# Breaking the Toxicity Barrier: Tumor Eradication in Pre-Clinical Models by Systemic Administration of a Multi-TLR, NOD and STING Agonist

HansonWade STING & TLR-Targeting Therapies Summit 2023


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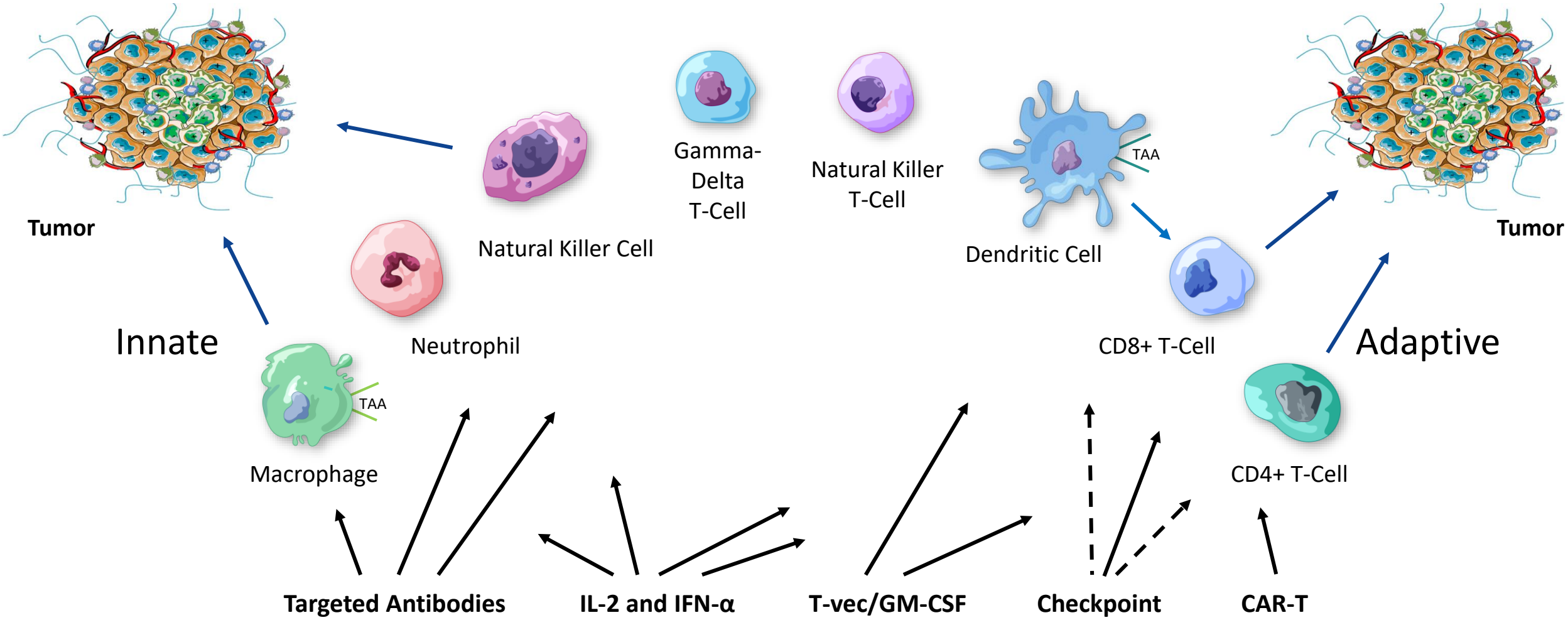
*Disclosure – Michael J. Newman is an employee, director and stockholder of Indaptus Therapeutics.*

# Current Cancer Immunotherapies: Low Percentage Cures for Most Advanced Cancers




Current immunotherapies only cure a very small percentage of advanced cancer patients, because they activate only one or a few innate or adaptive immune cell types

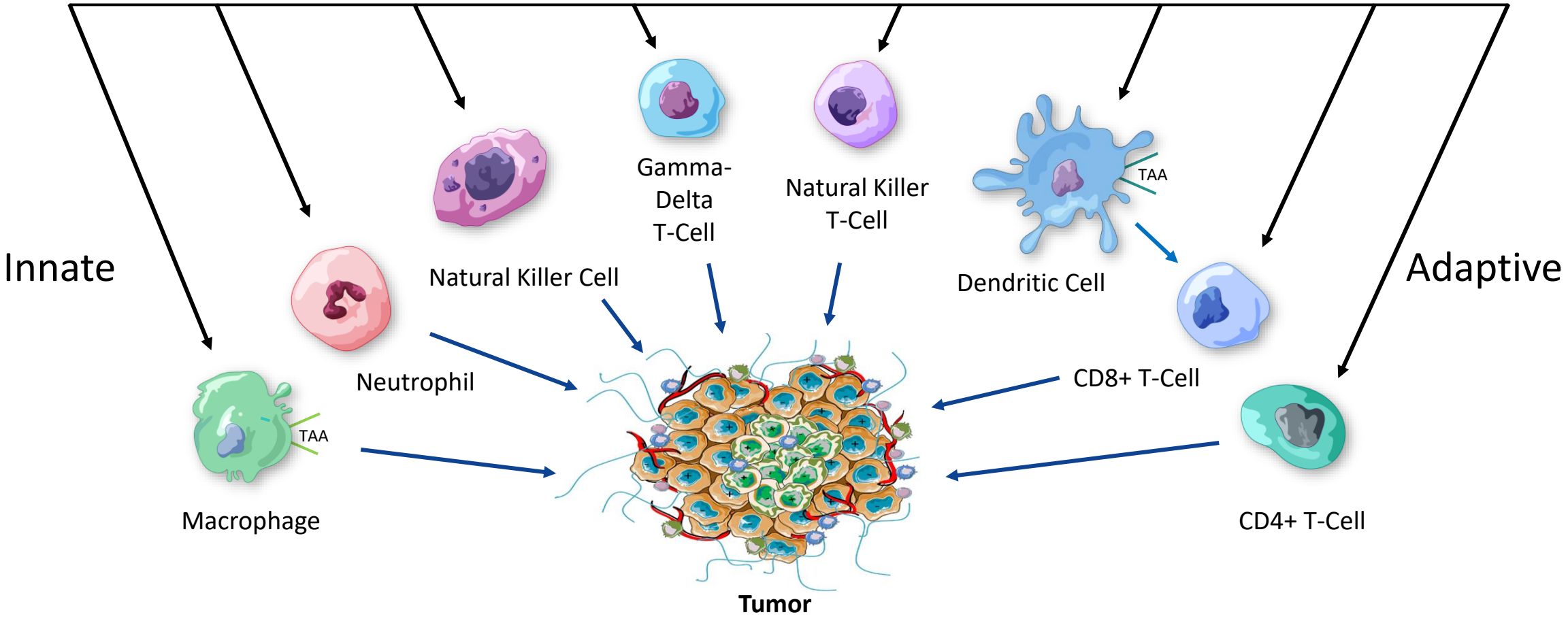
# Improving Cancer Immunotherapy - Indaptus Assumptions

- **We need to activate innate and adaptive immune pathways**
  - Innate and adaptive pathways complement/cooperate to produce maximum effect

- **We need to activate innate and adaptive immune pathways in tumor and lymphoid organs**
  - Most steps required for innate and adaptive immune responses take place outside of the tumor
  - Tumors negatively remodel entire systemic immune system
  - Systemic immunity is required for successful anti-tumor immunity
  - Hiam-Galvez Nature Rev Cancer 21 345 2021 for review

# Goal is Activation of Both Innate and Adaptive Cellular Pathways in Multiple Locations

No one has figured out how to do this safely




History provided a clue about how to do this

# World's First Immunotherapy: Coley's Toxins (CT)

- **Based on long-standing observation of spontaneous cancer regression in setting of infection**
  - Invented by Dr. William Coley at NYC precursor of Memorial Sloan Kettering in 1894
  - Composed of a heat-killed mixture of Gram-negative/positive pathogenic bacteria
- **Coley's Toxins produced durable responses with advanced cancer patients**
  - Mechanism of action was not known – impossible to optimize or standardize
  - Fifteen different protocols for manufacturing led to significant variability
  - Coley reported CT was likely most effective i.v., but was too toxic, so administered i.t. or s.c.
  - Chemotherapy and radiation therapy supplanted CT approach by mid-20$^{th}$ century
- **Despite lack of current use, historical observations provide clinical validation**
  - We now know that activation of the immune system can lead to durable anti-tumor responses
  - We also now know the mechanism of action of Coleys Toxins and why it was too toxic i.v.

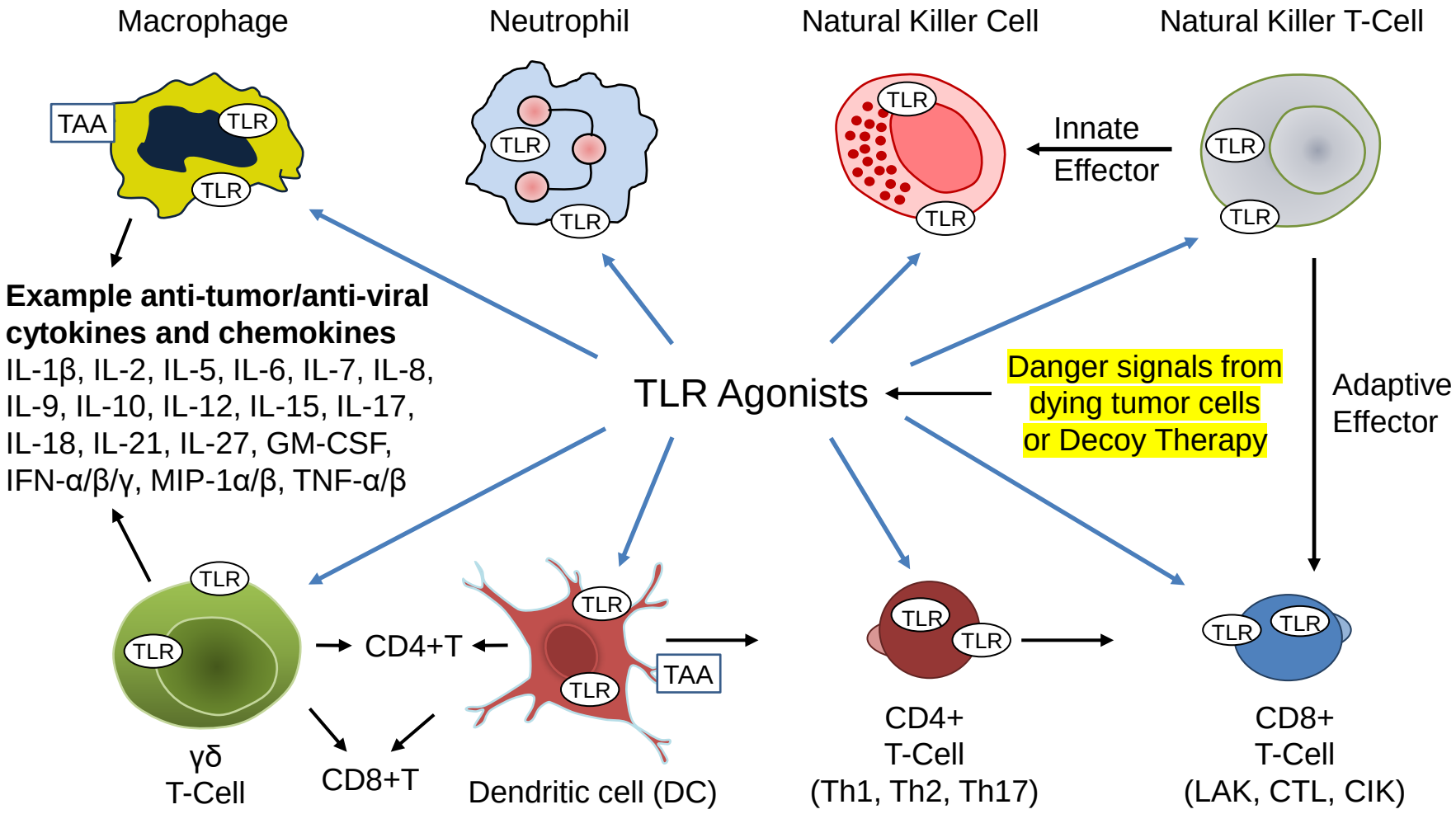
# Mechanism of Action of Coley's Toxins: Gram-Negative Bacteria Contain Immune System Stimulating Danger Signals

- **The most prominent danger signal family activates Toll-like receptors (TLR) on Immune Cells**

| Source | Danger Signal (TLR Ligand/Agonist) | Toll-Like Receptor on Immune Cells |
|---|---|---|
| Bacteria | Lipoproteins, Peptidoglycans | TLR2 (2/1, 2/6) |
| Viruses/Bacteria | Double Stranded RNA | TLR3 |
| Bacteria | Lipopolysaccharide (LPS-endotoxin) | TLR4 |
| Bacteria | Flagellin | TLR5 |
| Viruses/Bacteria | Single Stranded RNA | TLR7/8 |
| Bacteria | Unmethylated CpG DNA | TLR9 |

- **TLRs directly and indirectly activate essentially all immune cells (innate + adaptive)**
  - Indirect activation occurs via induction of secretion of cytokines and chemokines
- **Cytokines and chemokines are principal inducers of anti-tumor immune responses**
  - Innate - cell recruitment, macrophage activation, NK cell activation, γδT-cell activation, ↓Treg
  - Adaptive - cell recruitment, APC/DC activation, T-cell activation ($CD4_H$/$CD8_{CTL}$), ↓Treg
  - Cytokines can also (directly) kill tumor cells

# Toll-Like Receptor (TLR) Agonists from Bacteria Directly Activate Immune Cells and Indirectly Activate by Inducing Secretion of Cytokines and Chemokines




Immune cells can kill tumor or virus-infected cells or inhibit viral infection via cytokine secretion, cytotoxic granules, apoptosis, antibody-dependent cellular cytotoxicity (ADCC) and reactive oxygen/nitrogen species (RO/NS)

# Problem – IV Administered Gram-Negative Bacteria are Toxic

- **TLR4 agonist LPS-endotoxin constitutes ~75% of the Gram-negative outer cell membrane**
  - LPS is one of the most potent and broadly acting immune system danger signals
  - Limits the number of bacteria (and other danger signals) that can be administered i.v.

- **Two options – eliminate or reduce LPS (activator of TLR4)**
  - Elimination of LPS was tried (Vion Pharmaceuticals) – no anti-tumor activity in Phase 1
  - TLR4 is required for dendritic cell activation, antigen processing and presentation for anti-tumor immunotherapy (Fang Cell Mol Immunol 11 150 2014; Apetoh Nature Medicine 13 1050 2007)
  - LPS induces M1 Macrophage polarization, stimulates NK cells, maturation of APC/Dendritic cells, primes and amplifies T & B cell function and enhances Th1 immune responses (Buscher Nature Comm 8 16041 2017; Arenas Drug Targets 12 221 2012)
  - Better bet – reduce LPS by ~90%
    Remaining 10% might be enough and allow i.v. administration of more of everything else

# Indaptus Solution – Passively Targeted Pulse Priming (PTPP)

- **Hypothesis to produce an i.v.-safe and effective product**
  - Use a single, pure strain of non-pathogenic, Gram-negative bacteria
  - Selectively reduce LPS-endotoxin activity by ~90%
  - Kill and stabilize the bacteria so that they don't fall apart prior to immune cell clearance
- **Potential advantages of PTPP approach**
  - IV-administered bacteria are passively targeted to liver, spleen, (tumors?) and rapidly cleared (≤1 hr)
  - Innate and adaptive immune system priming or activation in lymphoid organs and tumor
  - Rapid clearance may reduce potential for systemic toxicities common with small molecule, protein and mammalian cell-based immunotherapies that depend on continuous exposure
- **Decoy products**
  - Frozen suspension of 100% killed, intact and stabilized bacteria
  - Broad and deep U.S./foreign issued patent coverage including compositions, methods and uses

# Patented Decoy Treatment Kills Bacteria and Significantly Reduces LPS-Endotoxin Activity and *In Vivo* Pyrogenicity

| Treatment | Live Bacteria | LPS Endotoxin Activity (LAL Assay) | Pyrogenicity Threshold (Rabbit Assay) |
|---|---|---|---|
| No Treatment | 100% | 44.7 Units / $10^6$ Bacteria | $3x10^4$ Bacteria |
| Decoy | 0% | 3.6 Units / $10^6$ Bacteria (92% reduction) | $9x10^5$ Bacteria (97% reduction) |

Decoy therapeutics are also 100 to 2,500-fold less toxic in mice ($LD_{50}$) than several live, attenuated bacterial products

# Decoy Treatment Reduces *In Vivo* Pyrogenicity and Toxicity, but Does Not Reduce (Most) Cytokine Secretion by Human Peripheral Blood Mononuclear Cells (PBMCs)

| **Secretion by Human PBMCs *In Vitro*** | **Untreated Bacteria** | **Decoy-Treated Bacteria (Decoy10) Research Strain** | **Decoy-Treated Bacteria (Decoy20) Drug Candidate strain** |
|---|---|---|---|
| Anti-Tumor Cytokine | 48 hr pg/mL peak (mean of triplicates) at same bacterial dose for each cytokine | | |
| GM-CSF | 1,094 | 1,197 | 1,695 |
| IFNγ* | 175,866 | 47,488 | 55,321 |
| IL-12p70 | 176 | 528 | 428 |
| TNFα | 49,782 | 77,919 | 99,247 |

*Same bacteria concentration for Untreated and Decoy-Treated, but doesn't represent the peak for Decoy10 or Decoy20

**Results suggest potential uncoupling of toxicity from anti-tumor activity**

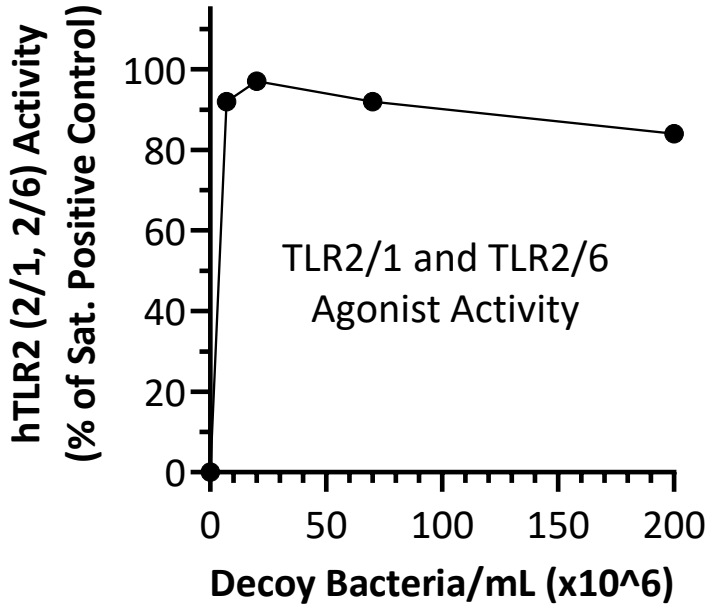
# Decoy Bacteria Induce Higher Levels of Cytokine Secretion by Human PBMCs Relative to Pure (Single) TLR Agonist Therapeutics

| | CpG ODN (TLR9) | Poly(I:C) (TLR3) | R848 (TLR8) | LPS (TLR4) | Decoy20 (TLR2,4,5,9) |
|---|---|---|---|---|---|
| Anti-Tumor Cytokine | 48 hr pg/mL (full titration peak / mean of triplicates) | | | | |
| GM-CSF | 0 | 0 | 87 | 175 | 1,695 |
| IFNγ | 7 | 103 | 31,324 | 29,416 | 75,530 |
| IL-12p70 | 4 | 18 | 253 | 109 | 428 |
| TNFα | 51 | 208 | 33,393 | 24,944 | 99,247 |

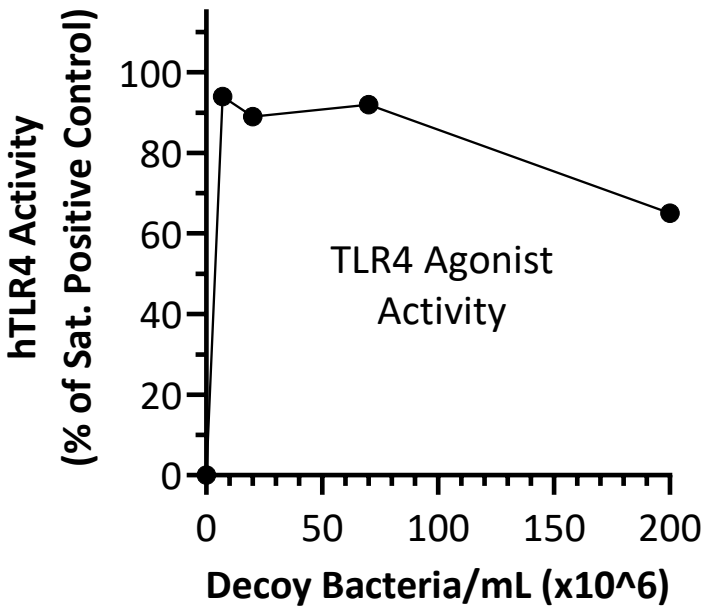
# Decoy Bacteria Contain TLR2 (2/1, 2/6), TLR4, TLR8, TLR9, NOD2 and STING Agonists

Decoy bacteria were tested in triplicate in Human Embryonic Kidney (HEK) reporter gene assays
Results are expressed as percent of saturating positive control activity

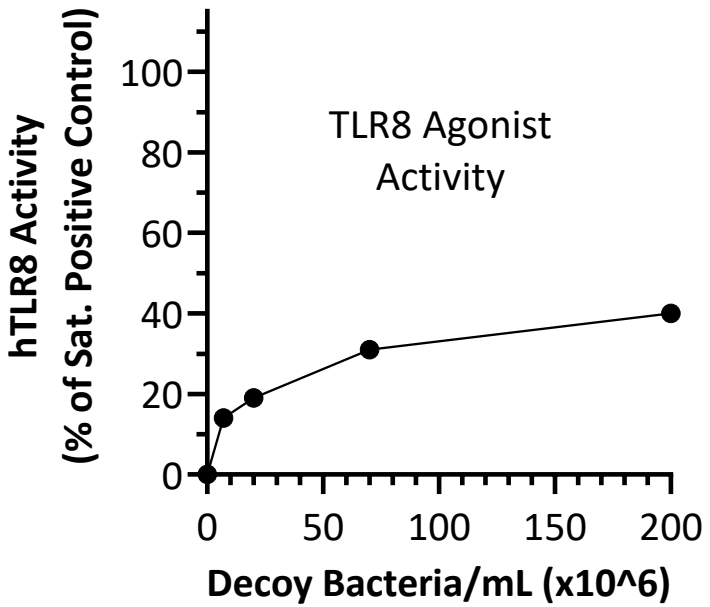
TLR2 control = Heat-Killed *L. monocytogenes*




TLR4 control = Purified *E. coli* K12 LPS




TLR8 control = TL8-506



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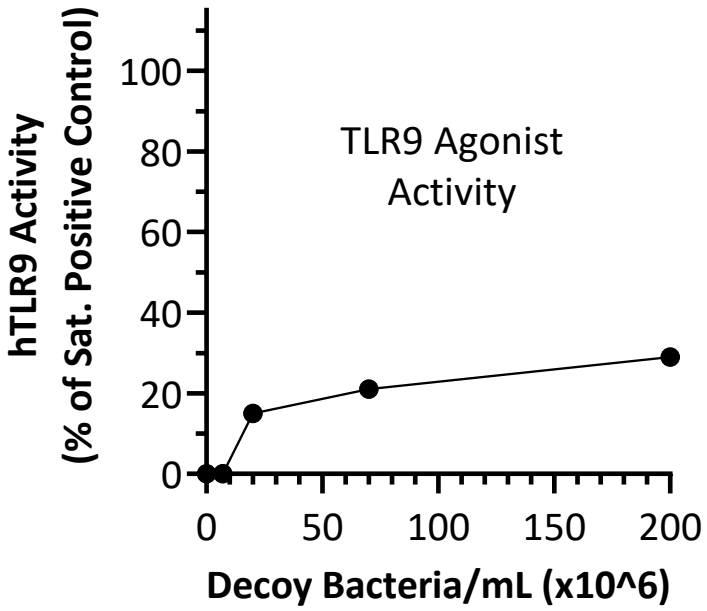
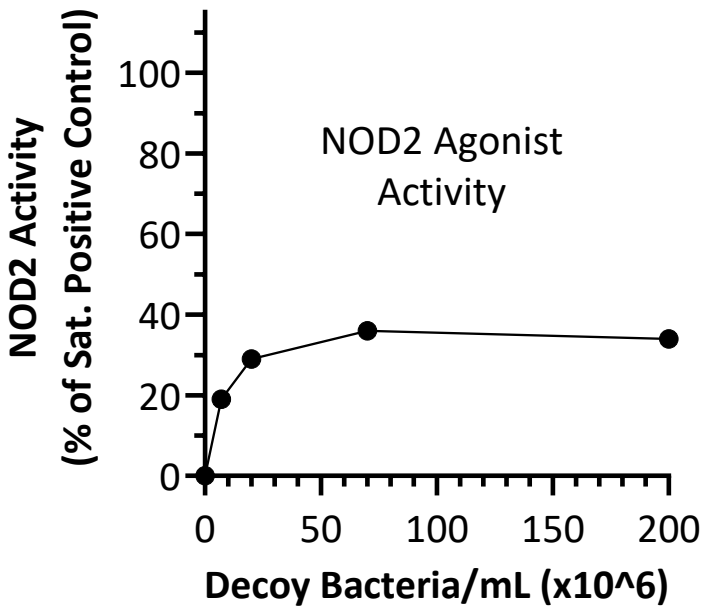
Decoy bacteria were tested in triplicate in Human Embryonic Kidney (HEK) reporter gene assays
Results are expressed as percent of saturating positive control activity

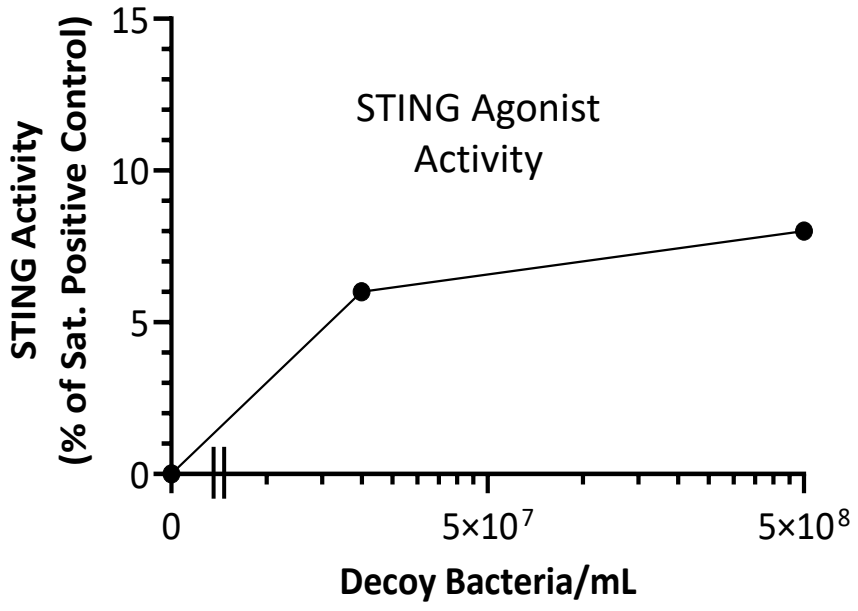
TLR9 control = CpG ODN 2006




NOD2 control = L18-MDP



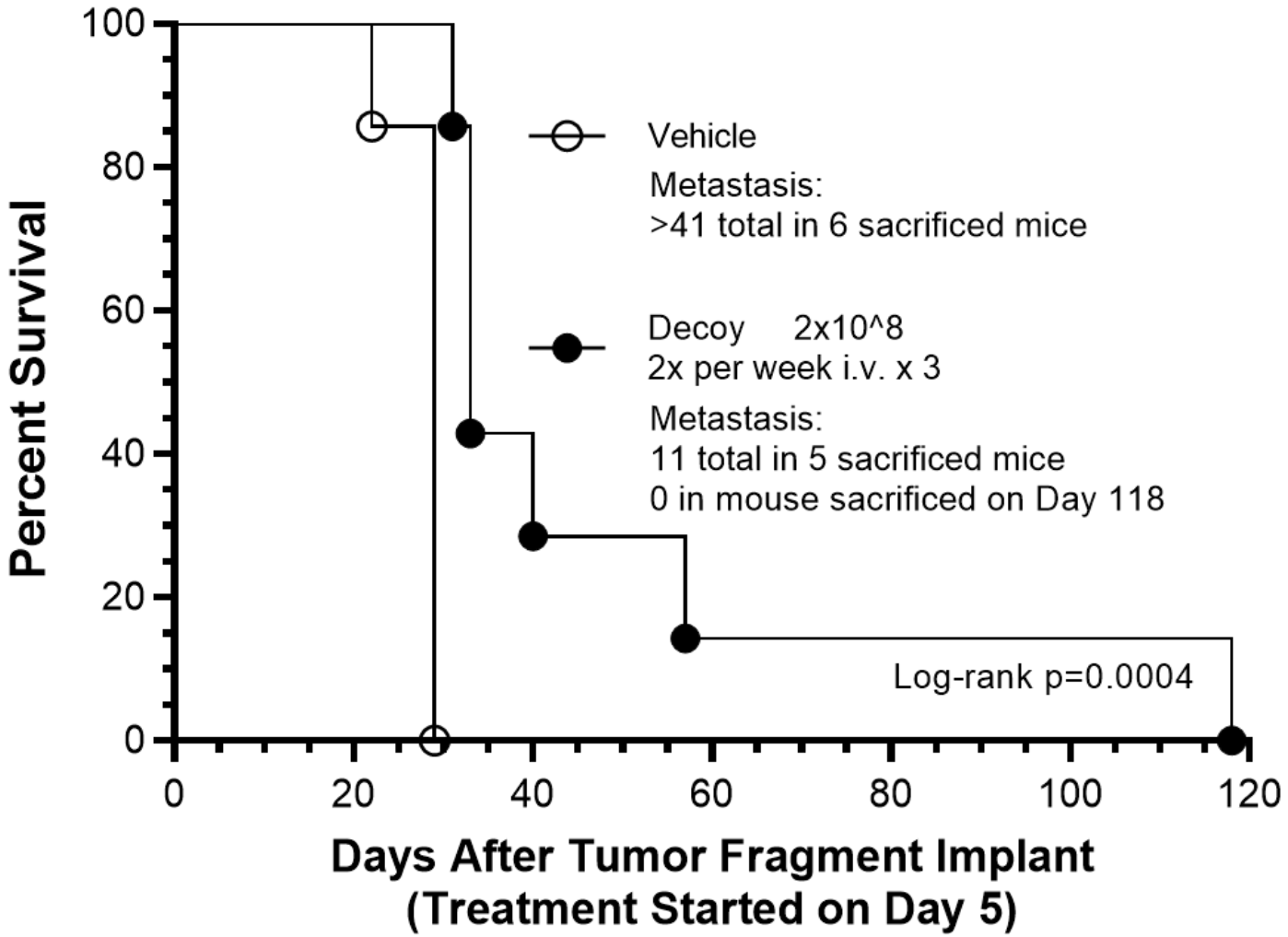

STING control = 2'3'-cGAMP



# Single Agent *In Vivo* Activity - Orthotopic CT26 Mouse Colorectal Carcinoma

Tumor fragments were sewn onto the cecum wall on Day 0 (7 mice/group)

Tumor cells express green fluorescent protein for metastasis imaging

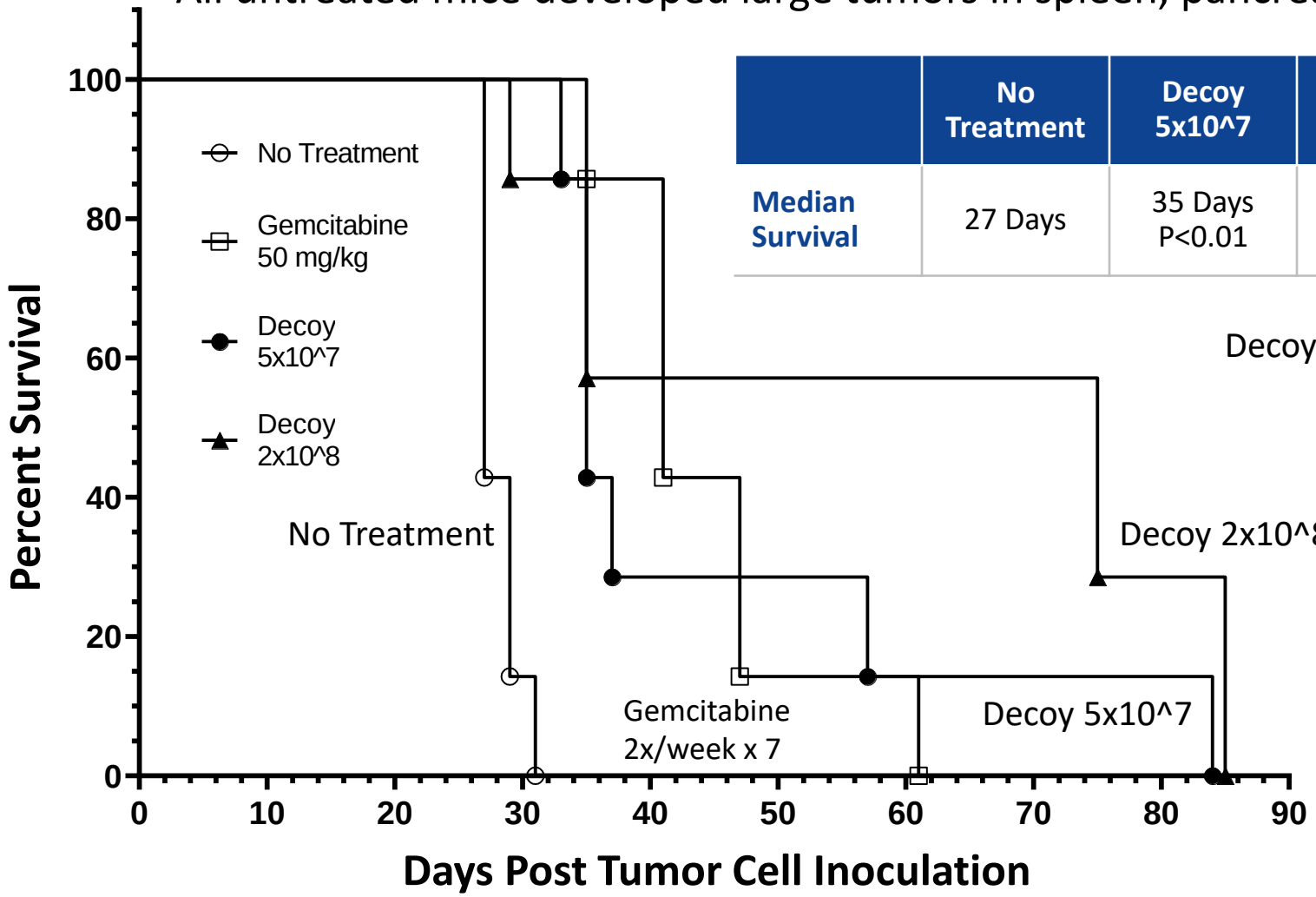


# Single Agent *In Vivo* Activity - Metastatic Pan02 Mouse Pancreatic Carcinoma

Pancreatic tumor cells were injected into the spleen on Day 0
All untreated mice developed large tumors in spleen, pancreas and liver

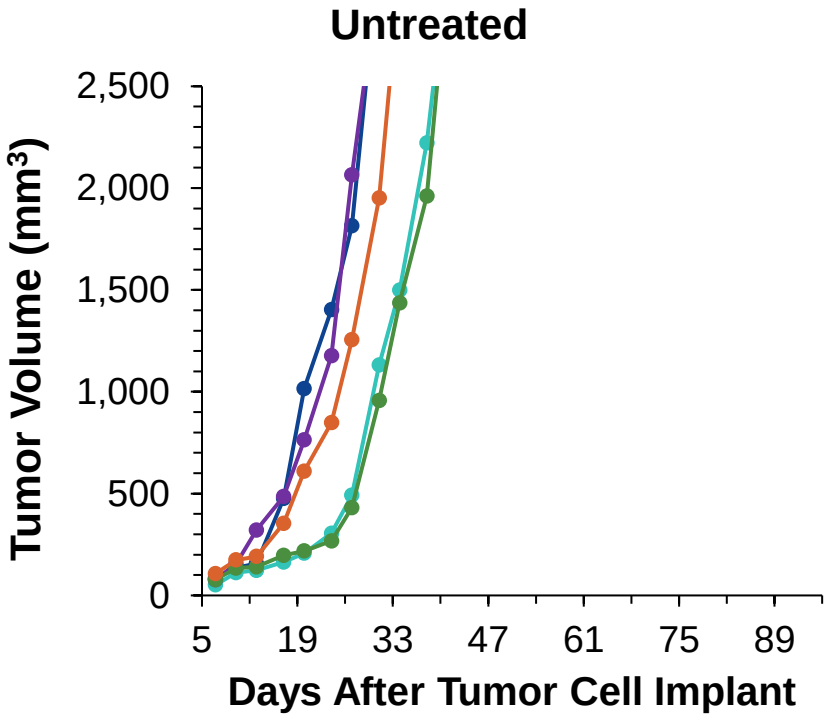
| | No Treatment | Decoy 5x10^7 | Gem | Decoy 2x10^8 |
|---|---|---|---|---|
| **Median Survival** | 27 Days | 35 Days P<0.01 | 41 Days P<0.01 | 75 Days P<0.01 |

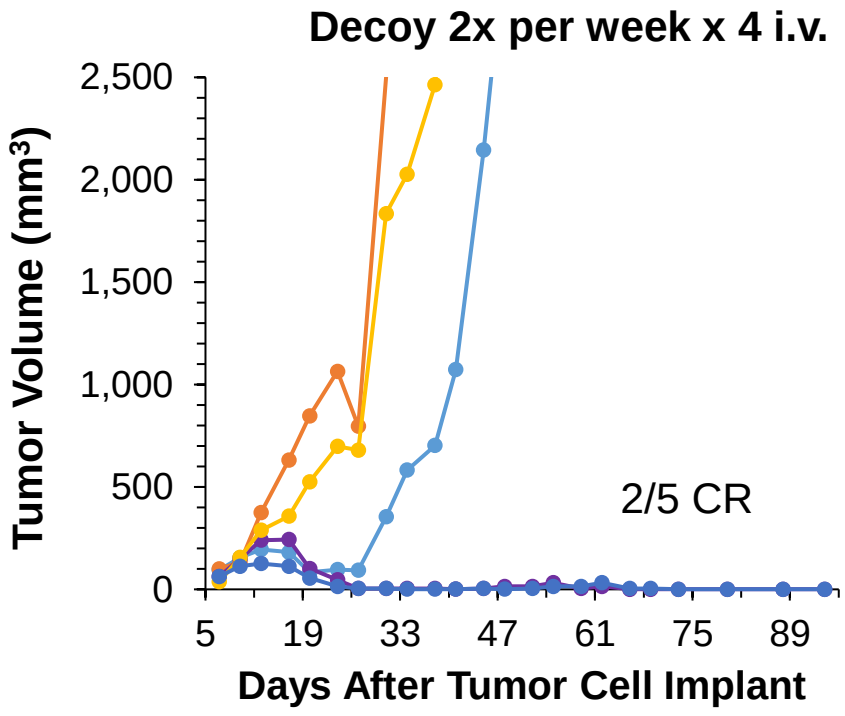
Decoy i.v. 2x per week x 3




(Treatment started on Day 5 with 7 mice/group)

# Single Agent-Mediated Regression of Established, EMT6-HER2 Antigen-Expressing, Mouse Breast Carcinoma Tumors by IV Decoy Bacteria



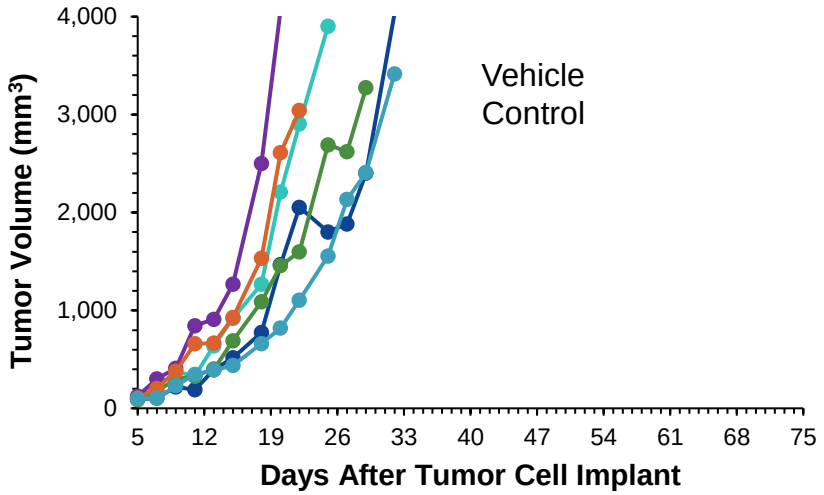



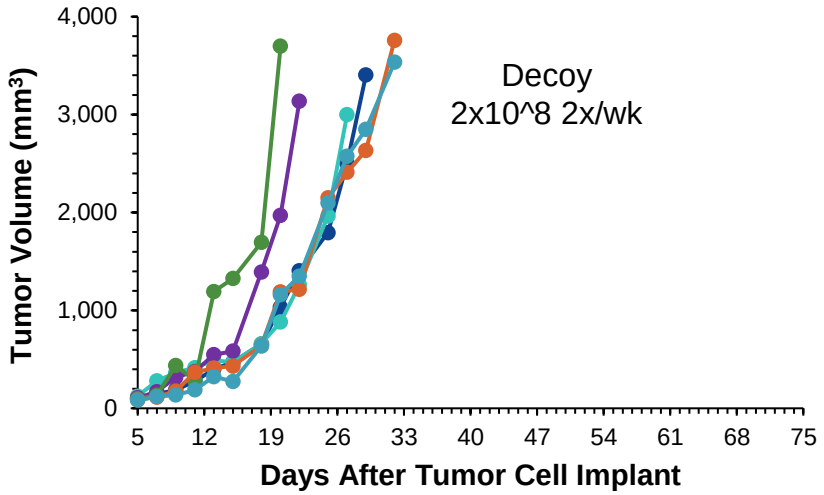

Decoy treatment started Day 12 with ~170 $mm^3$ tumors (5 mice per group)
Anti-PD-1 checkpoint therapy produced a similar result (3/5 CR, not shown)

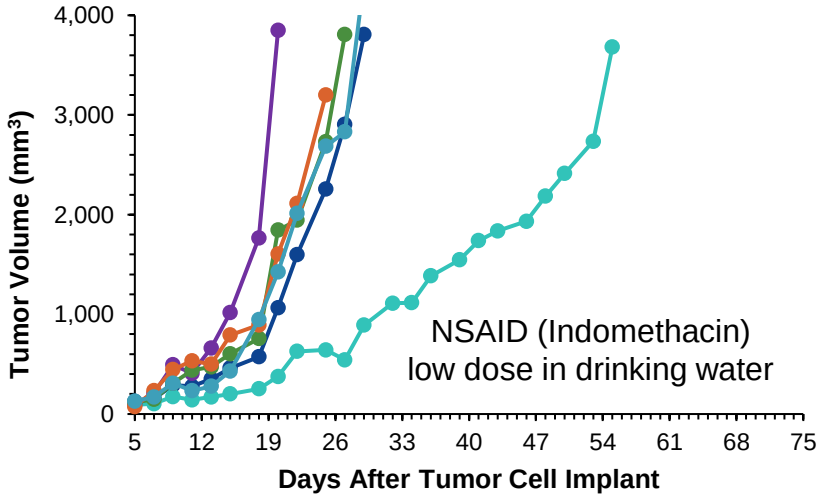
# Decoy Synergizes With a Non-Steroidal Anti-Inflammatory Drug (NSAID) to Safely Eradicate Subcutaneous H22 Mouse Hepatocellular Carcinomas (HCC)

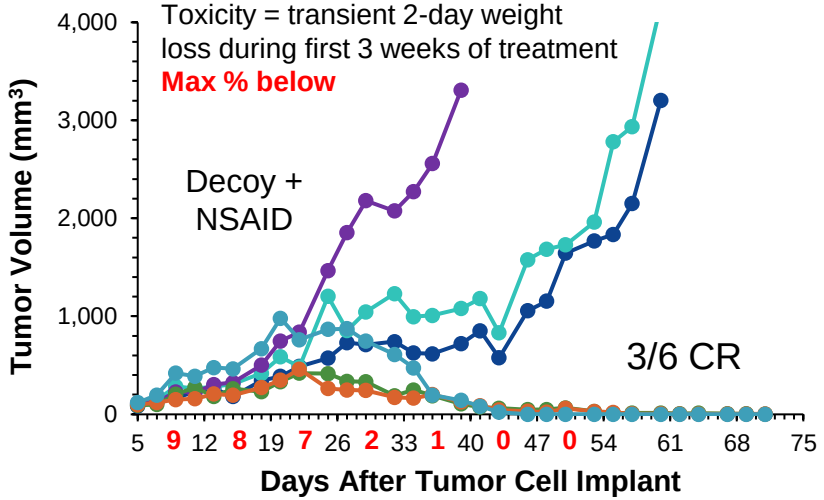
Treat 6 mice per group with Decoy 2x per week i.v. for 7 weeks / Start treatment at 103 $mm^3$

NSAIDS reduce myeloid-derived immune suppressive cells

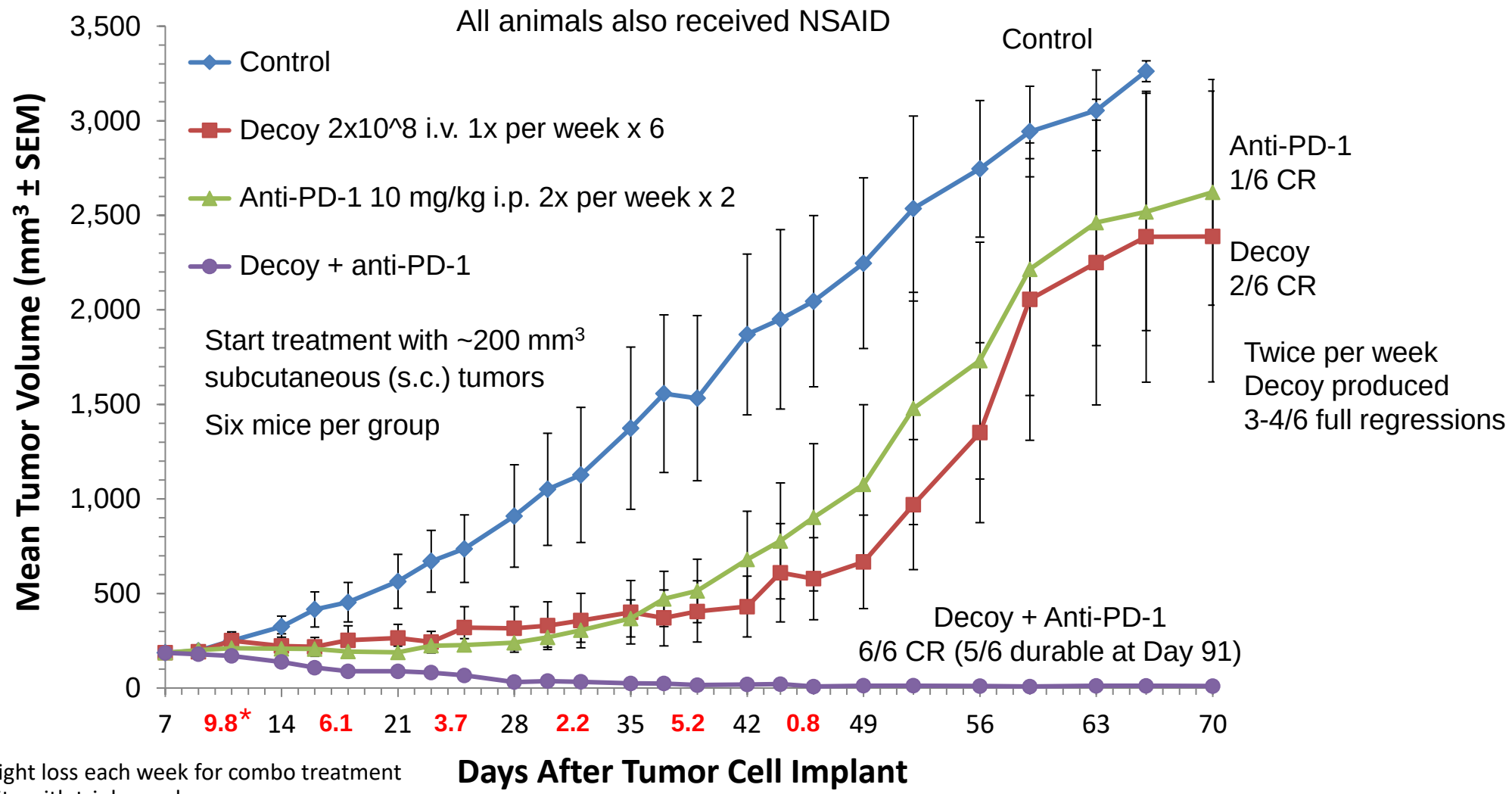


# Combination of Decoy With NSAID + Anti-PD-1 Checkpoint Therapy Produces 100% Complete Responses With H22 Hepatocellular Carcinoma

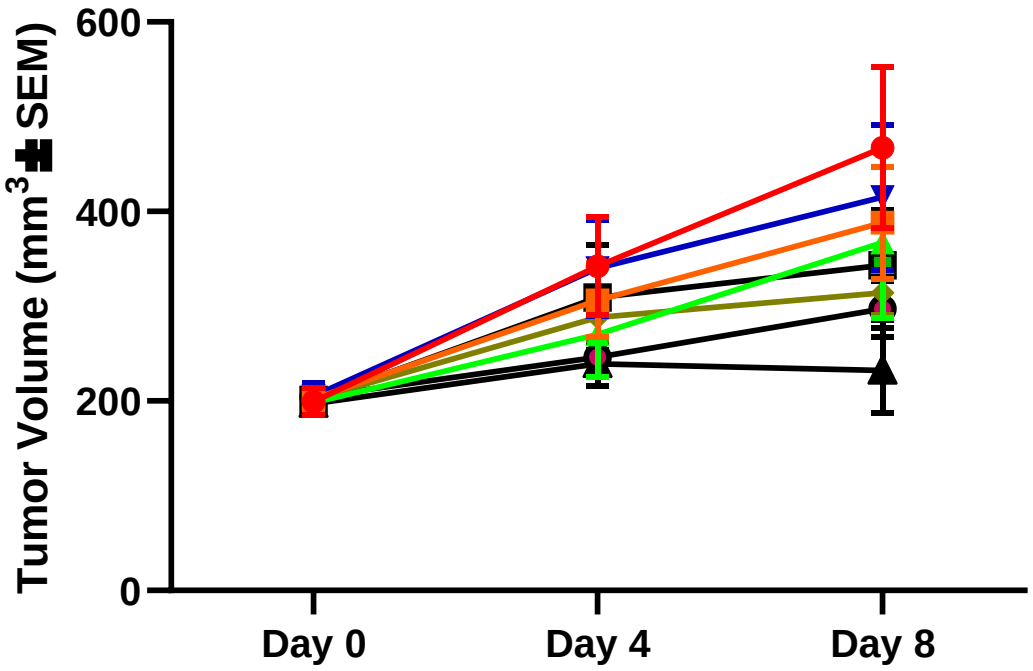



* Max % transient weight loss each week for combo treatment
No increase in toxicity with triple combo

# Tumor-Eradicating Combinations Transform “Cold” HCC Tumors to “Hot”

## HCC Model

**Treatment Effects on Tumor Volume**
**(RNA prepared on Day 8 for NanoString Analysis)**




**Fold increase in**
**Tumor Inflammation Signature (TIS)**
**“Cold” vs. “Hot” Tumor**

| | | |
|---|---|---|
| NT | | 0 / 6 |
| NSAID | 1.68 | 0 / 6 |
| Decoy | 1.61 | 0 / 6 |
| a-PD-1 | 2.30 | 0 / 6 |
| N + D | 2.21 | 2 / 6 |
| N + P | 2.92 | 1 to 2 / 6 |
| D + P | 3.60 | 2 / 6 |
| N + D + P | 5.89 | 5 to 6 / 6 |

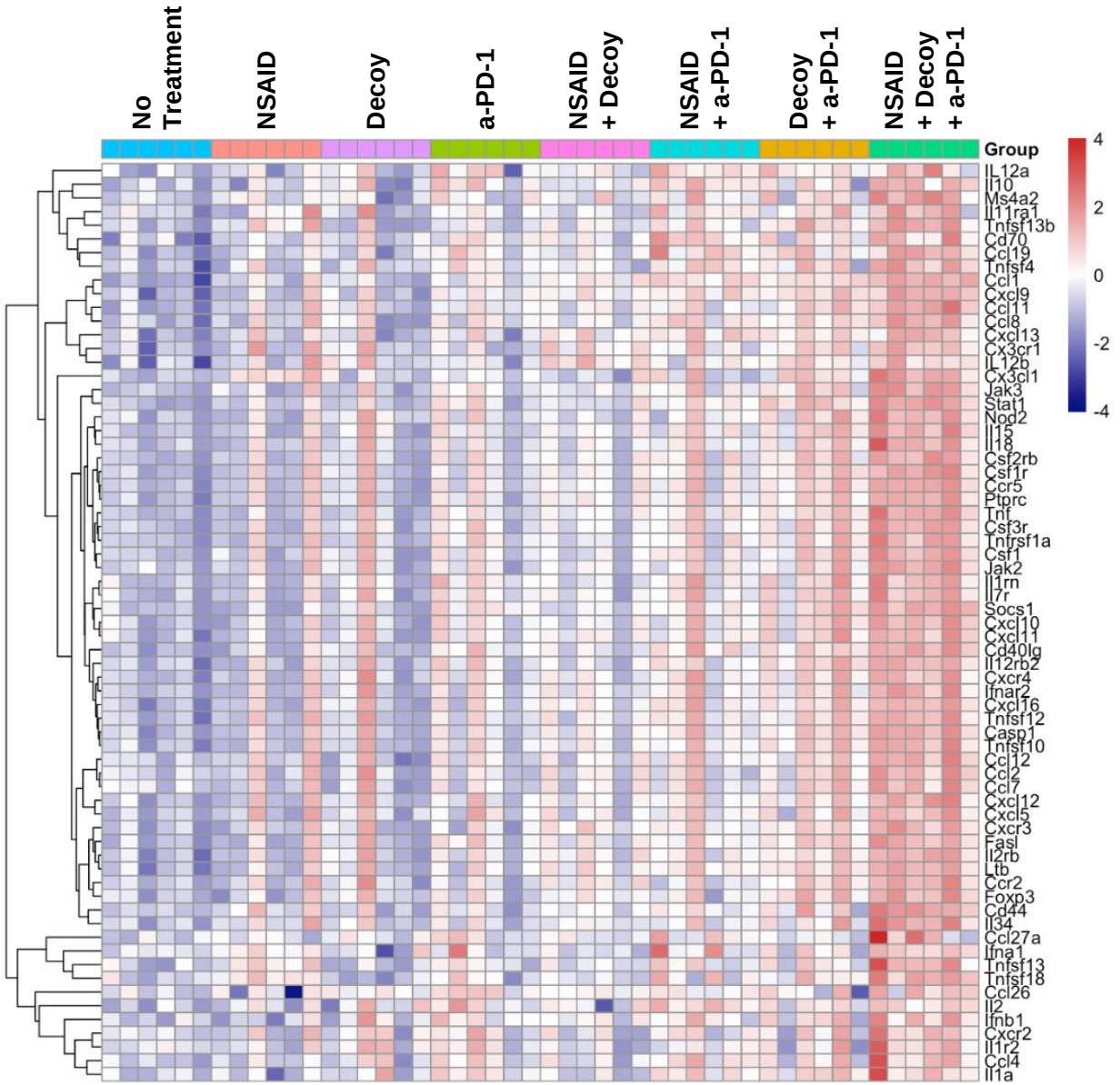
**Number of long-term regressions per group**

# Systemic Administration of Decoy Therapy (1 i.v. Dose), NSAID and Anti-PD-1 Induces **Cytokine** Immune Pathways in HCC Tumors

**HCC Model**

NanoString 770 gene expression analysis: Cytokines and cytokine pathways in tumor

Mice with 200 $mm^3$ tumors were treated for 1 week before tumor removal and RNA isolation/analysis



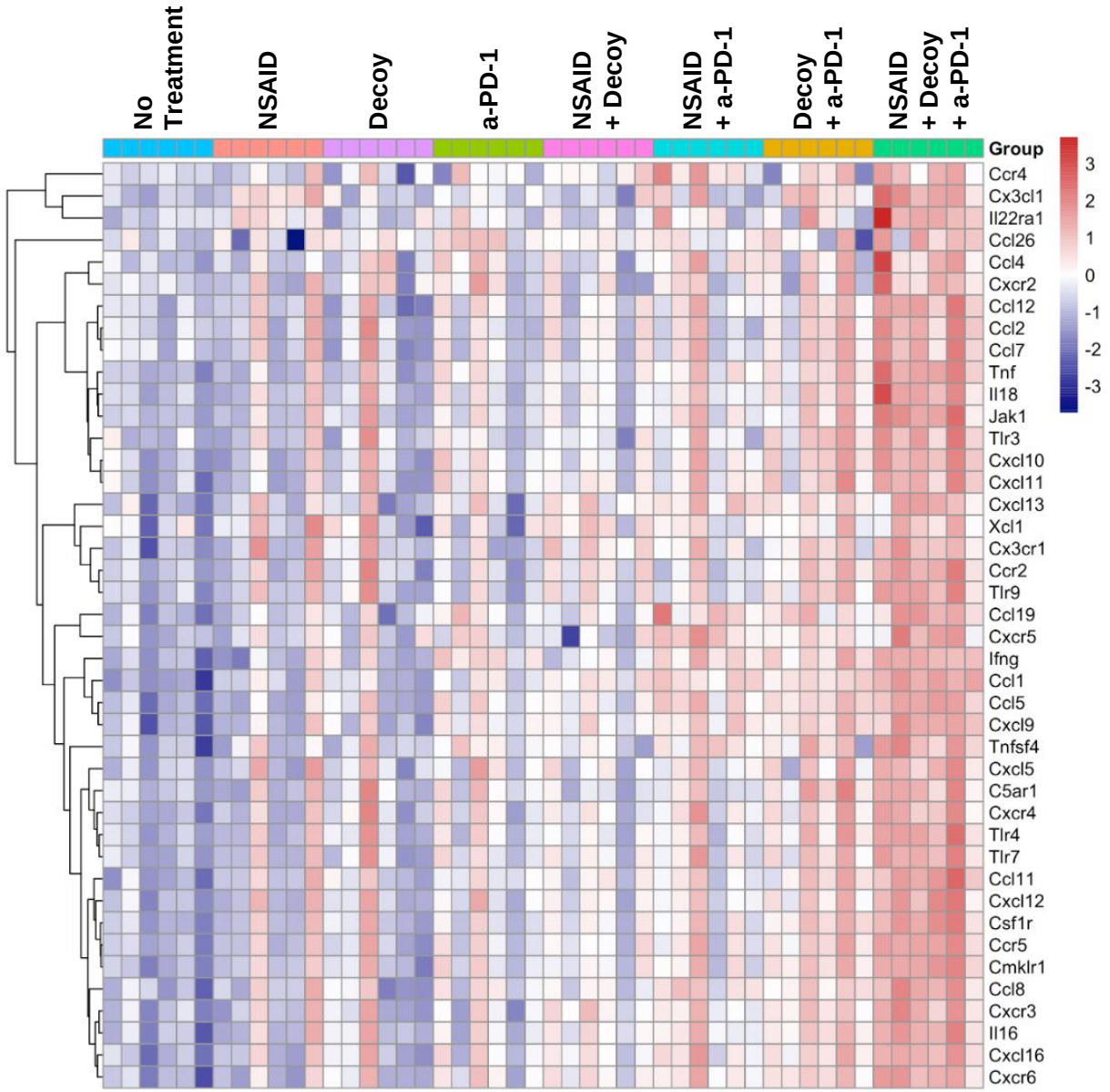

Each horizontal row represents a different cytokine or cytokine pathway gene (log base 2 scale)

# Systemic Administration of Decoy Therapy (1 i.v. Dose), NSAID and Anti-PD-1 Induces **Chemokine** Immune Pathways in HCC Tumors

**HCC Model**

NanoString 770 gene expression analysis: Chemokine or chemokine pathways in tumor

Mice with 200 $mm^3$ tumors were treated for 1 week before tumor removal and RNA isolation/analysis



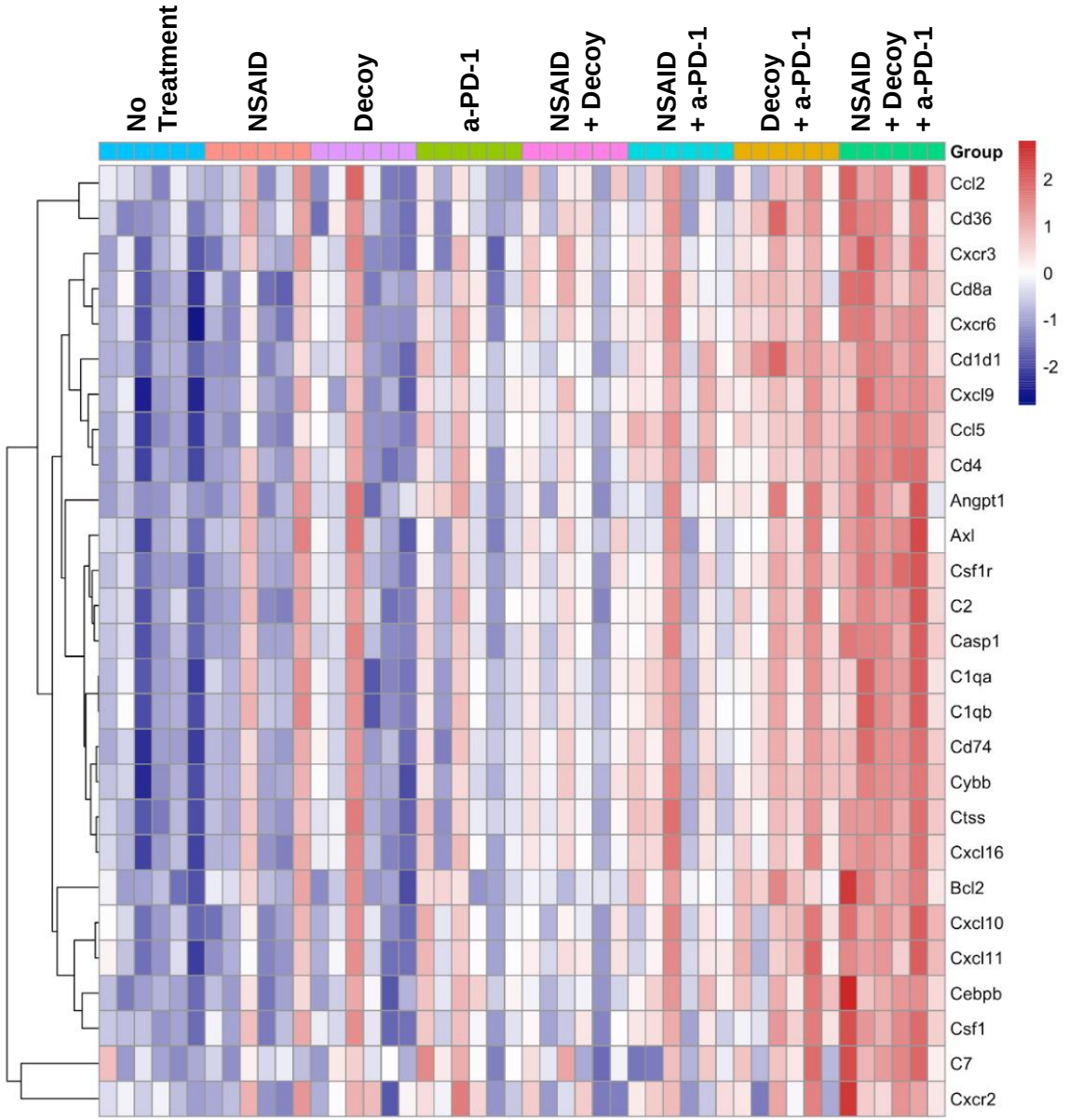

Each horizontal row represents a different chemokine or chemokine pathway gene (log base 2 scale)

# Systemic Administration of Decoy Therapy (1 i.v. Dose), NSAID and Anti-PD-1 Induces **Innate** Immune Pathways in HCC Tumors

**HCC Model**

NanoString 770 gene expression analysis: Innate immune pathways in tumor

Mice with 200 $mm^3$ tumors were treated for 1 week before tumor removal and RNA isolation/analysis



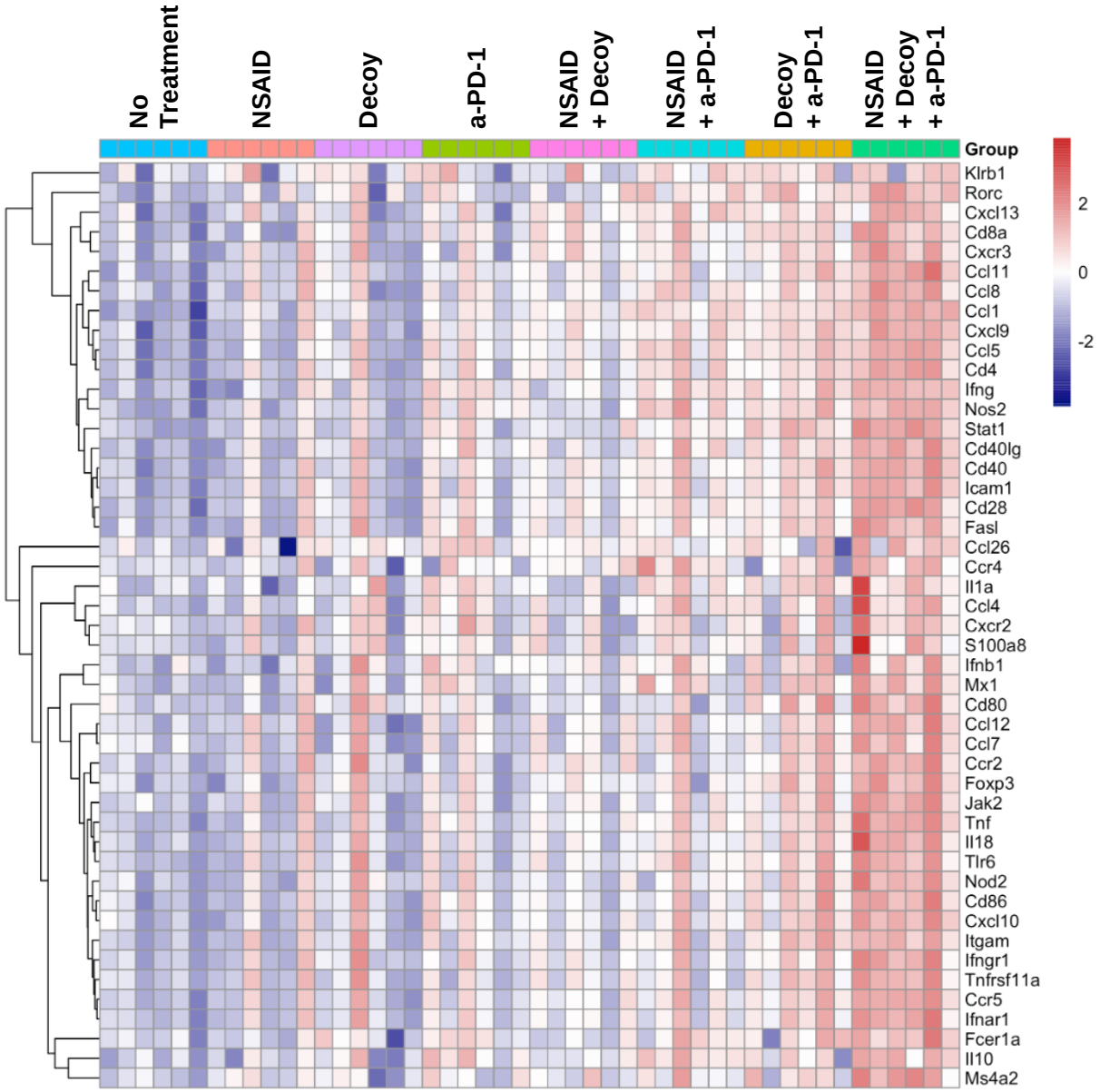

Each horizontal row represents a different innate immune pathway gene (log base 2 scale)

# Systemic Administration of Decoy Therapy (1 i.v. Dose), NSAID and Anti-PD-1 Induces **Adaptive** Immune Pathways in HCC Tumors

## HCC Model

NanoString 770 gene expression analysis: Adaptive immune pathways in tumor

Mice with 200 $mm^3$ tumors were treated for 1 week before tumor removal and RNA isolation/analysis

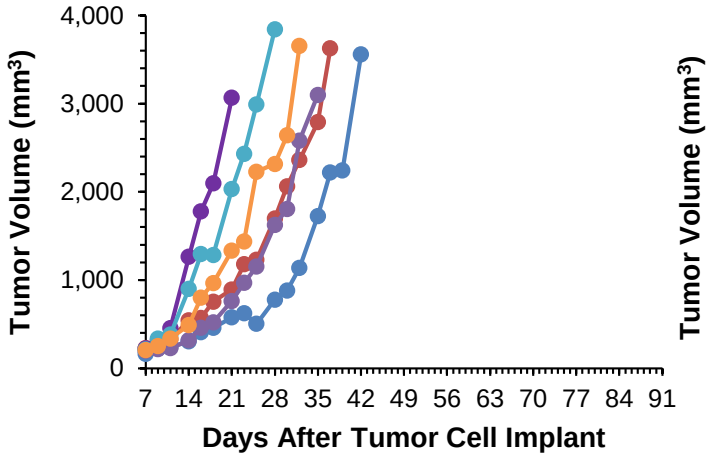



Each horizontal row represents a different adaptive immune pathway gene (log base 2 scale)

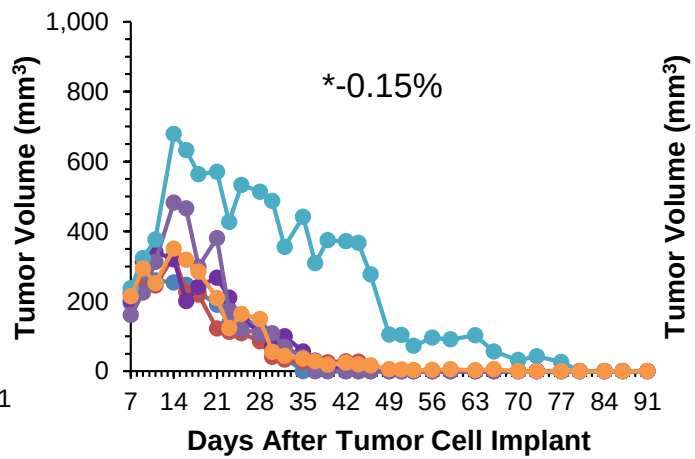
# Synergistic Eradication of H22 Murine HCC Exhibits a Very Wide Decoy Therapeutic Index (≥33-fold)

All Decoy-treated animals also received indomethacin + Anti-PD-1 therapy

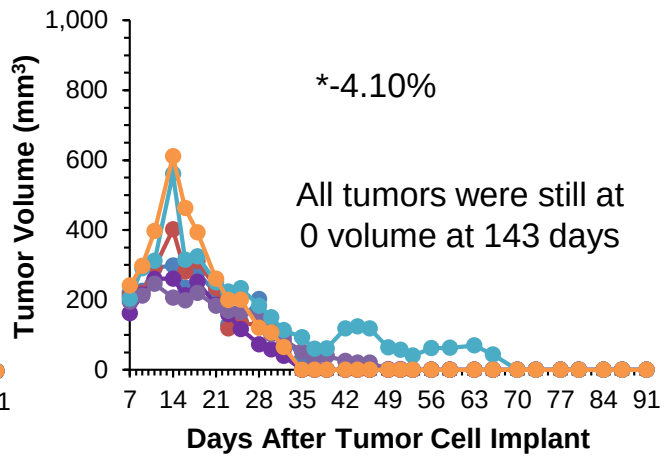
**No Treatment (6 mice per group)**




**3x10⁷ Decoy**

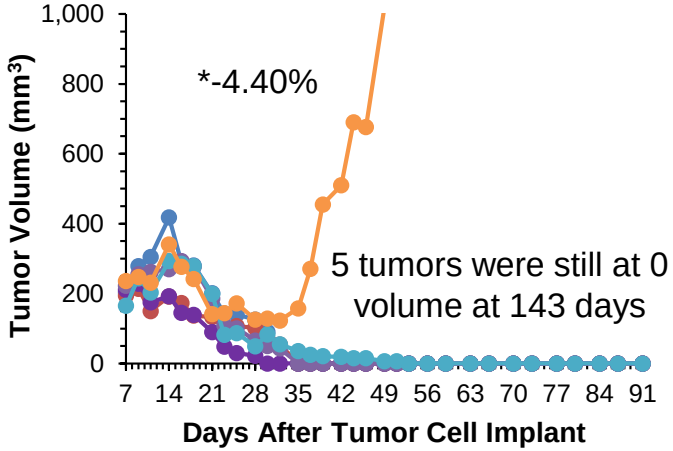



**1x10⁸ Decoy**

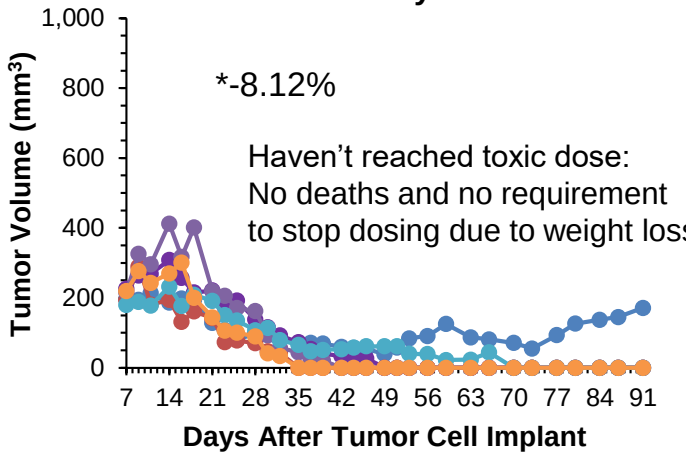



Start treatment at ~200 mm³

**3x10⁸ Decoy**



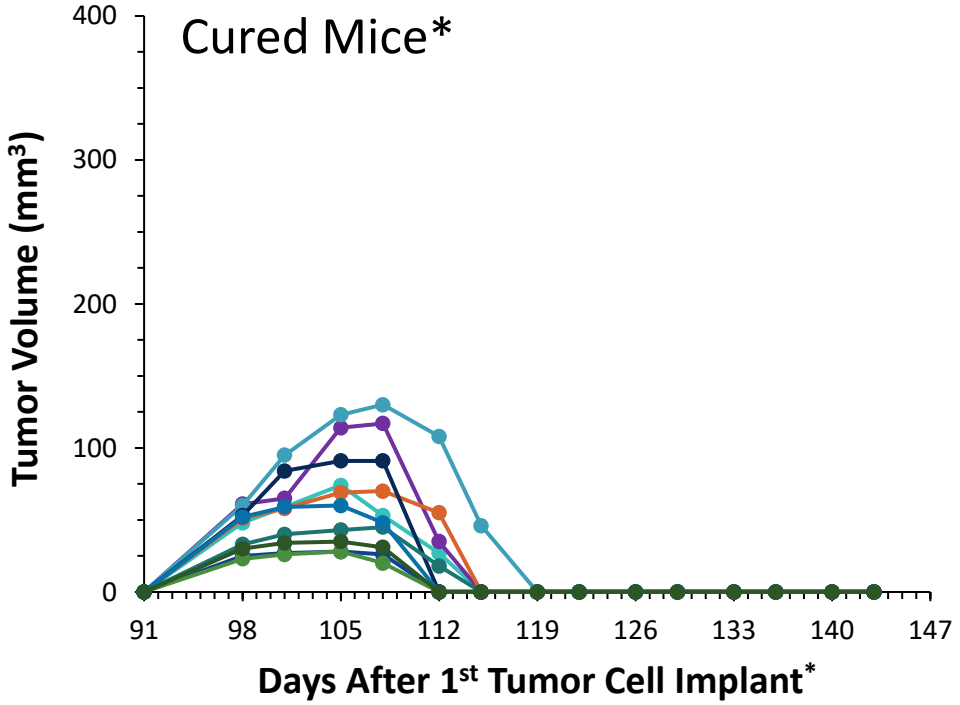

**1x10⁹ Decoy**




*Maximum transient body weight loss relative to start of treatment

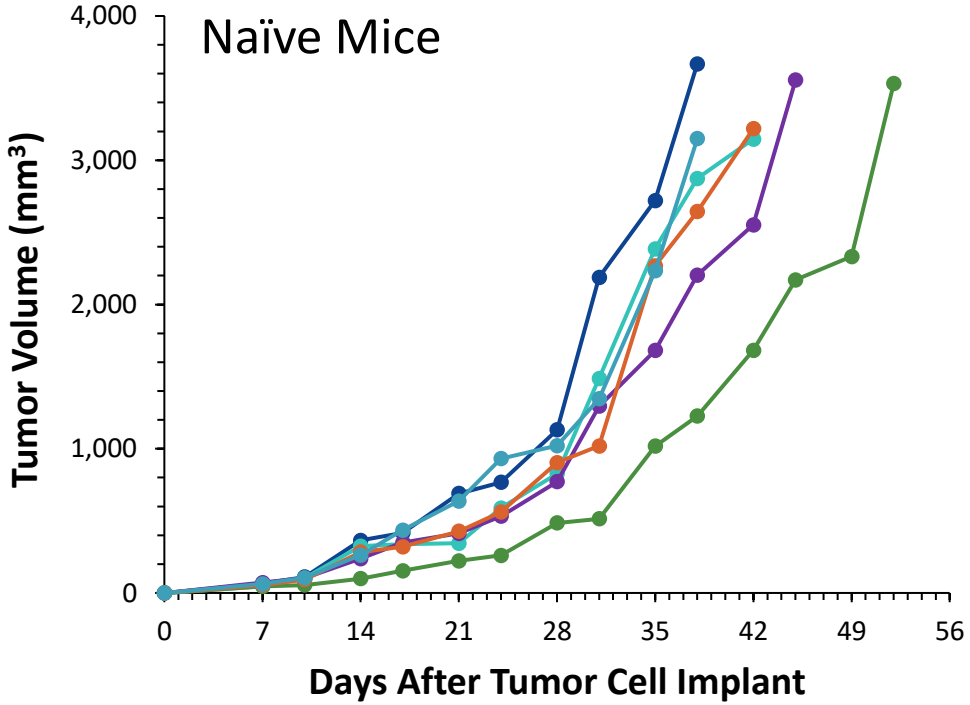
# Mice Cured by Decoy + NSAID + Anti-PD-1 and Re-Challenged with Fresh HCC Tumor Cells Reject the Tumors (Immunological Memory)

Eleven Cured Mice were Re-Challenged with Fresh HCC Tumor Cells on Day 91 on the Opposite Flank from the First Challenge



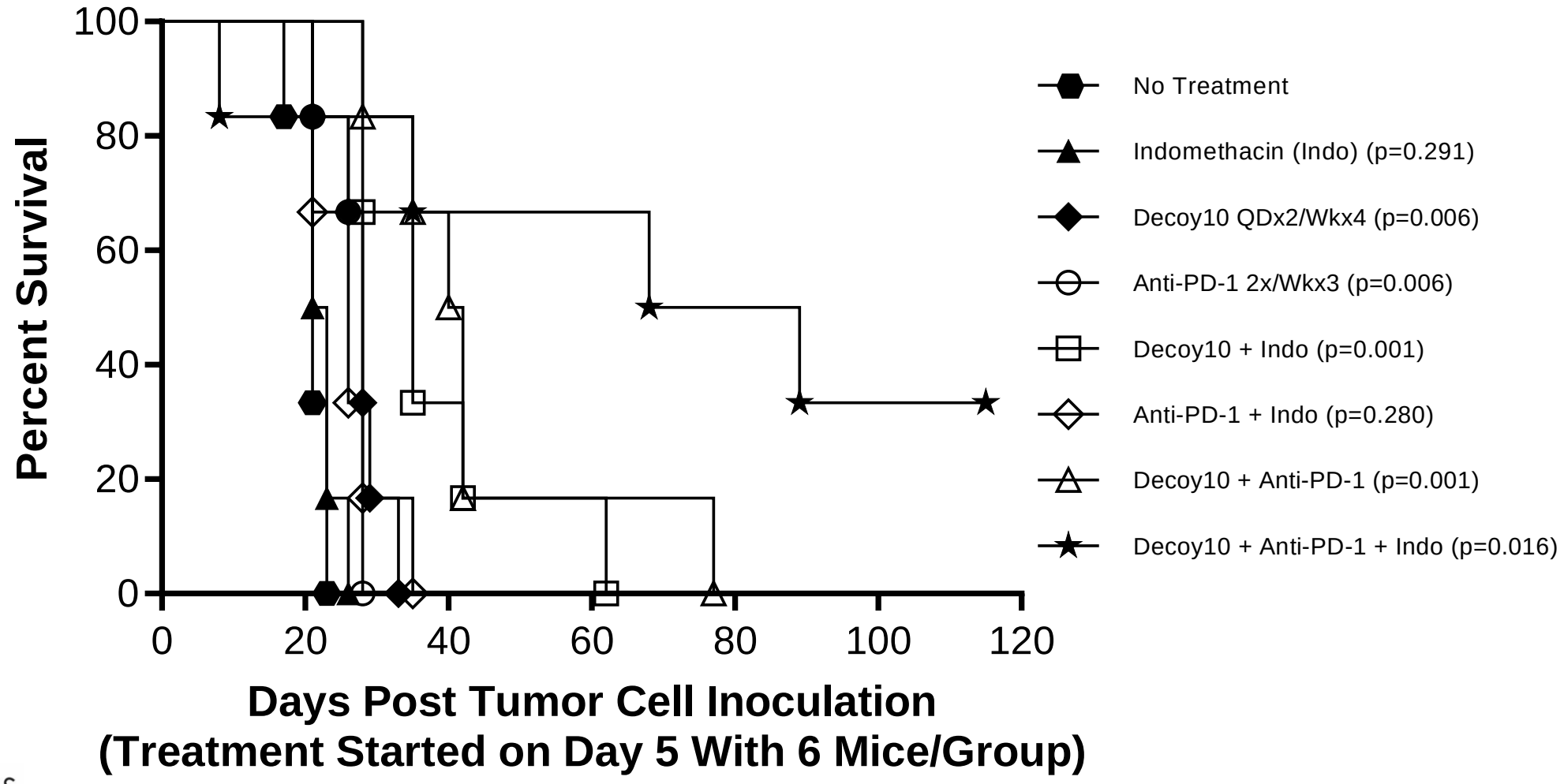

*All 1st challenge tumor sites remained tumor-free

Six Naïve Mice were Challenged with the Same Tumor Cells as the Cured Mice on the Same Day



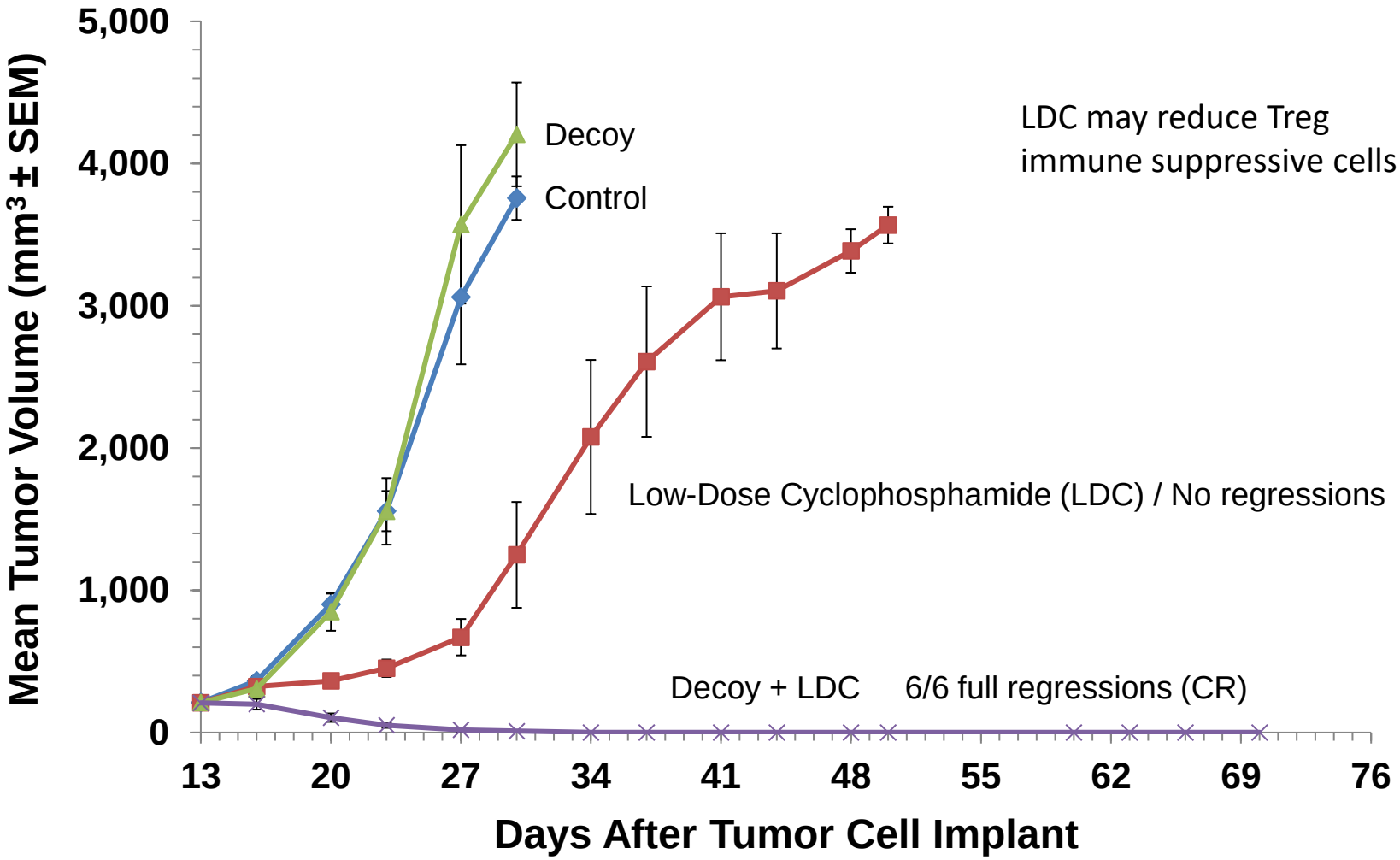
# Combination of Decoy + NSAID + Anti-PD-1 Checkpoint Therapy Extends Survival in a Metastatic Pan02 Mouse Pancreatic Carcinoma Model

Pancreatic tumor cells were injected into the spleen on Day 0
All untreated mice developed large tumors in spleen, pancreas and liver

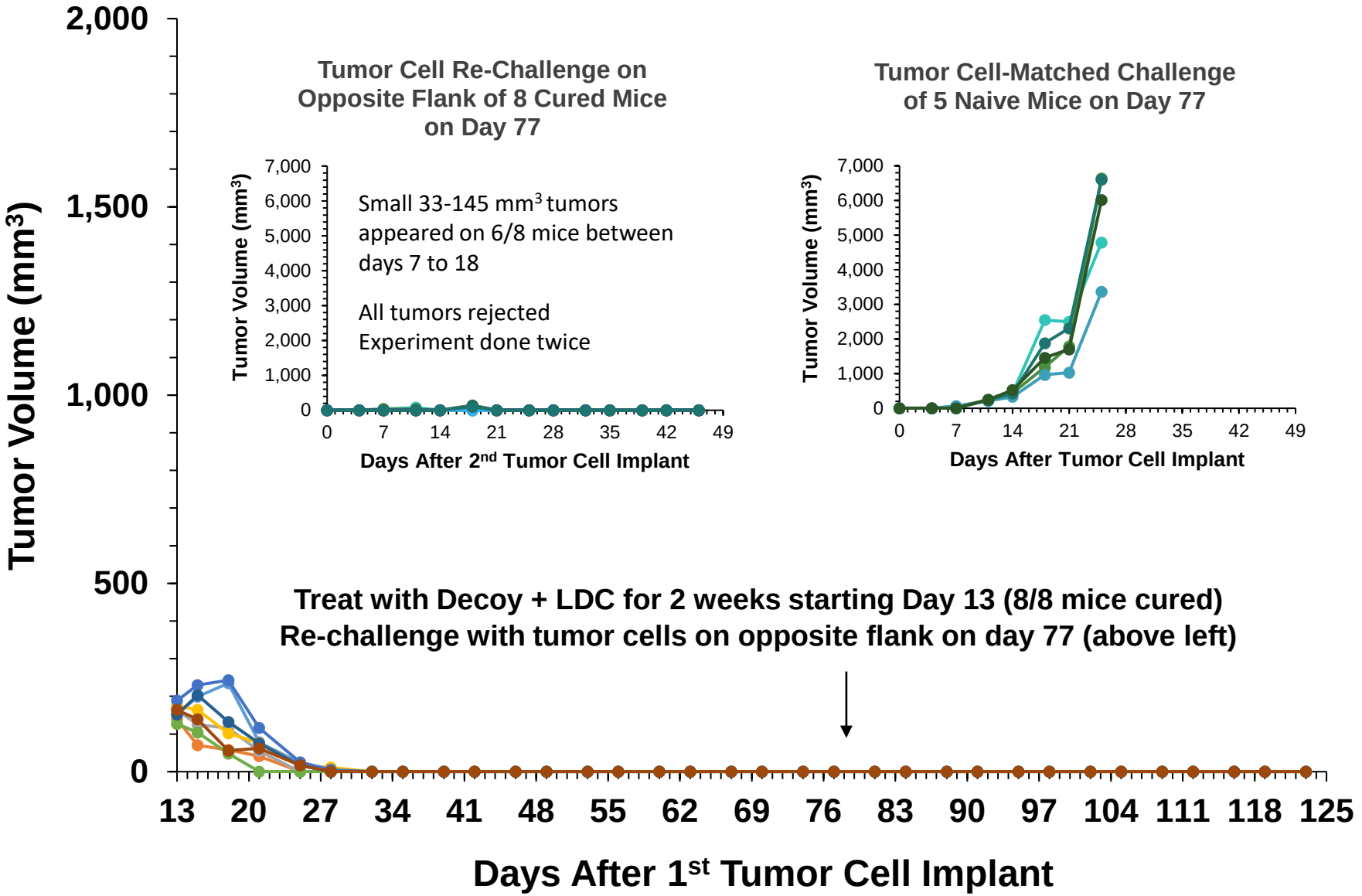


# Decoy Therapeutic Synergizes with Low-Dose Cyclophosphamide (LDC) to Safely Induce Regression of s.c. A20 Mouse Non-Hodgkin's-Lymphoma (NHL)

Treat 6 mice per group with Decoy i.v. 2x per week for 2 weeks / Start treatment at ~200 $mm^3$

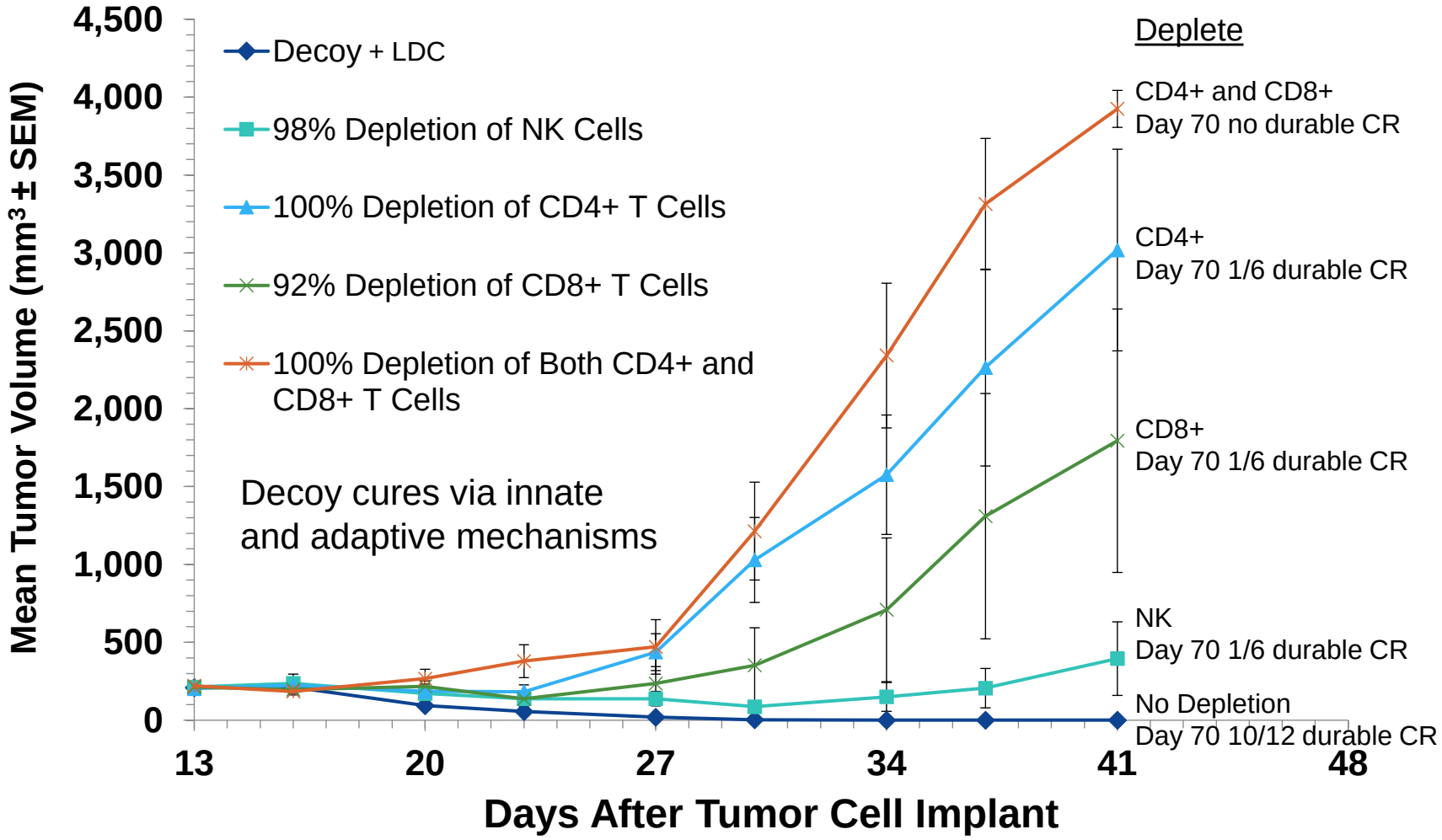


# Mice Cured by Decoy + LDC and Re-Challenged with Fresh NHL Tumor Cells Reject the Tumors (Immunological Memory)

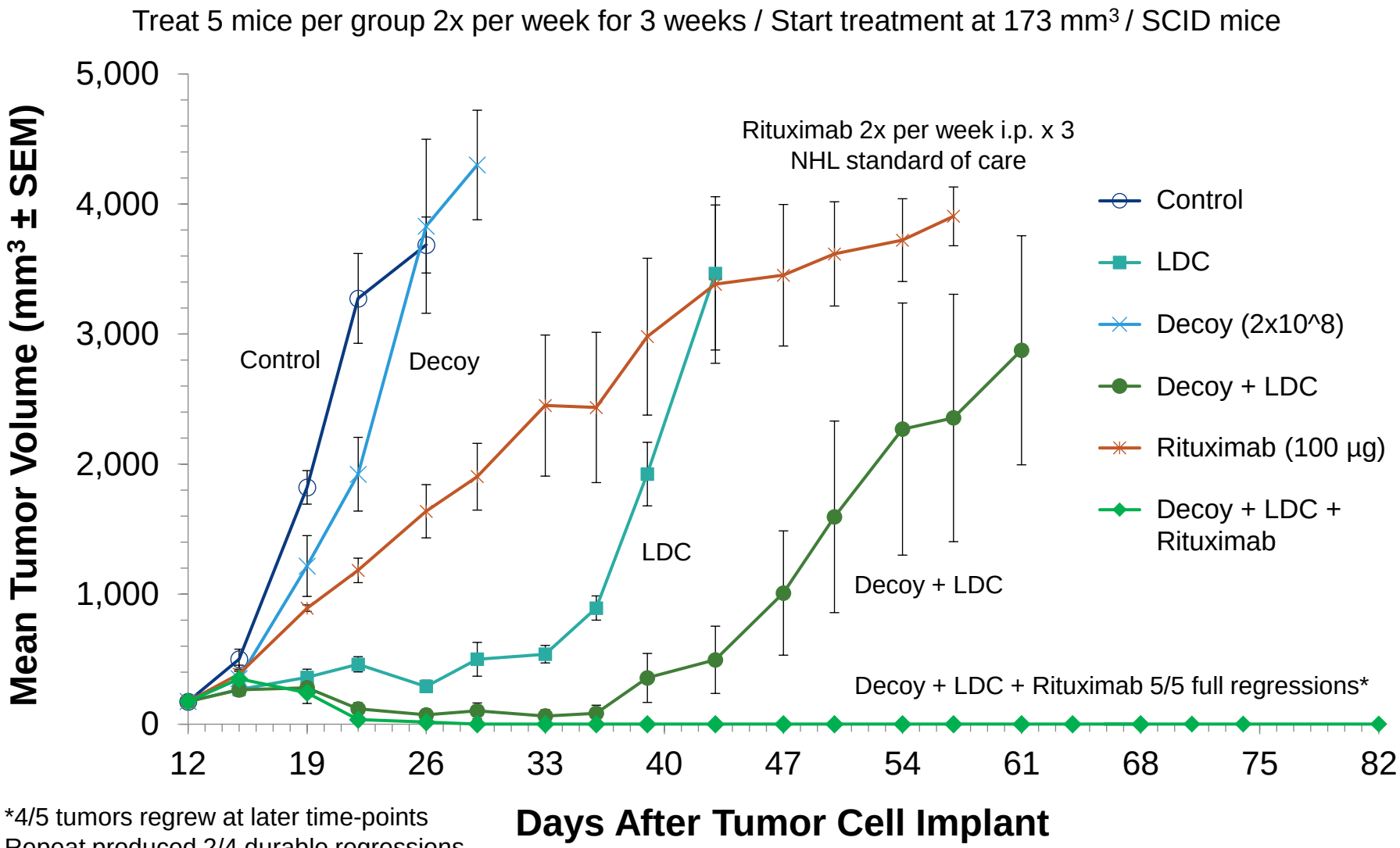


# High Percentage Eradication of s.c. NHL by Decoy + LDC Requires NK Cells and CD4+ and CD8+ T Cells (Innate and Adaptive Immunity)

Treat all groups (6 mice per group) with i.v. Decoy + LDC for 2 weeks / Start treatment at ~200 $mm^3$

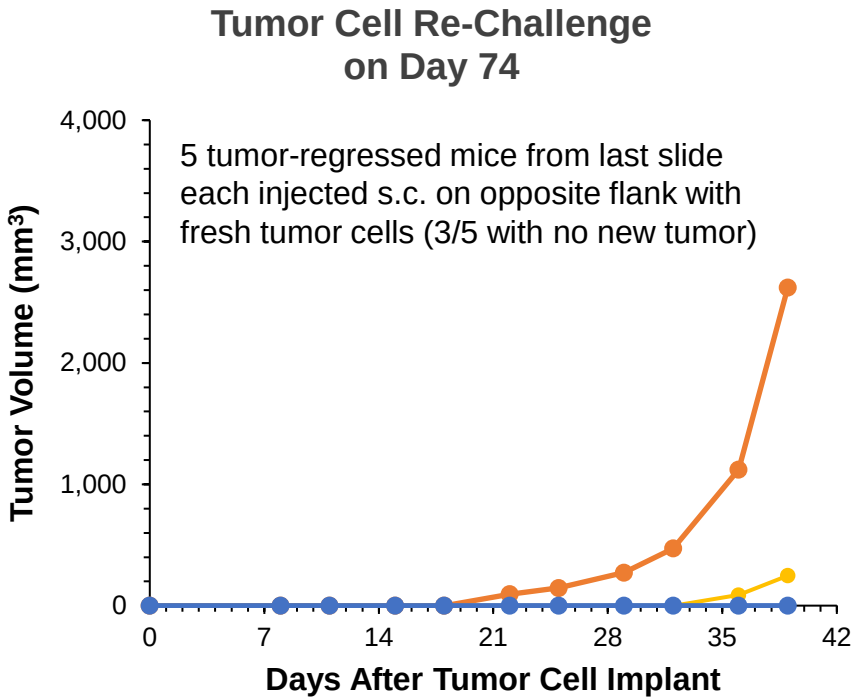


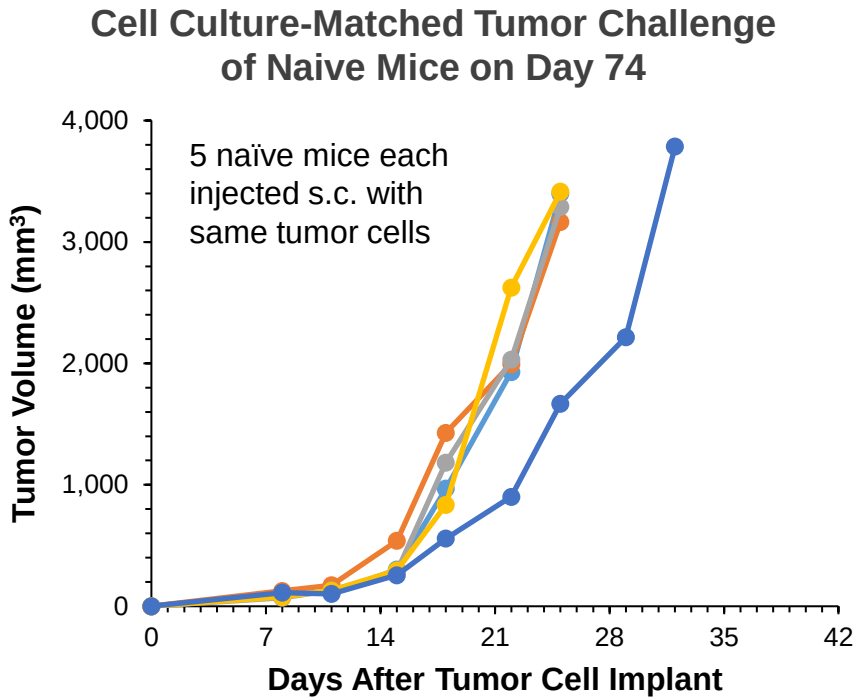
# Decoy + LDC Synergizes with a Targeted Antibody to Regress and Eradicate Established Ramos Human NHL Tumor Xenografts in SCID Mice




*4/5 tumors regrew at later time-points
Repeat produced 2/4 durable regressions

# Decoy Technology Can Induce Immunological Memory Via the Innate Immune System




- Tumor regression with immunological memory via the innate immune system alone is very rare in preclinical models, but consistent with a multiple danger signal mechanism
- Results suggest that Decoy technology may synergize with other marketed ADCC mechanism-based, targeted antibody therapeutics (~12 on market)

# Indaptus' Decoy Platform – Clinical Stage / Oncology Summary

- Single agent anti-tumor activity + tumor eradicating synergy with several different existing therapies
- Reduced toxicity and broad therapeutic index (no increase in toxicity with combinations)
- Safe induction of both innate and adaptive immune pathways (MoA) confirmed
- Innate and adaptive immunological memory leading to rejection of tumor re-challenge
- Efficacy in mouse syngeneic and human tumor xenograft models (Breast, CRC, HCC, Pancreatic, NHL)
- GMP batch of drug product produced (Decoy20) – stable at -70°C, -20°C (6 months at 5°)
- IND-enabling toxicology with GMP product – biomarkers of cytokine release syndromes not seen
- Solid tumor all-comer Phase 1 trial initiated with Decoy bacteria (Decoy20) NCT05651022
- Acknowledgements - AntiCancer, Crown Biosciences, Eurofins, InvivoGen, Molecular Diagnostic Services, Pacific BioLabs