Cryoablation as a Breast Cancer Treatment in a Murine Model

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Study Question and Background

The abscopal effect is the hypothesis that treatment of the primary tumor can prime immune cells against tumor antigens and therefore minimize distant metastasis. Breast cancer cryoablation is a minimally invasive procedure that can be performed in the clinic under general anesthesia. It involves ablation of the local tumor mass using freeze-thaw cycles. Patients return to work within a few days. We are testing the hypothesis that cryoablation of breast cancer may be better than surgical resection due to an abscopal effect in a murine model.

Murine Model

Here we present preliminary data for cryoablation of breast cancer in a mouse model. Five BALB/c mice were transplanted with the highly metastatic breast cancer cell line 4T1-12B-luc into the mammary fat pad. This cell line expresses luciferase allowing the primary tumor to be monitored and metastasis to be tracked by *in vivo* bioluminescence imaging (IVIS) (Figure 1).

Between 2 and 3 weeks post-transplant, the primary tumor was cryoablated. The procedure involved resecting the skin to prevent frostbite and freezing (< -150 ^{o}C) the tumor using a freeze/thaw/freeze cycle (Figure 1). Mice were continually monitored post-procedure and imaged using IVIS to track progression of disease (Figure 2).

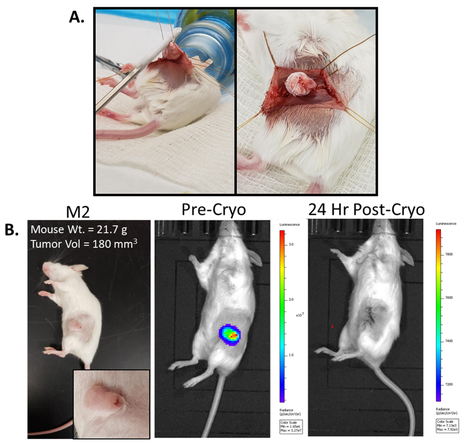

Figure 1. Mouse model for cryoablation of breast cancer. A. Cryoablation procedure: skin retracted and tumor exposed, cryoablation of the tumor, completely frozen tumor. B. *In vivo* imaging before cryoablation and 24 hours post-cryoablation.

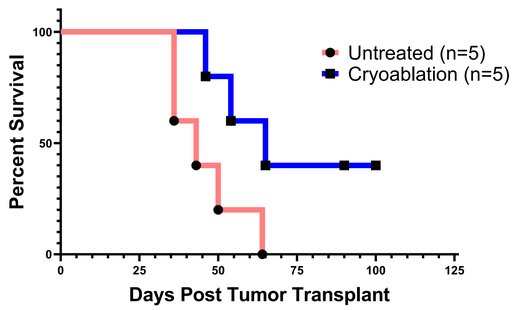

Figure 3. Mouse survival curve. Comparison of mice treated with cryoablation to untreated mice transplanted with 4T1-12b-luc.

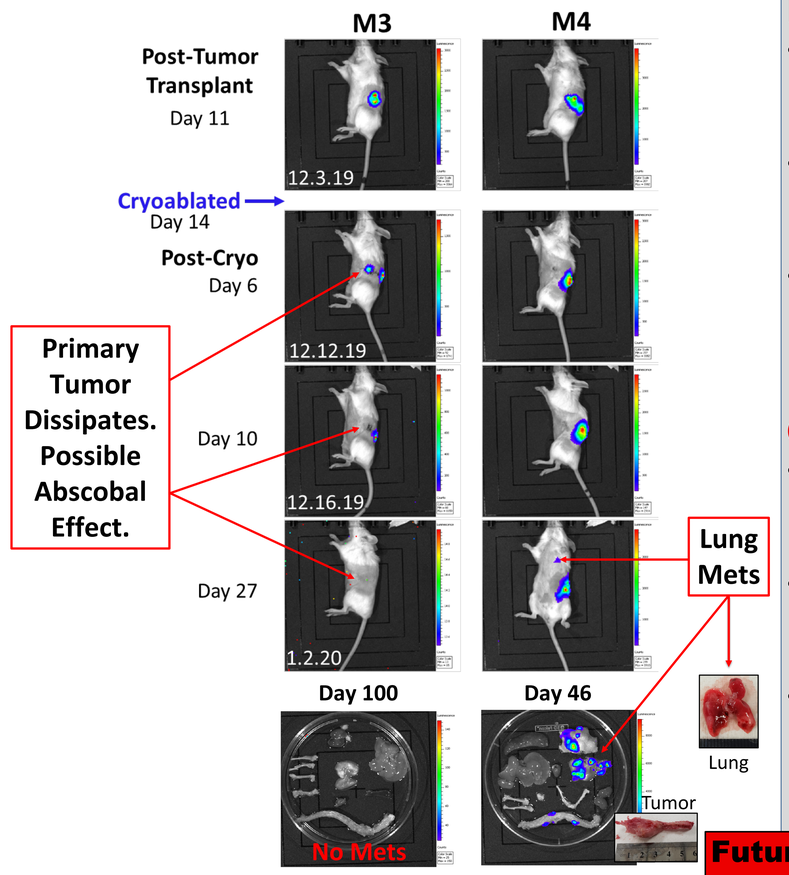

Figure 2. Monitoring mice for disease progression. Mice were palpated and imaged weekly by IVIS for tumor and metastasis. (*Ex-vivo* imaging for M4).

Mouse	Tumor size pre-cryo (caliper)	Palpation description (before cryoablation)	Cryoablation Freeze/Thaw/Freeze Cycle	Days survival post-transplant	Tumor present (IVIS &/or palpation)	Mets by IVIS (Ex-vivo imaging)
Experiment 1						
M1	Vol = 709 mm3 (3 week)	Unifocal, spherical – ulceration starting	51/60/51	48 days	Vol = 1531 mm^3	NO
M2	Vol = 180 mm3 (3 week)	Unifocal, spherical – ulceration starting	75/85/90	63 days	NO	NO
Experiment 2						
M3	Vol = 130 mm3 (2 week)	Unifocal, spherical	56/30/48	100 days	NO	NO
M4	Vol = 228 mm^3 (2 week)	Multifocal – 3 lobes 2nd tumor on spine	47/38/34	46 days	Vol = 4756 mm^3	(Mets at Day27) Tumor – spine, lung
M5	Vol = 45 mm^3 (2 week)	Multifocal – flat, peritoneum embedded	40/34/26	67 days	Vol = 1905 mm^3	NO

Table 1: Disease monitoring for experiments. Tumor characteristics, cryoablation conditions, days survival, metastasis.

Results

- As seen in table 1, 2/5 mice treated with cryoablation had no primary tumor recurrence.
- 4/5 mice showed no signs of metastasis by IVIS or *ex-vivo* organ imaging.
- Mice treated with cryoablation survived longer than mice not treated. (Figure 3)

Conclusion

- Cryoablation appears more effective in smaller, unifocal tumors with longer freeze/thaw/freeze cycles.
- We demonstrated metastasis can be monitored *in vivo* by IVIS in this model.
- Cryoablation may produce an abscopal effect. No metastasis in 4 out of 5 mice by IVIS or at necropsy.

Future Directions

- Tissue histology will be performed for metastasis and T cell infiltrates.
- We are in the process of standardizing the mouse model to improve the cryoablation technique for uniform freezing of the primary tumor.
- Future studies will combine cryoablation with adjuvant pharmaceutical therapies to evaluate synergistic effects of available drugs.
- Preliminary data will be used to benefit current human cryoablation trials.

Acknowledgements

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