

Anti-Tumor Potential of Attenuated Salmonella Containing the Human IL-2 Gene

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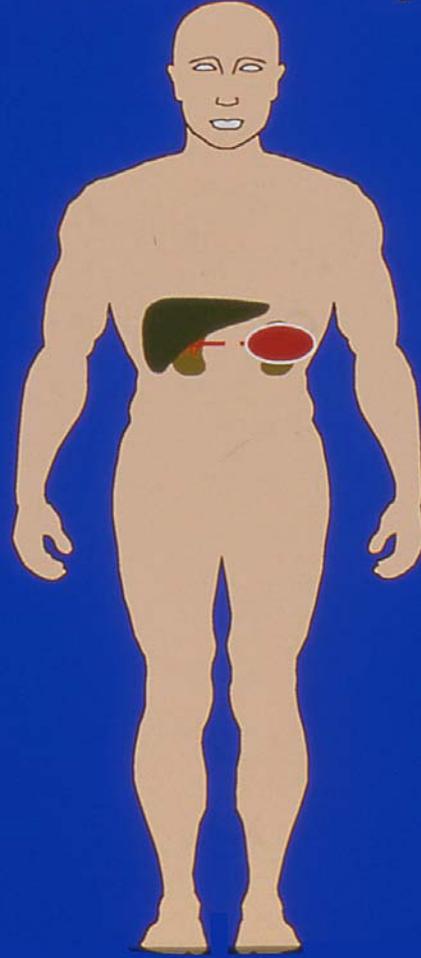
Interleukin-2

- 15-kD cytokine produced by CD4+T cells
 - Lymphocyte proliferation
 - Enhances the cytolytic function
 - CD8+ T cells
 - Natural killer (NK) cells.
- Advanced renal cell carcinoma and melanoma

Interleukin-2: Metastatic Cancers

- IL-2 effectiveness requires high systemic doses
 - fever, malaise, hypotension, renal dysfunction, hepatic dysfunction, capillary leak syndrome, and death (1-2%)
- Attempts to diminish the adverse effects
 - Altering the dose, route and formulation unsuccessful
 - Theorized that local delivery is less toxic

Salmonella Naturally Tracks to the Liver and Spleen



Hare Brained Idea (Hypothesis)

Can Salmonella carry IL-2 to the liver and tumors to yield an anti-cancer effect?

Wait a minute!!!!

Salmonella found in unopened Schwan's container

By Gordon Slovut
Staff Writer

Minnesota health officials for the first time have found salmonella contamination in an unopened container of ice cream from the Schwan's Sales Enterprises plant in Marshall.

Buddy Ferguson, spokesman for the state Health Department, said Tuesday that the Minnesota Department of Agriculture laboratory in St. Paul recently confirmed salmonella contamination in three containers of Schwan's ice cream — one still sealed and two opened — that came from homes.

Laboratory personnel last week isolated salmonella germs from an opened container of Schwan's, which had been in the refrigerator of a per-

son with food poisoning. In instances where containers have been opened, it is virtually impossible to know when the contamination occurred.

Schwan's products from the Marshall plant have been linked to thousands of cases of salmonellosis food poisoning in most of the 48 contiguous states. Schwan's products are home-delivered by trucks in every state except Hawaii and Alaska.

In a news release, Eldon Redalen, state agriculture commissioner, described the finding of contamination in the sealed container as "significant."

The Schwan's plant has been closed since Oct. 7, when Michael Osterholm, state epidemiologist, warned that there appeared to be an associa-

tion between an outbreak of salmonella enteritidis and Schwan's products from the Marshall plant.

Dave Jennings, manager of intergovernmental relations for Schwan's, said that to his knowledge, state and federal investigators have not found a source of contamination at the Schwan's plant or in its raw materials or its transportation and storage systems. They have given no indication of when the plant, a major employer in Marshall, will be allowed to reopen, he said.

He said that the sealed container that was contaminated was manufactured on the same day as the opened first sample with confirmed salmonella.

The three new samples are being tested to see if they have salmonella

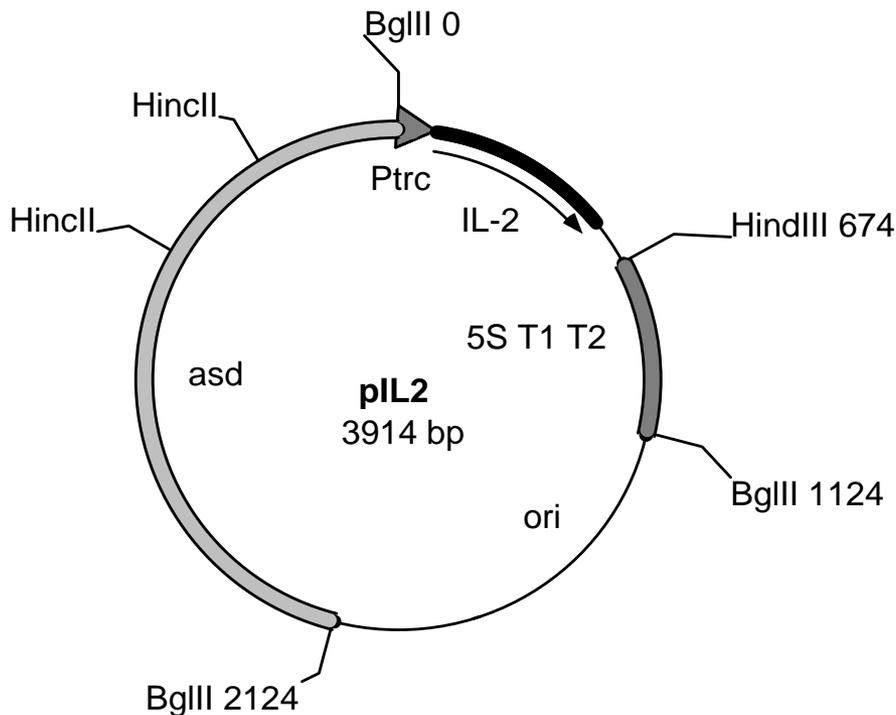
enteritidis, the second most common strain of salmonella detected in foodborne outbreaks in the United States. There are 2,000 known strains of the bacteria, about 200 of which are detected in the United States in any given year.

Schwan's, which issued a voluntary recall of its Marshall products, has agreed to recondition and repasteurize all existing inventories of ice cream premix, make thorough, multiple cleanings of its plant and equipment and establish procedures to ensure that tankers used to transport its ice cream products are used solely for that purpose, the Food and Drug Administration (FDA) said. "In addition," the FDA said, "the company has agreed that it will institute rigorous sampling and testing procedures."

Attenuated *Salmonella typhimurium*

- Deletion mutant of cAMP and cAMP receptor protein (Δcya , Δcrp)
 - Avirulent and highly immunogenic
- Deletion mutant of aspartate semialdehyde dehydrogenase (Δasd)
 - Diaminopimelic acid: gram negative cell wall
 - Mutants die rapidly after invading mucosa and lymphoid tissue

Attenuated *S. typhimurium*



- Plasmid
 - Interleukin-2 gene
 - ASD gene
- Transformants that lose plasmid die without ASD gene
 - *Salmonella*-pIL2
 - *S. typhimurium*

Hepatic Metastases Model



Day 0
• Laparotomy
• Splenic Injection of
MCA-38 Tumor cells

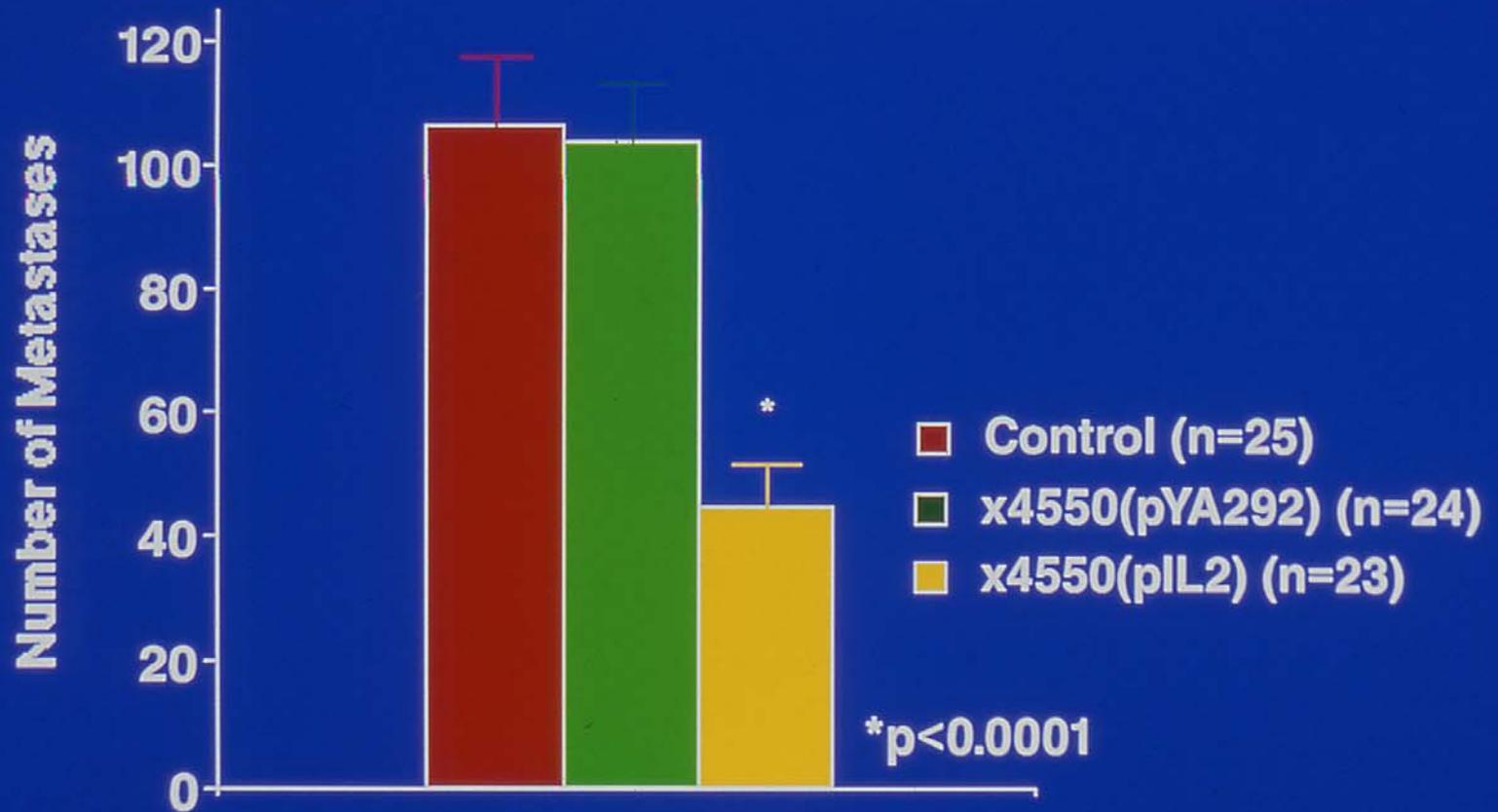


Day 3
• Gavage feed
Salmonella-IL2



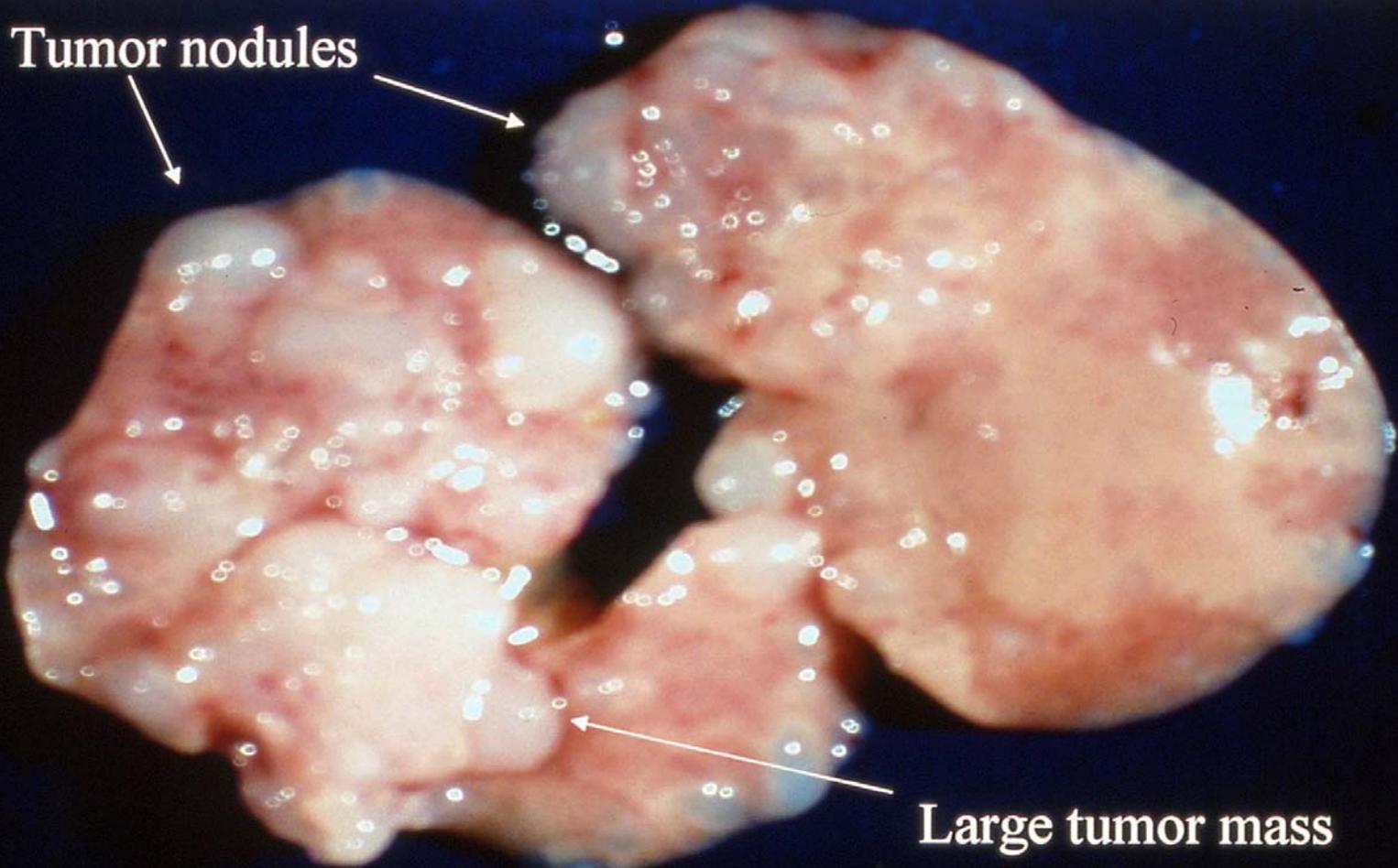
Day 12
• Inject Ink Soln
into SMV
• Hepatectomy
• Count Mets
• ANOVA

χ 4550(pIL2) Significantly Reduces Hepatic Metastases



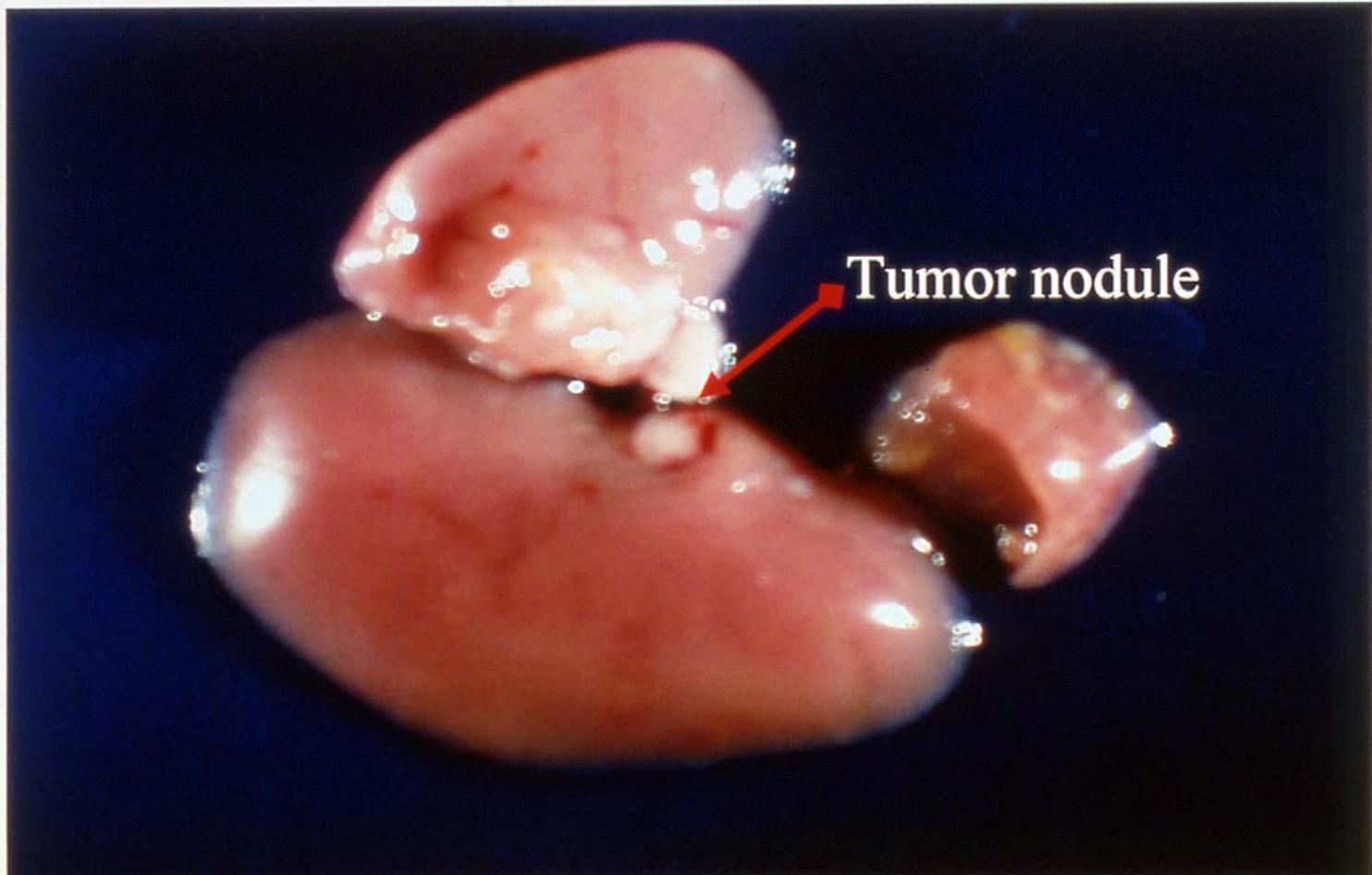
Saline Control Livers

Tumor nodules

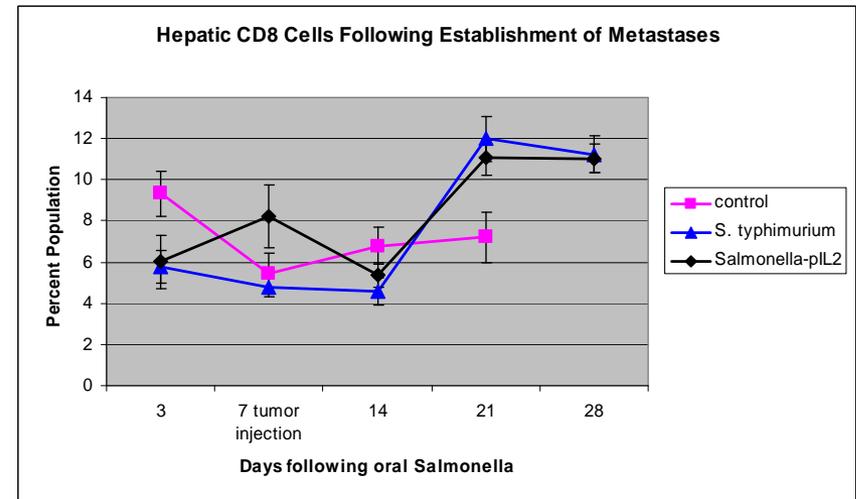
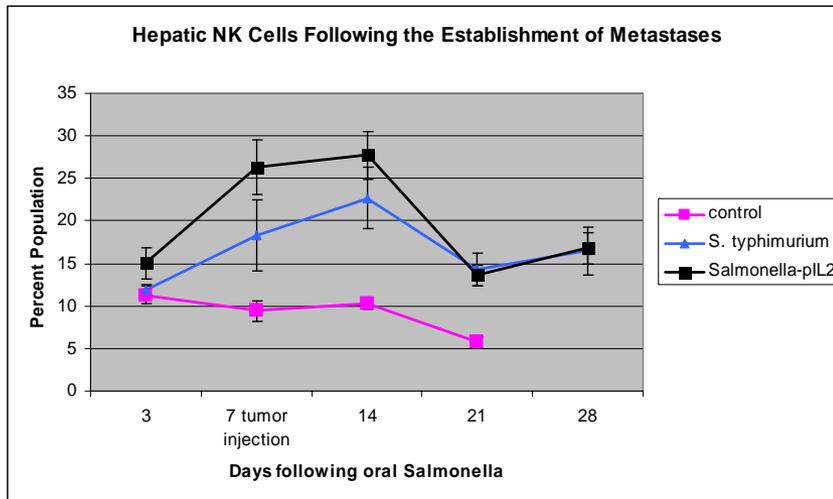


Large tumor mass

Salmonella typhimurium
Treated Liver

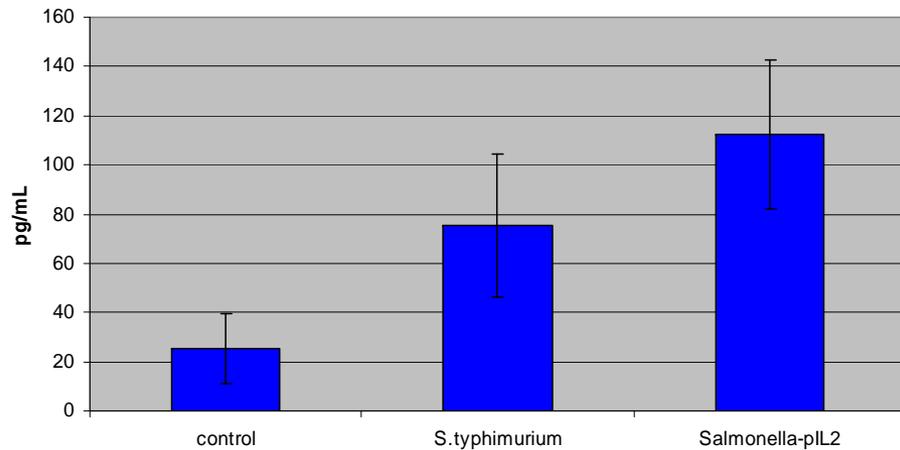


NK and CTL Cells Responsible for Anti-tumor Effect

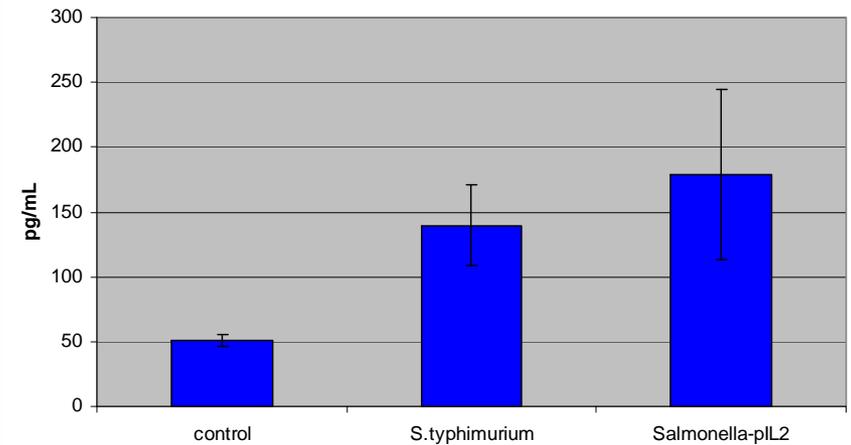


Elevation of CTL and CTL/NK Promoting Cytokines

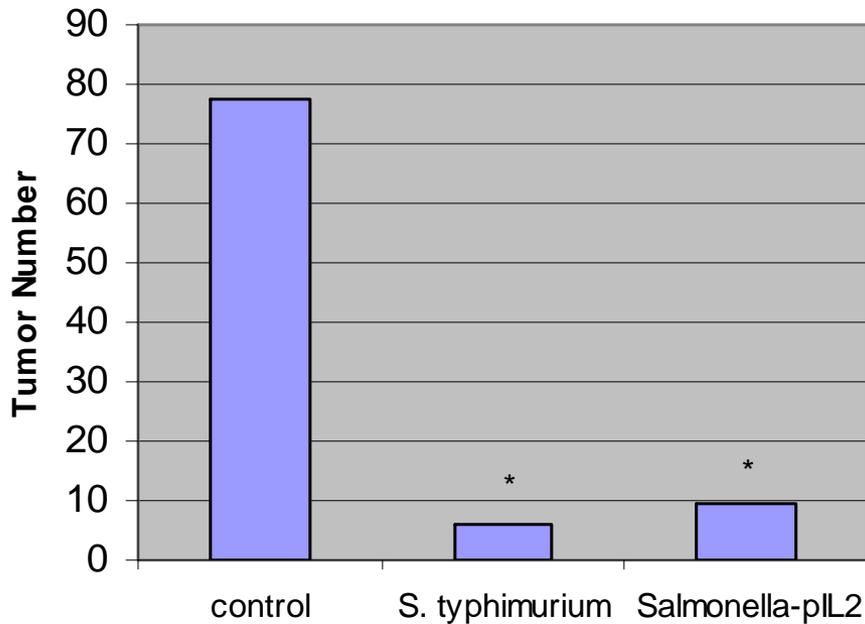
Serum INF γ : 7 Days Following Hepatic Metastases



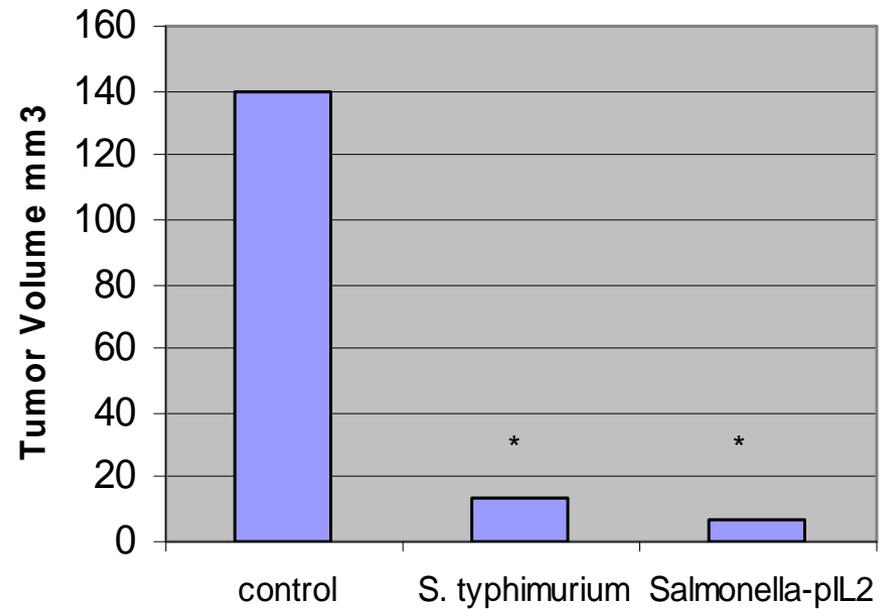
Serum IL-6: 7 Days Following Hepatic Metastases



Attenuated Salmonella Prevents Establishment of Tumor in Liver

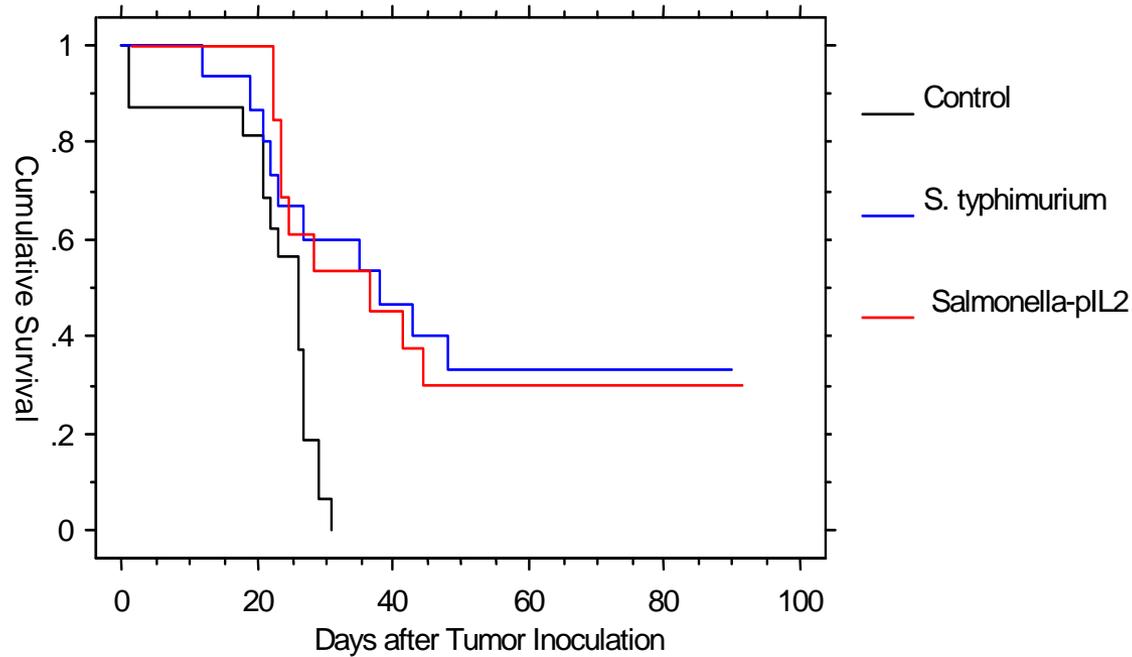


* p<0.0001; Fisher's exact



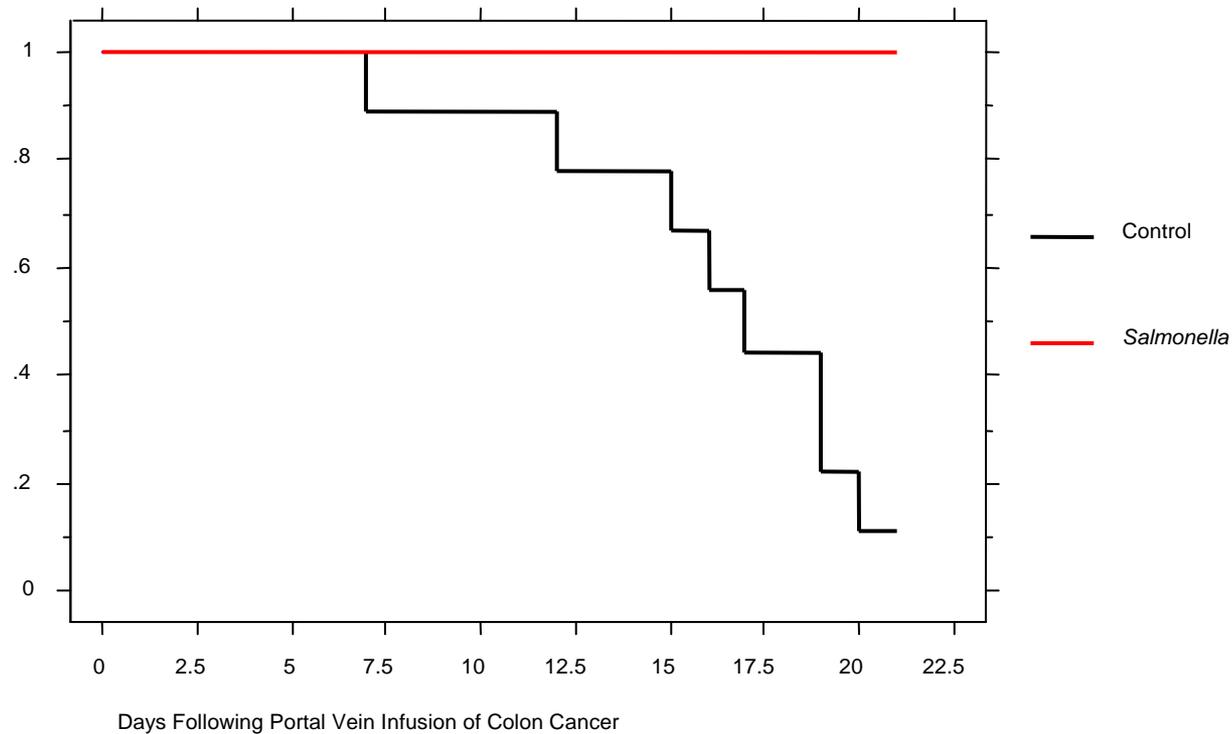
*p<0.0001; Fisher's exact

40% Complete Prevention



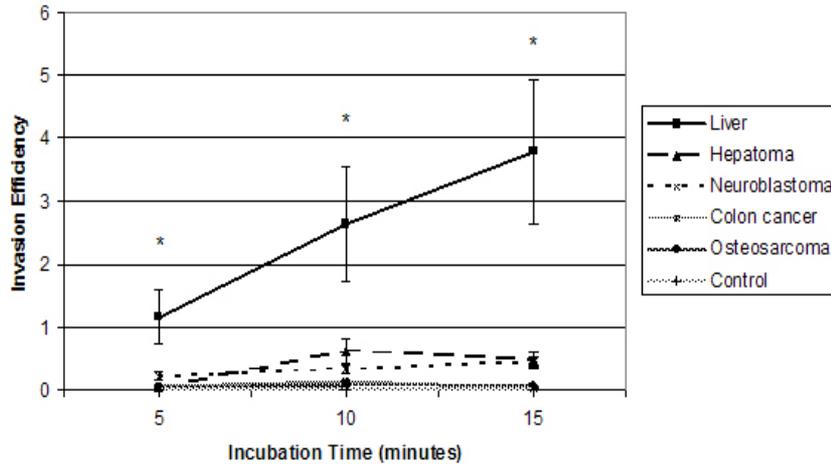
100% Survival with Tumor Re-challenge

Kaplan-Meier Survival Following Re-challenge of Hepatic Metastases



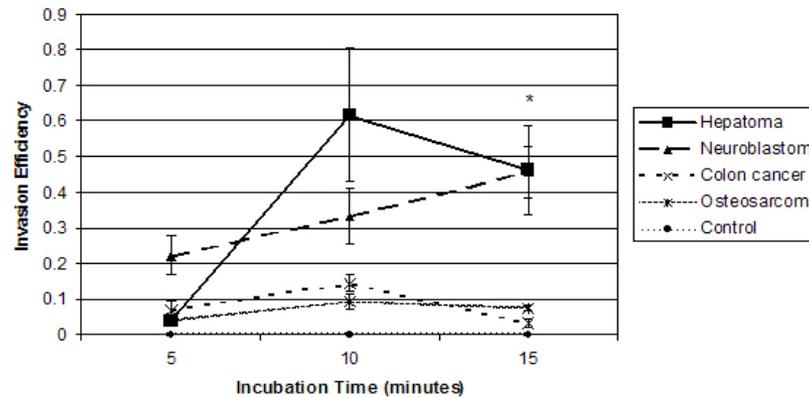
In vitro Invasion

Salmonella-pIL2 Invasion

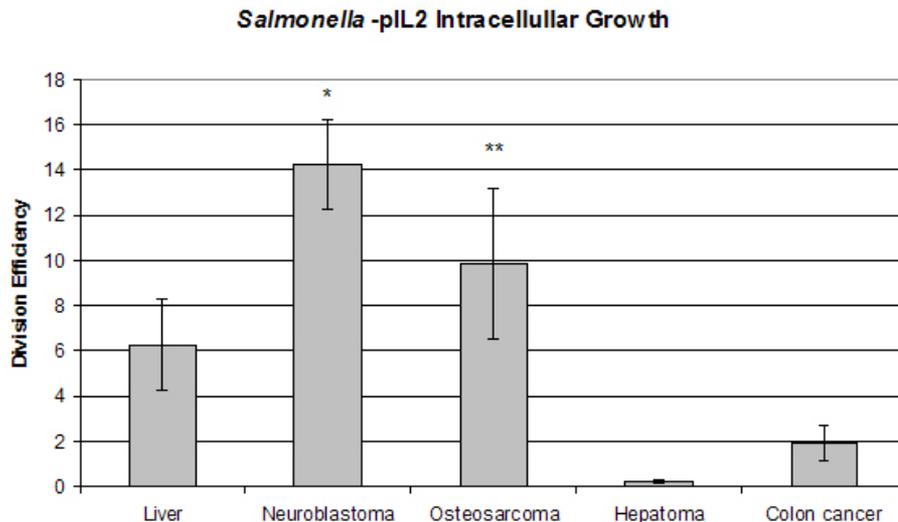


- Invasion efficiency represents the percentage of intracellular bacteria recovered compared to inoculum size

Salmonella-pIL2 Tumor Invasion



Division Efficiency



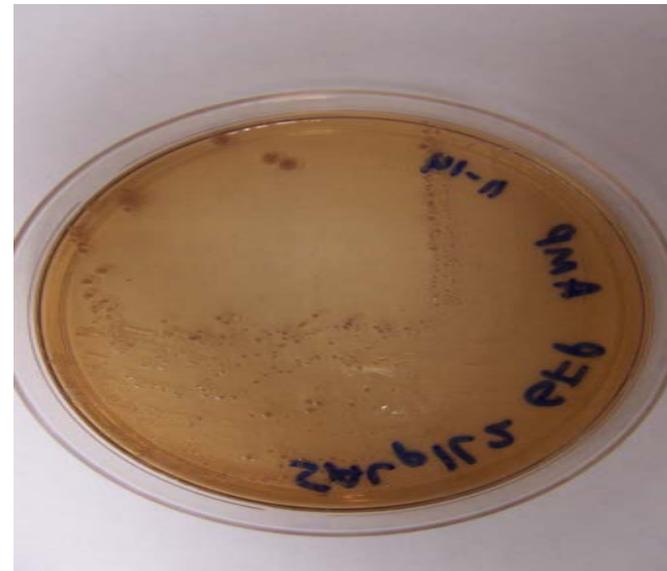
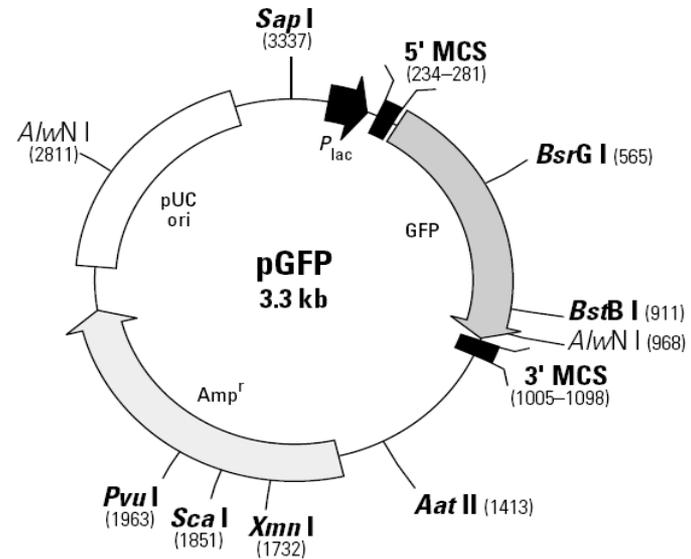
- Division efficiency represents the number of intracellular divisions in a 24 hour period
 - Intracellular division is higher in neuroblastoma ($p < 0.01$) and osteosarcoma ($p < 0.09$) than in hepatocytes, colon cancer, or hepatoma

Neuroblastoma

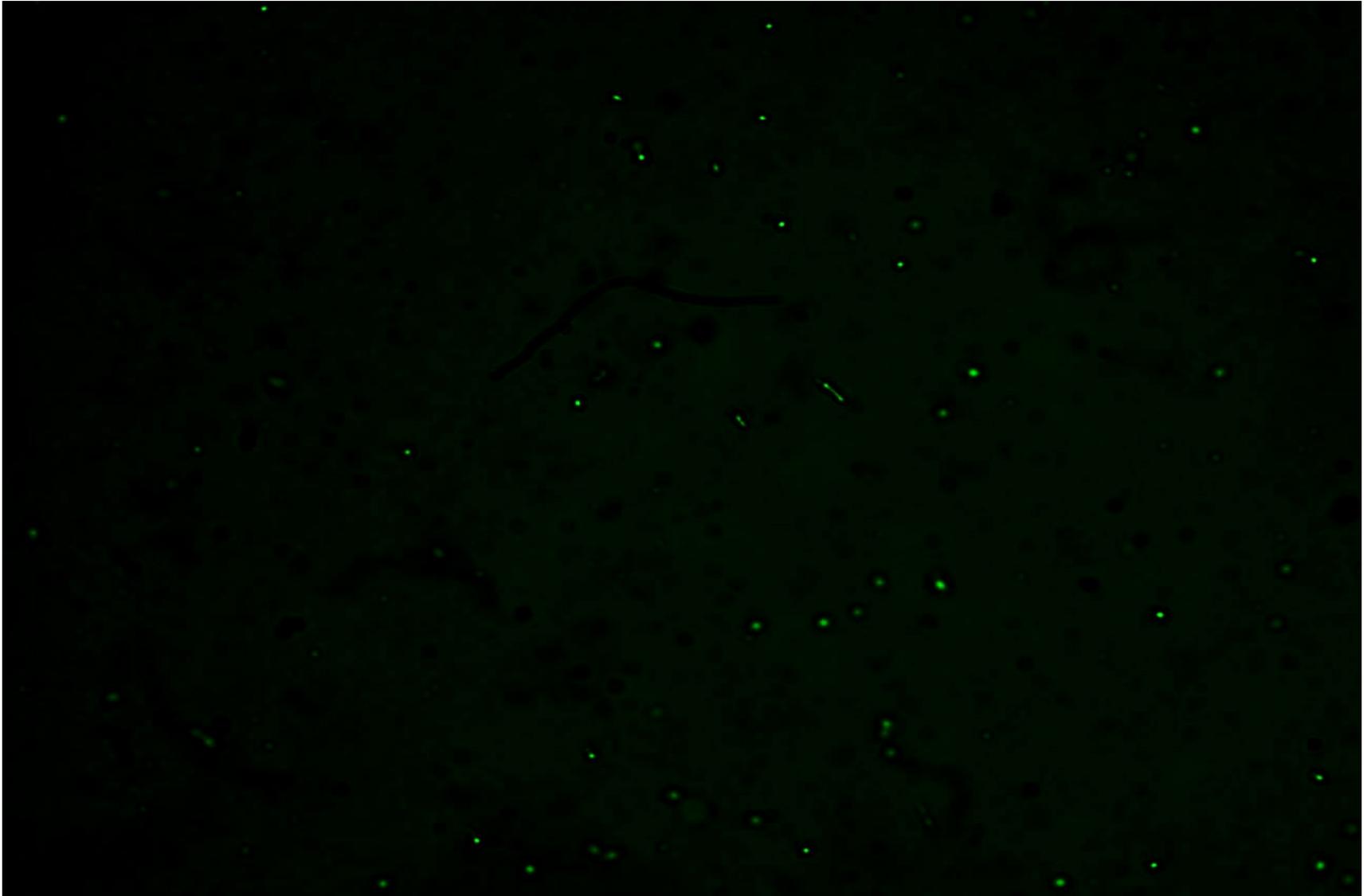
- Neuroblastoma is the most common extracranial tumor in children.
- The survival rate 5 years from diagnosis is approximately
 - 83% for infants
 - 55% for children aged 1-5 years
 - 40% for children older than 5 years

In vivo Invasion

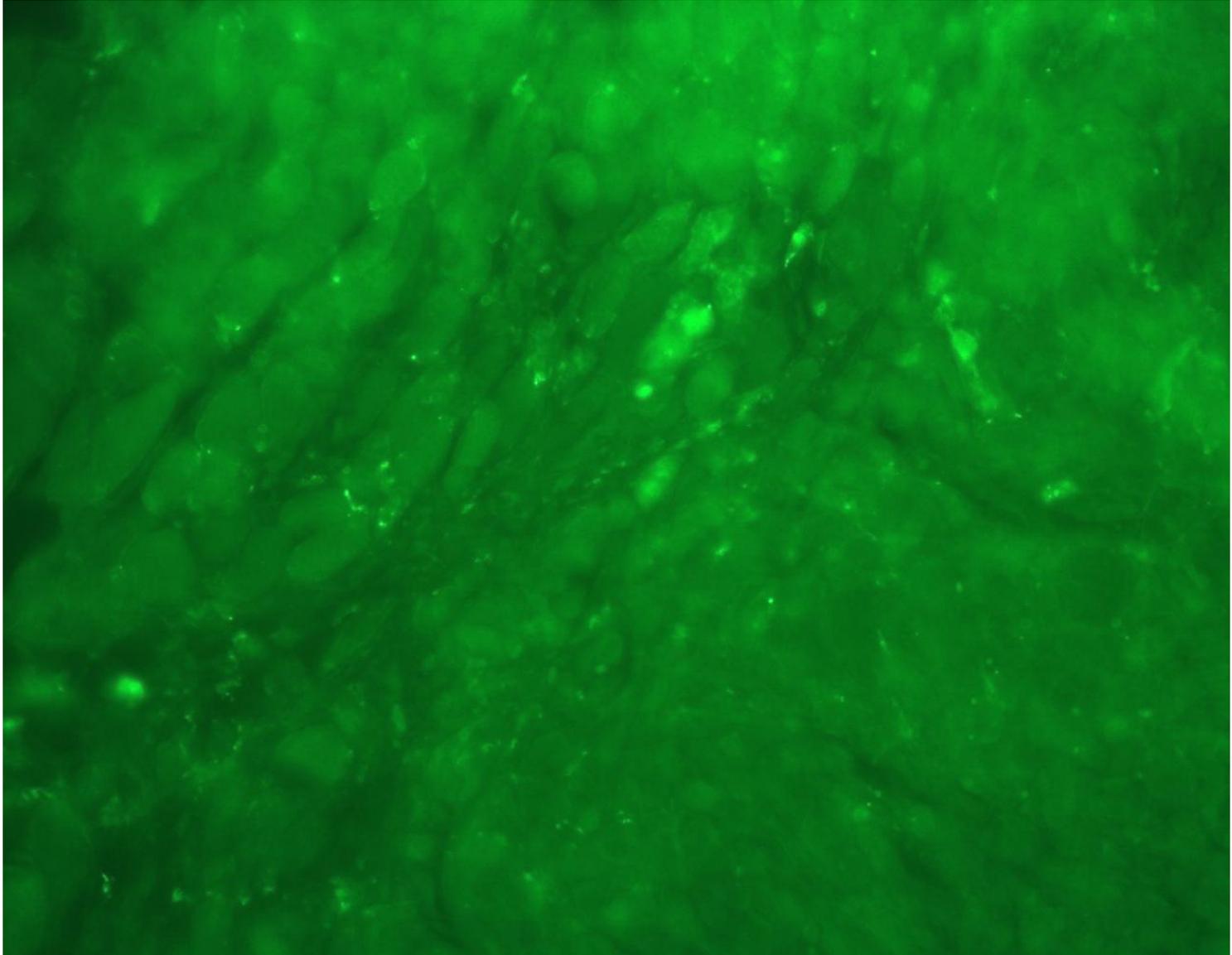
- Green Fluorescent Protein (GFP) reporter gene incorporated into *Salmonella* via electroporation



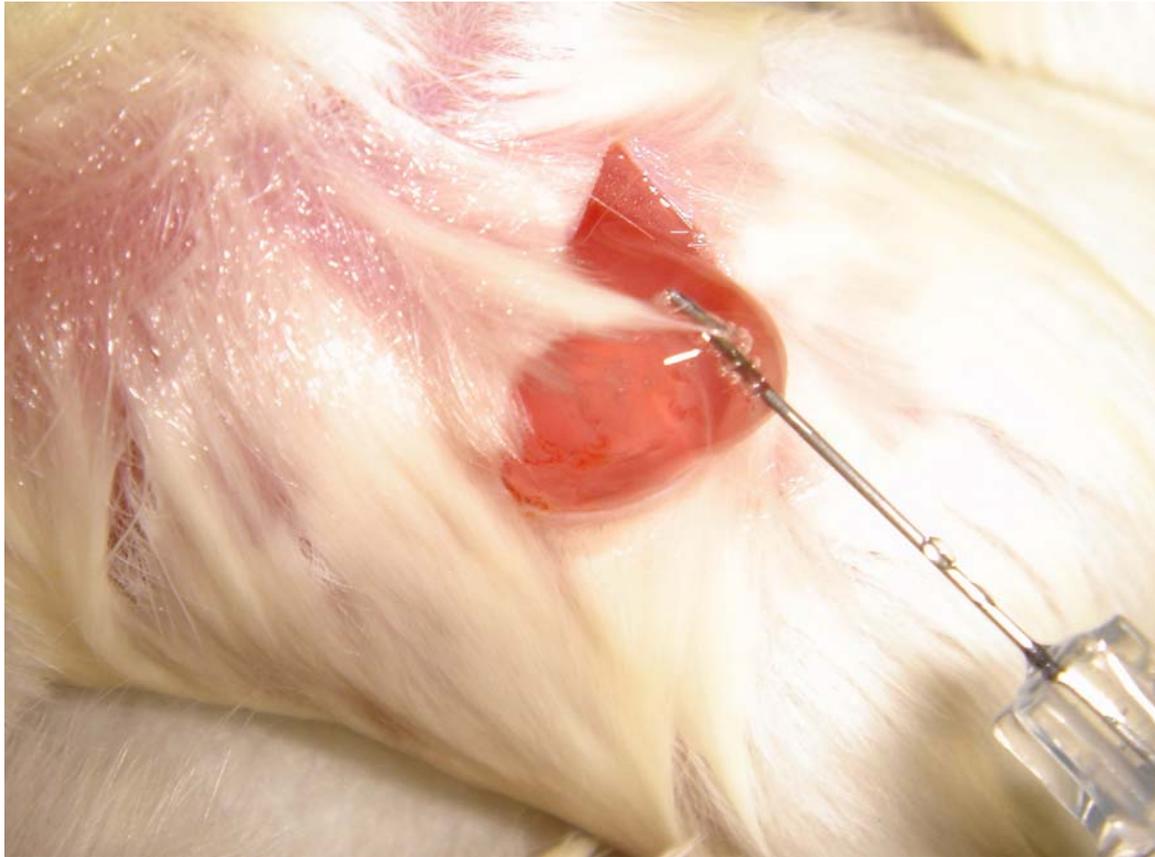
In vitro Invasion



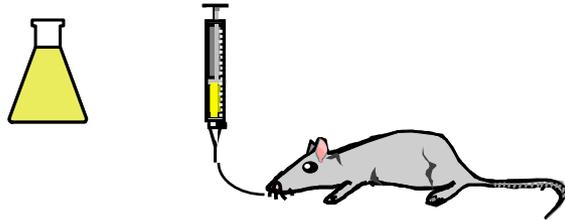
In vivo Invasion



Injecting Neuroblastoma



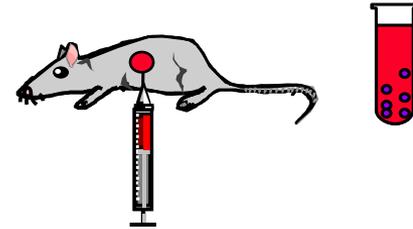
14 Day Treatment Studies



Day 7
Gavage 10^8 CFU
Sal-pIL2, Sal-NG or
Saline (control)



**Animals are monitored,
those that die are examined
for tumor growth**

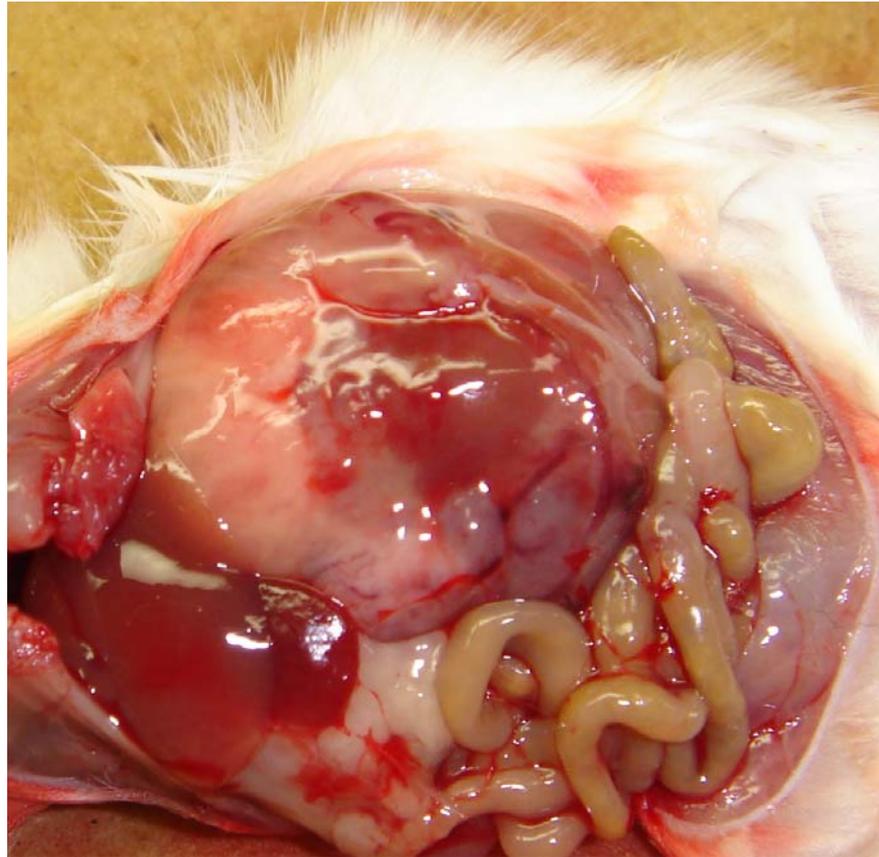


Day 0
Left Suprarenal
injection of
 1×10^6 N2a cells

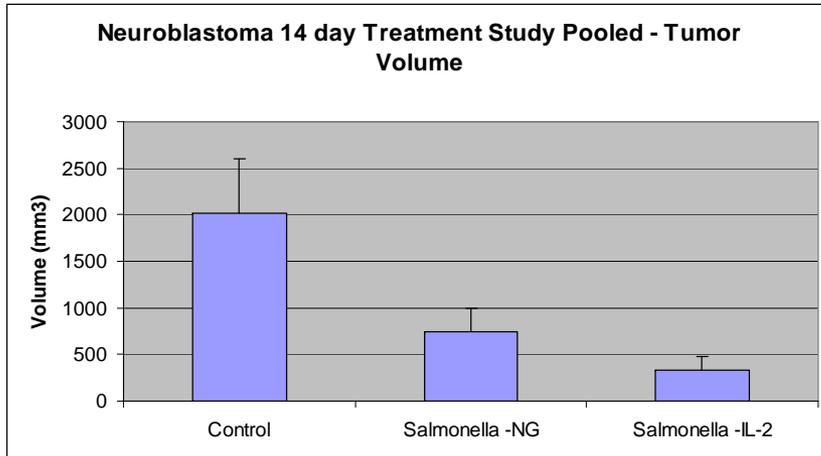


**Day 21, remaining animals
are sacrificed and tumors
enumerated and examined**

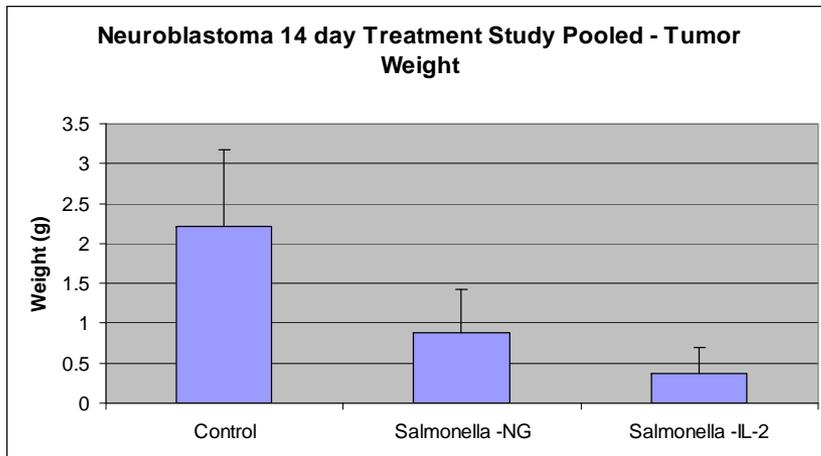
Neuroblastoma



14 Day Treatment Studies ONE ORAL DOSE



- Reduction of tumor size and weight by **63%** and **84%** respectively when compared to controls ($p < 0.001$).



Metastatic Osteogenic Sarcoma

- Most common bone cancer in teenagers and young adults
- Overall survival is 70%
- Up to 80% will develop lung metastases
- With lung mets – survival is 30%

Gross Examination

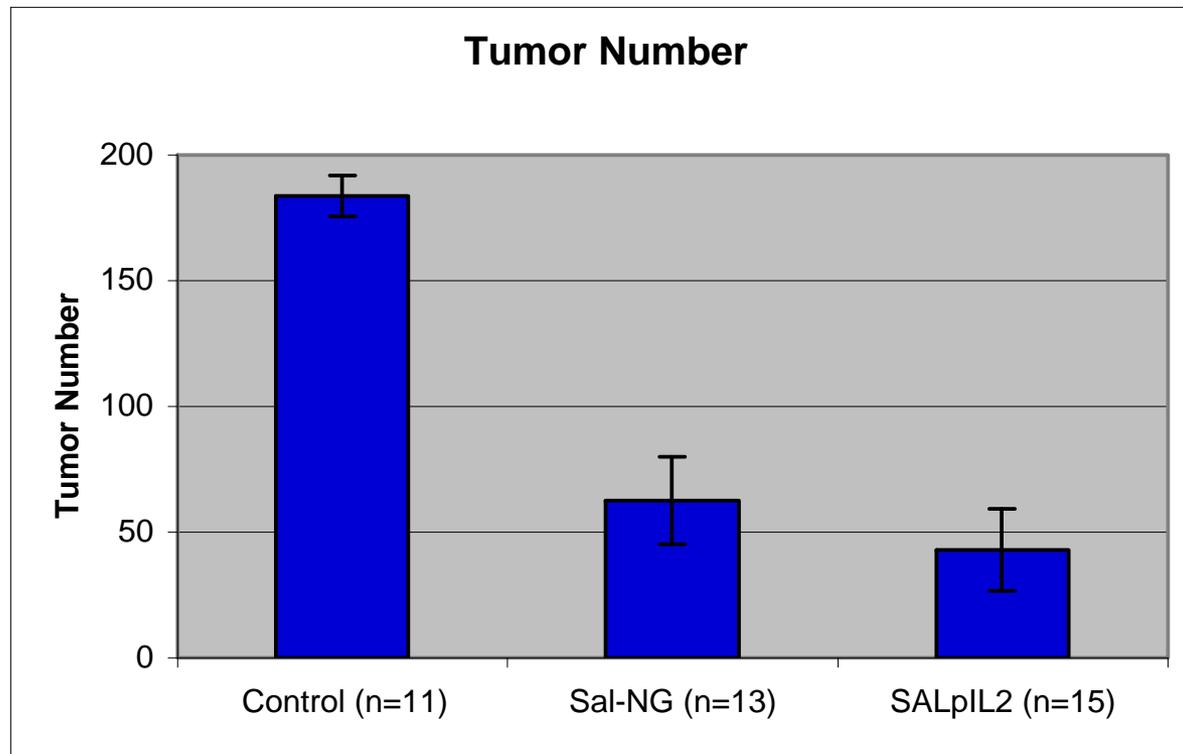


**Control:
Saline Gavage**

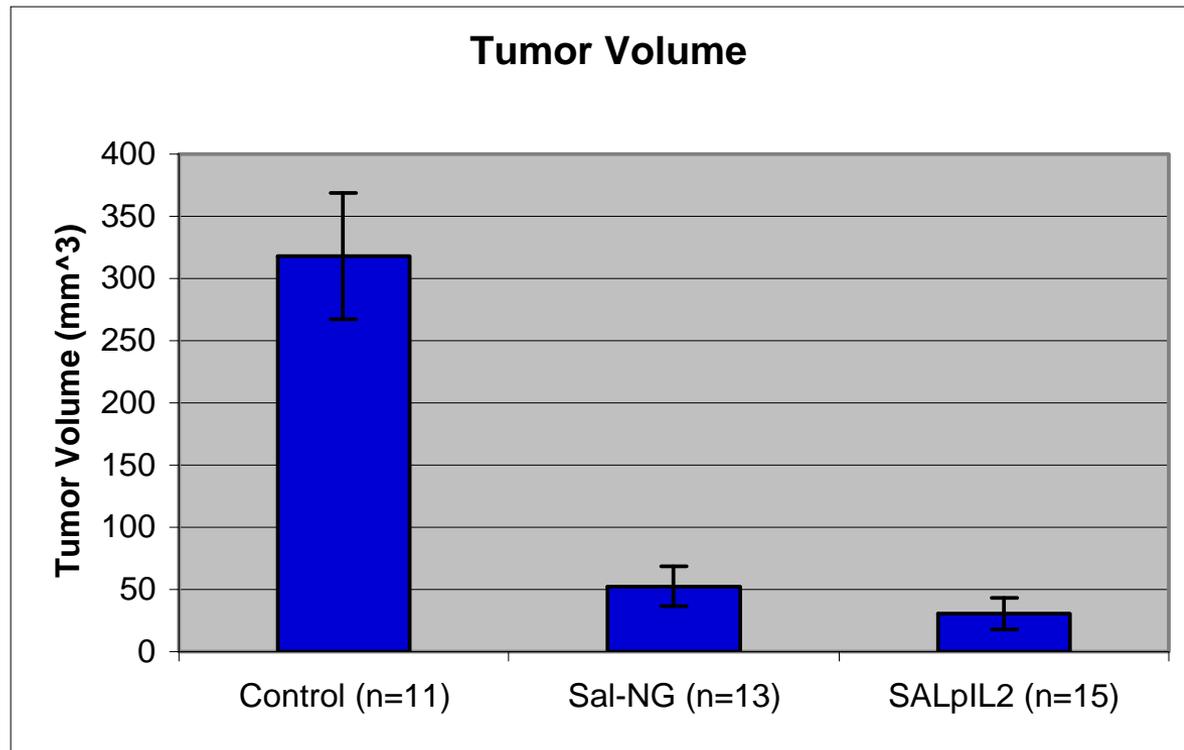


Gavage *SalpIL2*

Osteogenic Sarcoma Lung Metastases



Osteogenic Sarcoma Lung Metastases



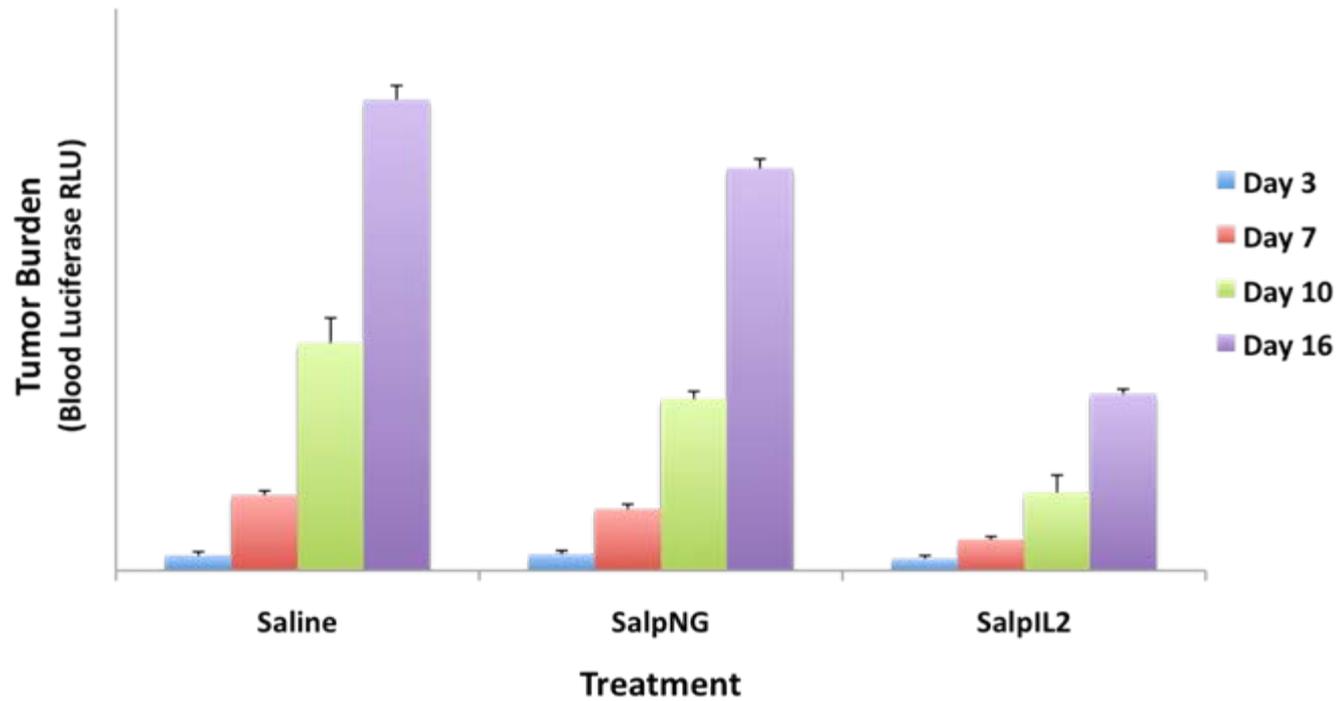
NCI R-21 Award

Pancreatic Cancer Studies

Preliminary Results

- Study of anti-tumor properties of Salmonella-IL2 in a murine model of primary pancreatic adenocarcinoma
- Model in immunocompetent mice
- Developed a luciferase expressing tumor line
- Limit need of sacrificing animals

Pancreatic Cancer Model



Untoward Effects

13 Published Studies

- In short – NONE
 - No deaths
 - No weight loss
 - No change in activity
 - Normal food and water intake

Comparison of the Safety and Immunogenicity of $\Delta aroC$ $\Delta aroD$ and Δcya Δcrp *Salmonella typhi* Strains in Adult Volunteers

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Conclusions

1. Salmonella-IL2 may represent a novel form of *in vivo* biotherapy.
2. Salmonella may be applied as a method to PREVENT metastases in those patients who are risk.
3. Attenuated *Salmonella typhimurium* accumulates preferentially in liver tumors, neuroblastoma, osteosarcoma, and recently shown pancreatic carcinoma.

Conclusions Continued

5. Salmonella accumulates within the tumor microenvironment (intracellular and extracellular)
6. Multifaceted anti-tumor mechanisms
 1. Immune mediated mechanisms – NK and CTL cell
 2. Anti-angiogenic mechanisms
 3. Depletion of metabolic support

Future Directions

- Phase I Trial
- Pancreatic Cancer
 - Developed a model in immunocompetent mice
 - National Cancer Institute Grant R21
- Develop a form of Salmonella carrying a more potent form of IL-2 with secretion capabilities
- Increase the functional expression of IL-2
- Study rare and common cancers
- Deduce mechanisms

Laboratory Accolades

- American Surgical Association Foundation Award
- American Society of Colorectal Surgeons, 2004 – Top Research Award
- American Academy of Pediatrics, 2005 – Top Research Award for Neuroblastoma
- Association for Academic Surgery, 2006 – Best Research Award

