Mechanisms of Chitosan/Interleukin-12 Immunotherapy for the Treatment of Bladder Cancer

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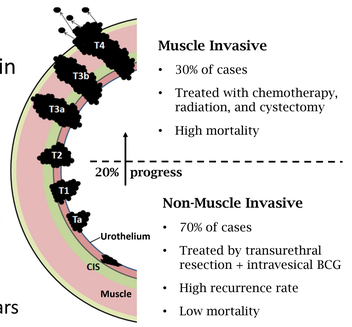
OBJECTIVES

1. Determine immune subtypes vital for tumor rejection and protection after intravesical treatment with CS/IL-12.
2. Identify the necessity of each treatment number.
3. Understand immune cell kinetics locally and systemically throughout a course of treatment.

INTRODUCTION

BLADDER CANCER

- 5th most common cancer in United States
  - 76,960 new cases
  - Prevalence above 570,000
  - 16,390 deaths in 2016
  - **Highest rate of recurrence**
    - 75% recur within 10 years
- Intravesical BCG
  - Standard of care for 40 years
  - Does not promote tumor specific memory



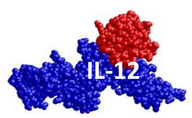
Muscle Invasive
- 30% of cases
- Treated with chemotherapy, radiation, and cystectomy
- High mortality

Non-Muscle Invasive
- 70% of cases
- Treated by transurethral resection + intravesical BCG
- High recurrence rate
- Low mortality

BCG does not adequately address bladder cancer recurrence.

INTERLEUKIN-12 (IL-12)

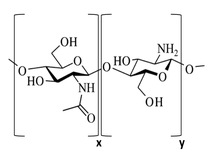
- Proinflammatory cytokine produced by dendritic cells and macrophages.
  - Hallmark T_H1 cytokine
  - IFNγ production
  - T-cell and NK-cell proliferation
  - Adaptive cell-mediate immunity
- Large (70 KD) protein
  - Delivery strategy needed to penetrate urothelium



IL-12 promotes an adaptive T_H1 polarized response.

CHITOSAN (CS)

- Biopolymer derived from the shells of crustaceans
- Soluble and cationic in mild acids
  - Suitable for delivery of labile proteins
- Enhances intravesical delivery
  - **Mucoadhesive**: Extends contact with the urothelium
  - **Viscous**: Prevents expulsion
  - **Absorption Enhancer**: Transiently opens tight junctions

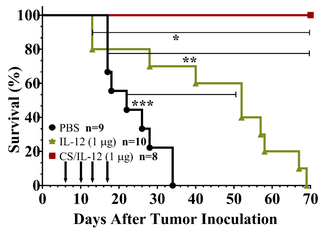


Structure of Chitosan

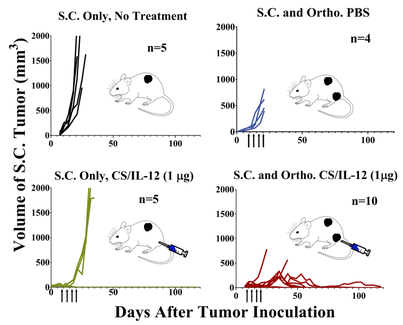
IL-12 delivered intravesically in chitosan solution is a simple coformulation designed to prevent recurrence by promoting a prolonged T-cell response at the tumor site.

RESULTS

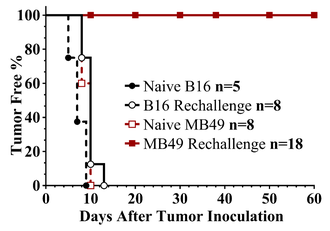
INTRAVESICAL CS/IL-12 ELIMINATES LOCAL AND DISTANT LESIONS AND INDUCES TUMOR-SPECIFIC SYSTEMIC IMMUNITY



Chitosan/IL-12 eliminates orthotopic MB49 tumors.



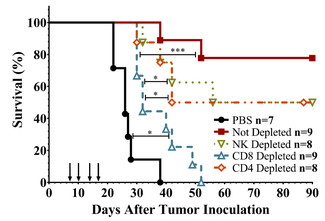
Intravesical CS/IL-12 best primes adaptive responses to distant lesions when also targeting orthotopic disease.



Cured mice reject distant (subcutaneous) inoculations in a tumor-specific manner.

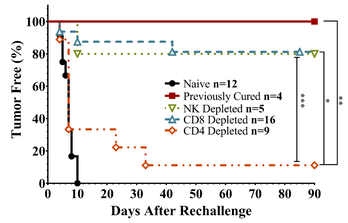
EFFECTOR CELLS DIFFER FOR INITIAL AND MEMORY ANTI-TUMOR RESPONSES

Depletion during treatment



CD8+ T-cells drive the anti-tumor response during treatment.

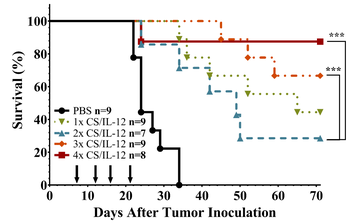
Depletion before distant rechallenge



CD4+ T-cells drive the memory response against rechallenge.

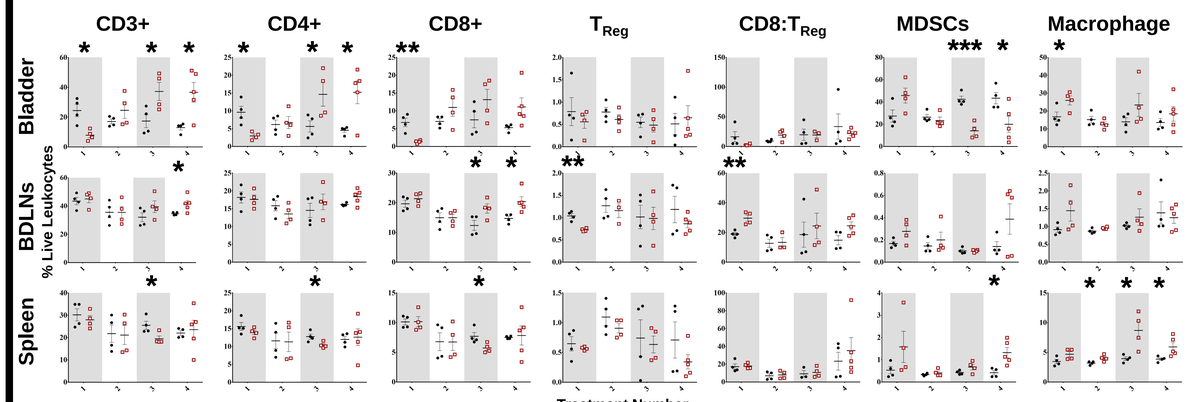
NUMBER OF TREATMENTS IMPACT CS/IL-12 EFFICACY

Applying 1,2,3 or 4 treatments

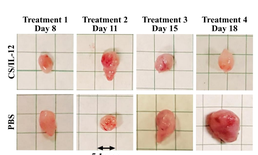


Increasing treatment number increases effectiveness of intravesical CS/IL-12.

TREATMENT NUMBER AFFECTS LOCAL AND SYSTEMIC IMMUNE CELL INFILTRATION



Bladder health during treatment



Treatment 1
- *Macrophage infiltration in the bladder*
- *Granulocyte infiltration in the bladder*
- *Increased CD8:T_{Reg} ratio in the BDLNs*

Treatment 2
- *Transition state with few detectible population shifts*
- *Increased naïve T-cells in the spleen*

Treatment 3
- *Increased CD3+, CD4+, and CD8+ cells in the bladder*
- *Reduced MDSCs in the bladder*
- *Increased macrophages in the spleen*

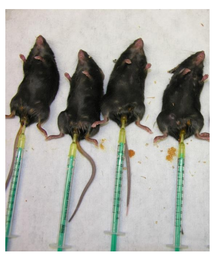
Treatment 4
- *Solidified T-cell infiltration in the bladder and BDLNs*
- *Shift to effector/memory phenotype*
- *Continued reduction of MDSCs in the bladder*

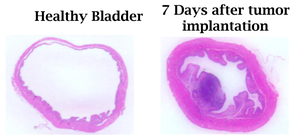
CONCLUSIONS

1. **Intravesical Chitosan/IL-12 immunotherapy is superior to BCG and engages systemic adaptive immunity against MB49.**
   i. Eliminates established orthotopic lesions in 80-100% of mice.
   ii. Eliminates established distant lesions.
   iii. Cured mice reject local and distant rechallenges up to 18 months post-cure.
2. **CD8+ T-cells are the primary drivers during the initial antitumor response, although CD4+ T-cells and NK cells also play measurable roles.**
3. **CD4+ T-cells are the dominant memory cells involved in rejecting subsequent rechallenge.**
4. **Even a single treatment eliminates 50% of tumors, but 3 or 4 treatments are more effective.**
5. **The first treatment induces macrophage and granulocyte infiltration as well as increased CD8:T_{Reg} ratio in the draining lymph node.**
6. **Subsequent treatments solidify T-cell infiltration and reduction in MDCS in the bladder .**

METHODS

Our models imitate orthotopic, superficial bladder cancer.





- MB49 Tumor cells (75K) are instilled intravesically for 40 minutes after a 20min poly-L-lysine wash.
- Tumors are multi-focal and superficial.
- >95% exhibit hematuria and palpable tumors within 7 days

CS/IL-12 Treatment

- CS/IL-12 preparation
  - Chitosan glutamate dissolved in PBS to form a 1% w/w solution.
- Add IL-12 to concentration
- 100 μl instilled via catheter
  - Dwell for 40 minutes

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