Cohort 2
 3×10^7 cfu

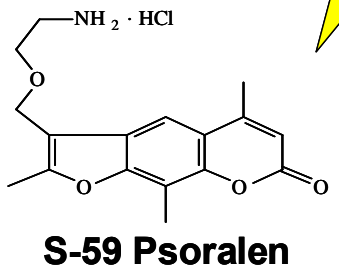
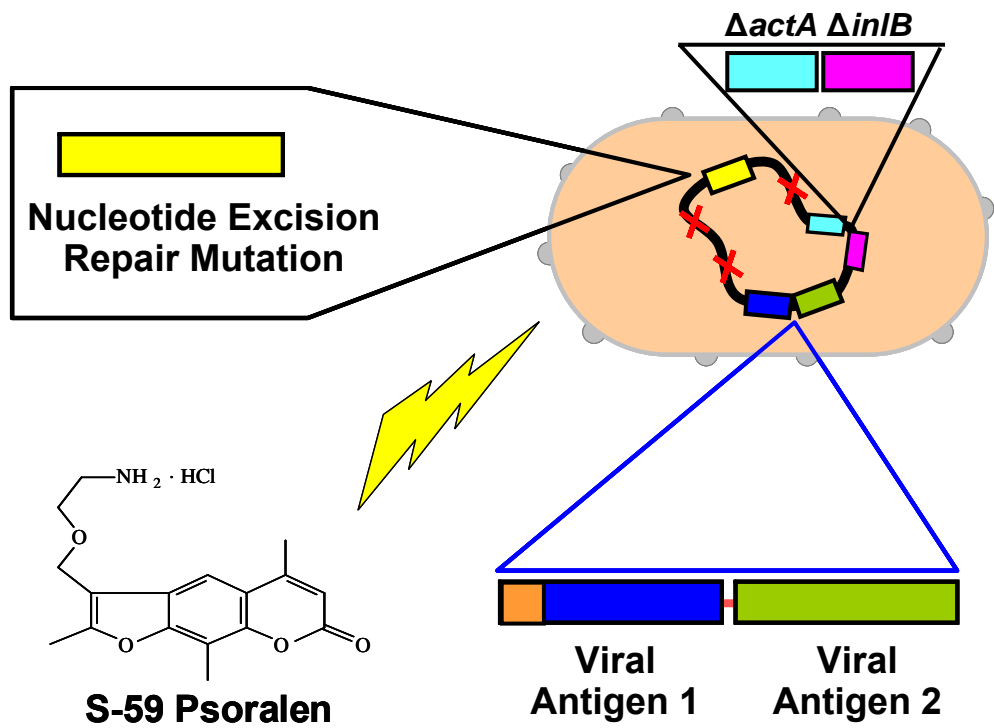
NK Cell Activation



Killed **B**ut **M**etabolically **A**ctive (KBMA) Vaccines



Killed But Metabolically Active (KBMA) Vaccines

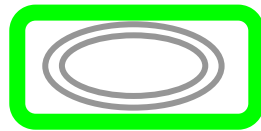


- ### Features
- Derived from attenuated *Listeria* clinical strain
 - Killed but able to express genes
 - Host response similar to live organism
 - Activates immune system
 - Microbial propagation blocked
 - Elicits protective T cell immunity
 - Can expressive several Ags

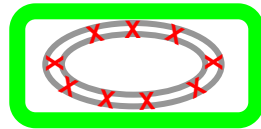
KBMA Vaccines Mechanism of Action

Conventional Inactivation

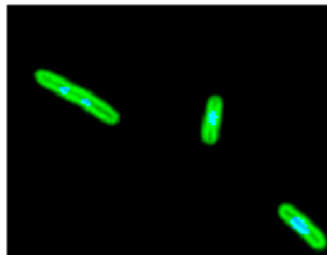
Wild-Type
Microbe



Many Crosslinks
To Inactivate

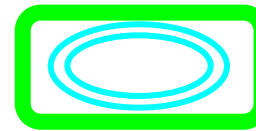


Gene Expression Diminished

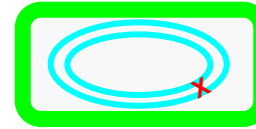


KBMA Immunotherapy

DNA Repair
Deficient Strain

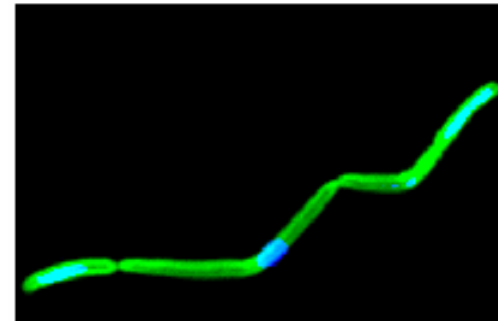


Photochemical Treatment



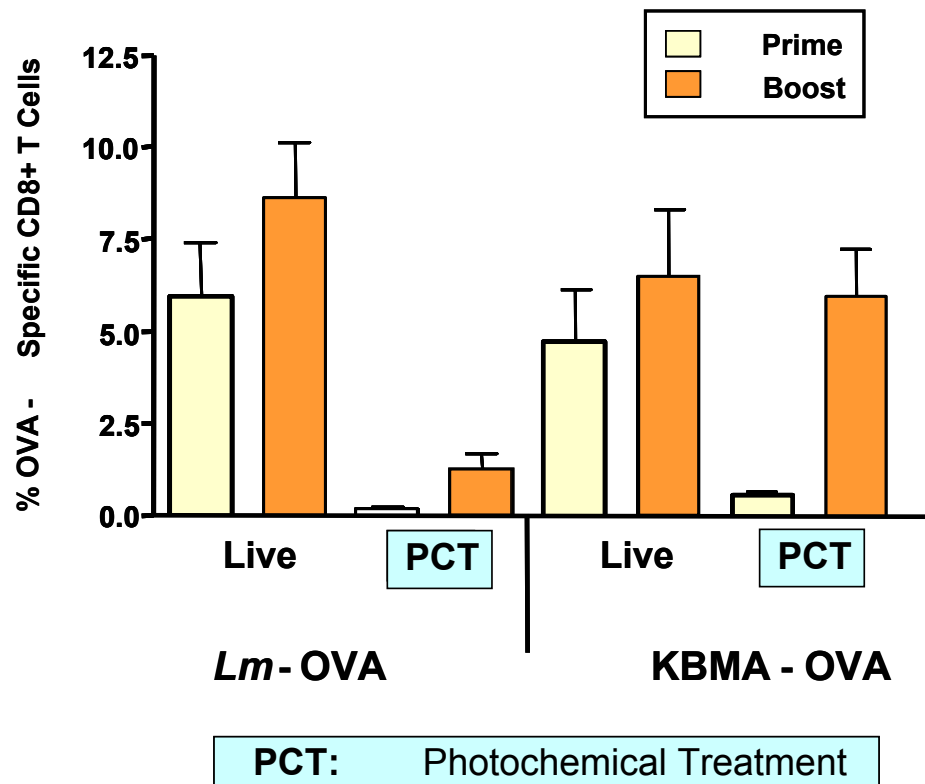
Single Crosslink
Inactivates

Gene Expression Preserved

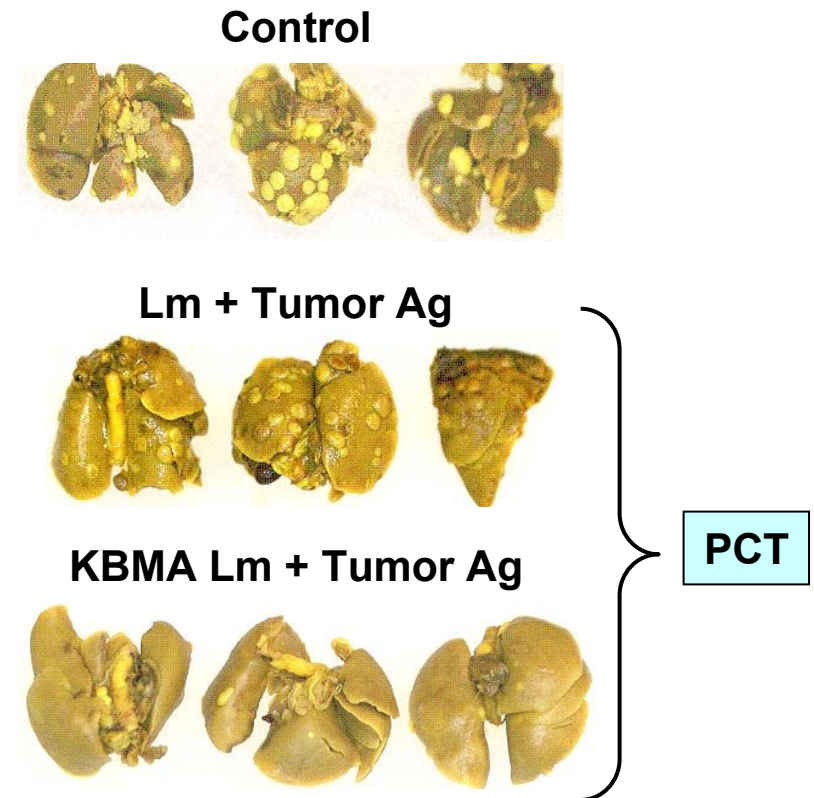


Potent Activity of KBMA Vaccines

Immunogenicity



Therapeutic anti-tumor efficacy



Summary

- We have developed live-attenuated and KBMA vaccine platforms based on *L. monocytogenes*
- Recombinant *Listeria* vaccines are efficacious in the presence of protective immunity and can be repeat administered
- GMP *Listeria* vaccine preparations can be cheaply and efficiently produced
- The live-attenuated vaccine platform is currently being evaluated in two Phase 1 FDA-approved clinical studies
- We are providing HIV & SIV Gag vaccines on the live-attenuated and KBMA strain backgrounds for CAVD consortium (McElrath PI)

Acknowledgements

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