Hydrogel-Mediated Local Anti-CTLA-4 Delivery Potentiates Immune Activation After Breast Tumor Cryoablation

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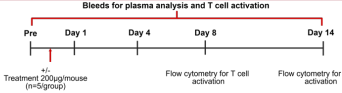
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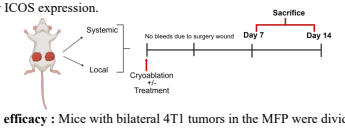
INTRODUCTION

Triple-negative breast cancer (TNBC) is highly aggressive with few treatment options and a significant risk of recurrence and metastasis owing to immune suppressive signals. Cryoablation offers dual advantage of local tumor necrosis while simultaneously stimulating anti-tumor immune responses via releasing tumor-associated antigens offering systemic control. Combining cryoablation with immune checkpoint inhibitors (ICIs) fosters stronger anti-tumor immunity, including abscopal effects. Nevertheless, systemic administration of ICIs like anti-CTLA-4 lead to widespread immune activation and immune-related adverse events (irAEs). To enhance the efficacy of immunotherapy while reducing systemic toxicity, we developed an injectable crosslinked chitosan-alginate hydrogel for localized and sustained delivery of anti-CTLA-4 – vinylated Poly(vinyl alcohol) conjugate in the tumor bed. We hypothesize that localized, controlled release of immune checkpoint inhibitors (ICIs) near the tumor can minimize the required dosage and reduce adverse side effects.

MATERIALS AND METHODS



Pharmacokinetic studies: naïve BALB/c mice received 200 µg anti-CTLA-4 either systemically (intraperitoneal; IP) or locally (hydrogel in mammary fat pad, MFP). Mice were bled on days 1, 4, and 8, and were sacrificed on day 14 for necropsy, during which plasma and mammary fat pad tissues were analyzed for anti-CTLA-4 concentrations using ELISA. The peripheral blood was further examined for T cell ($CD4^+$, $CD8^+$) activation by ICOS expression.



Therapeutic efficacy : Mice with bilateral 4T1 tumors in the MFP were divided into 2 groups. The left tumor was cryoablated and mice were administered with 200 ug of anti-CTLA-4 systemically (IP) (n=6) or locally (cryoablated tumor) (n=6) and 3 mice/group sacrificed at 7 and 14 days for analysis. Tumor size, hydrogel retention, T cell activation, and systemic anti-CTLA-4 concentrations were assessed post-treatment on days 7 and 14.

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RESULTS

Gel Formulation

- The gel is composed of two key components: sodium alginate and 50% quaternized chitosan.
- Chitosan (1 mg/mL) was prepared in a solution of vinylated PVA and antibody.
- Sodium alginate (1 mg/mL) was prepared in deionized (DI) water.
- To form the gel, equal volumes (120 µL) of each component were mixed.
- Gels were prepared using needles or within a 1 mL syringe compartment.

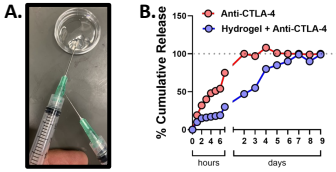


Figure 1. Injectable Chitosan Hydrogel Enables Controlled Antibody Release. A) Chitosan hydrogel can be delivered through a standard needle and solidifies upon contact with physiological saline. **B)** anti-CTLA-4 is gradually released from the hydrogel into saline, demonstrating sustained delivery capability.

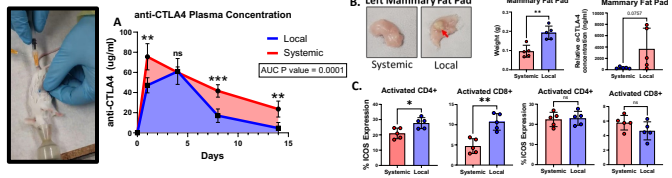


Figure 2. Local Gel-Formulated Anti-CTLA-4 Reduces Systemic Exposure While Maintaining T Cell Activation. A) Local treatment resulted in significantly lower levels of circulating anti-CTLA-4 in plasma compared to systemic administration. **B)** Locally treated MFP are bigger and had higher levels of anti-CTLA-4 relative to systemic controls. **C)** By day 8, local treatment increased activated $CD4^+$ and $CD8^+$ T cells relative to systemic treatment, indicating intact T cell activation. By day 14, no significant differences were observed. (Student T-test with significance considered $*p < 0.05$)

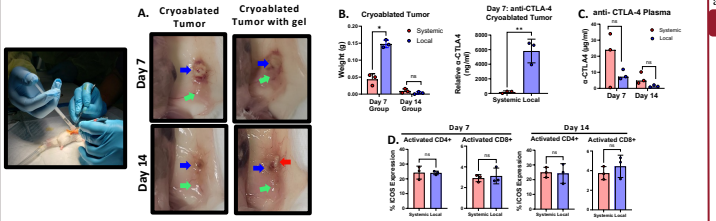


Figure 3. Local Anti-CTLA-4 Delivery Enhances Tumor Retention, Reduces Systemic Exposure, and Maintains T Cell Activation. A). By day 7, gel anti-CTLA-4 was incorporated in the left cryoablated tumor in MFP and was larger compared to systemic treatment. By day 14, the cryoablated tumors in both groups were almost completely reabsorbed with only small residual scar tissue remaining. [Blue arrow = tumor site, Green = LN, Red = residual gel-antibody formulation] **B)** Locally treated tumors weighed significantly more at day 7 and showed a significant increase of anti-CTLA-4 compared to the systemic treated mice. By day 14 little to no tumor tissue was present for either treatment group. **C)** Locally treated mice had lower concentration of anti-CTLA-4 in the plasma compared to systemic treatment. **D)** Flow cytometry analysis showed no significant differences in T cells at either time point analyzed. (Student T-test with significance considered $*p < 0.05$)

KEY RESULTS

- **Reduced systemic exposure:** Local gel-formulated anti-CTLA-4 injection into the mammary fat pad (MFP) significantly lowered circulating antibody levels compared to systemic (IP) administration in non-tumor bearing mice ($p < 0.05$).
- **Enhanced local retention:** At day 14, local treatment yielded a ~10-fold increase in anti-CTLA-4 concentration and greater tissue mass in the MFP versus systemic controls ($p < 0.05$).
- **Effective T cell activation:** Flow cytometry at day 8 revealed elevated ICOS expression on $CD4^+$ and $CD8^+$ T cells in both groups, confirming immunostimulatory activity; no significant differences were seen by day 14.
- **Improved tumor targeting:** In a bilateral 4T1 breast cancer cryoablation model, local anti-CTLA-4 delivery at the cryoablated site achieved higher tumor antibody retention and lower systemic exposure than IP administration.
- **Tumor response:** Locally treated tumors were larger with higher antibody levels at day 7 due to gel retention. By day 14, tumors were nearly fully reabsorbed in both groups.
- **Consistent systemic activation:** Peripheral blood showed lower plasma anti-CTLA-4 levels in the local group, with comparable ICOS expression on T cells at days 7 and 14, indicating equivalent systemic T cell activation.

CONCLUSIONS

Local delivery of anti-CTLA-4-vinylated PVA conjugates using a chitosan-alginate hydrogel after cryoablation enhanced retention of anti-CTLA-4 in the tumor and provided equivalent activation of T cells in the blood, reducing systemic antibody exposure. This strategy presents a promising approach to minimize irAEs without compromising therapeutic efficacy. Moreover, this hydrogel-based method requires additional validation with lower doses of anti-CTLA-4 to further de-escalate treatment-associated toxicity. It supports its future application in clinical settings to enhance combinatorial cryoablation and ICI therapy.

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