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Cryoablation Combined with Immune Checkpoint Inhibitors Enhances Conventional Dendritic Cell Activation in Abscopal Tumors and Tumor-Draining Lymph Nodes

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INTRODUCTION

Immune checkpoint inhibitors (ICI) have shown clinical efficacy in triple-negative breast cancer (TNBC) but are associated with immune-related adverse events. These limitations underscore the need for combination strategies that enhance therapeutic outcomes and reduce ICI dosing requirements. Cryoablation, a non-surgical procedure, "kills" the tumor through freeze-thaw cycles leading to tumor necrosis while preserving tumor-associated antigens (TAAs) and releasing damage-associated molecular patterns that trigger antitumor immune responses and may induce the abscopal effect - systemic immune response against distant tumors. We hypothesize that cryoablation combined with immune checkpoint inhibitors (ICIs) will stimulate systemic T cell–mediated antitumor immunity, improving control of both local and distant tumors.

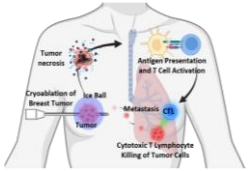


Figure 1. Breast cancer cryoablation and the abscopal effect. Through a small skin incision, a cryoprobe is inserted directly into the tumor. The subsequent freeze-thaw cycles induce tumor necrosis, leading to the release of intact tumor antigens and inflammatory molecules. These molecules are captured by antigen-presenting cells which stimulate the activation of anti-tumor T cells capable of recognizing and attacking both local and distant tumor cells. This phenomenon where a localized treatment elicits a systemic anti-tumor response is called the abscopal effect.

MATERIALS AND METHODS

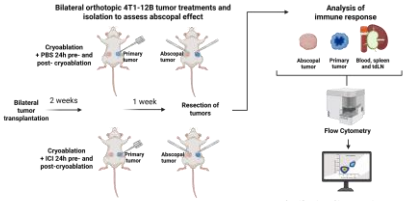


Figure 2. Schematic of the experimental approach. BALB/c mice were bilaterally transplanted in the mammary fat pad with 4T1-12b-luciferase-expressing BC cells. Two weeks after transplant, left tumors were cryoablated, and PBS or ICI (anti-CTLA-4, PD-1, or PD-L1) were administered 24h pre- and post-cryoablation by intra-peritoneal injection. The right tumors were not manipulated and represented distant metastatic tumors to examine the immune abscopal effect (abscopal tumors). Mice were sacrificed one-week post-cryoablation; right and left tumors, blood, spleen, and tumor-draining lymph nodes (TdLN) were collected and processed to obtain isolated cells for flow cytometry analysis.

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RESULTS

1. Inhibition of anti-CTLA-4 is a better combination to cryoablation than blockade of PD-1/PD-L1 axis

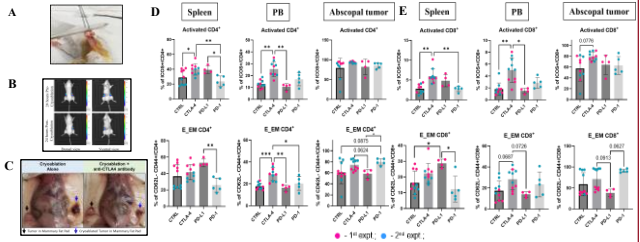


Figure 3. Characterization of cryoablation + ICI effects. A. Representative cryoablation of a mouse breast cancer tumor. **B**. Representative images of mouse tumors by IVIS before and after cryoablation. **C.** Representative pictures from the primary and abscopal tumors, after mice sacrifice one-week post-cryoablation. **D.** Frequency of ICOS$^+$ CD4$^+$ T cells in the spleen, peripheral blood and abscopal tumor. **E.** Frequency of ICOS$^+$ CD8$^+$ T cells in the spleen, peripheral blood and abscopal tumor of mice treated with cryoablation monotherapy or combined with ICI. CTRL = control (cryoablation monotherapy); CTLA-4 = cryoablation plus anti-CTLA-4; PD-L1 = cryoablation plus anti-PD-L1; PD-1 = cryoablation plus anti-PD-1. E_EM = effector/effector memory; CM = central memory. p<0.05 (*), p<0.01 (**)

2. Combination of cryoablation to anti-CTLA-4 also affects distant tumors

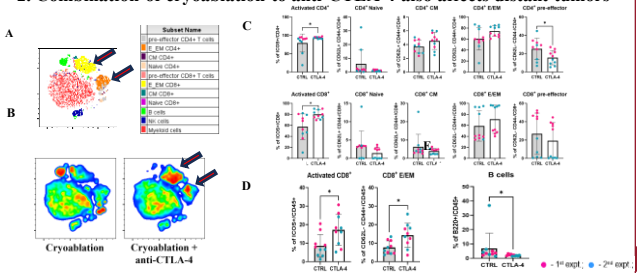


Figure 4. Cryoablation plus CTLA-4 blockade increases the frequencies of activated T cells at the abscopal tumors. A. Representative tSNE map from one experimental repeat, showing the clustering of different lymphoid populations at the abscopal tumors based on flow cytometry parameters' similarity. **B.** Representative tSNE heatmap from one experimental repeat, made from concatenated abscopal tumors from cryoablation monotherapy versus cryoablation plus anti-CTLA-4. Red arrows point to E_EM populations. **C.** Subpopulations of T cells, analyzed as frequency of the parent (CD4$^+$ or CD8$^+$), at the abscopal tumors. **D.** Subpopulations of T cells (activated and effector/effector memory CD8$^+$ T cells), analyzed as frequency of the live immune cells (CD45$^+$), at the abscopal tumors. **E.** Parent immune populations, analyzed as frequency of the live immune cells (CD45$^+$), at the abscopal tumors. CM = central memory; E_EM = effector/effector memory; CTRL = control; CTLA-4 = anti-CTLA-4. n = 10 per group. p<0.05 (*).

3. Frequency and activation of conventional dendritic cells in the tumor-draining lymph nodes are enhanced by combination of cryoablation with anti-CTLA-4

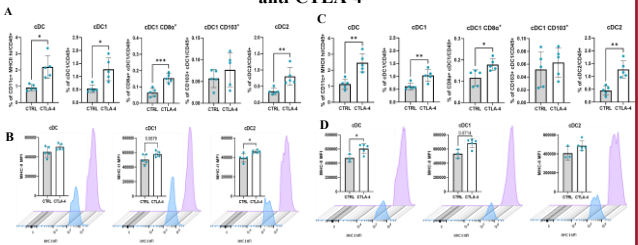


Figure 5. Blockade of CTLA-4 leads to enhancement of dendritic cell frequencies at the TdLNs when combined with cryoablation. **(A, C)** Frequency of conventional dendritic cells and their subpopulations, analyzed as frequency of the live (CD45$^+$), at the primary **(A)** and abscopal **(C)** TdLN. **(B, D)** From left to right, MHC class II mean fluorescence intensity (MFI) per count in cDC, cDC1, and cDC2, from the primary **(B)** abscopal **(D)** TdLN. Histograms were generated with concatenated samples from one representative experiment**.** Unpaired Student's t-test for normally distributed data or Mann-Whitney test for non-normally distributed data was performed comparing cryoablation monotherapy to combination with ICI, with p<0.05 (*) and p<0.01 (**) considered significant. cDC = conventional dendritic cell; CTRL = control; CTLA-4 = anti-CTLA-4. n = 5 per group for frequency analysis; n = 3-5 per group for MFI analysis.

CONCLUSIONS

- Combining cryoablation with anti-CTLA-4 therapy enhances systemic antitumor immunity more effectively than cryoablation alone or with PD-1 or PD-L1 inhibition.
- Cryoablation with anti-CTLA-4 increased frequencies and activation of cDC1 and cDC2 in the TdLNs of abscopal tumors, likely contributed to the elevated numbers of activated CD4$^+$ and CD8$^+$ T cells in the abscopal tumor microenvironment.
- Cryoablation with anti-CTLA-4 combination strategy suggests long-term potential to prevent tumor recurrence and metastasis.

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