Abstract
Immunotherapy has emerged as a promising treatment for metastatic cancer. However, immunotherapy for advanced stage breast carcinoma remains an unmet need. Here we demonstrate that the novel immunomodulator alpha-tocopherylxyacetic acid (αTEA) synergizes with radiation therapy (RT) in a metastatic murine mammary carcinoma model to improve tumor control, and we propose a mechanism based on the stimulation of type I interferons by αTEA.

Materials/Methods
For in-vivo studies, BALB/c mice bearing 4T1 murine mammary carcinoma lesions were treated with dietary αTEA, ionizing radiotherapy, or the combination of αTEA/RT. Immune activation in animals treated with dietary αTEA was determined by analyzing the percentage of proliferating CD4 and CD8 T cells using a Ki-67 assay. Pulmonary metastatic burden was determined using a clonogenic metastasis assay. For in-vitro studies, mammary carcinoma cell lines were treated with αTEA or LPS and whole cell lysates were analyzed by Western blot. Expression of interferon regulation factor 3 (IRF3) pathway proteins, including phospho-TANK binding protein kinase 1 (TBK1) and stimulator of interferon genes (STING) were determined. Reactive oxygen species were detected using a DCFDA assay and treatment with 30-60 uM αTEA.

Radiotherapy
Radiotherapy performed using an Xstrahl small animal radiation research platform
Animals received 20 Gy x 2 or 12 Gy x 5 fractions (BED$_{10}$ of 120-132)

Results
Combination therapy with αTEA and radiation resulted in improved tumor control compared to monotherapy

4T1 in-vivo: αTEA +/- 20 Gy x 2 RT

![Tumor size (mm²)]

- No Treatment
- TEA Only
- RT Only
- TEA + RT

Combination therapy was effective with alternate fractionation schemes

4T1 in-vivo: αTEA +/- 12 Gy x 5 RT

![Tumor size (mm²)]

- No Treatment
- TEA Only
- RT Only
- TEA + RT

Treatment of 4T1 bearing mice with oral αTEA results in increased T cell activation and decreased pulmonary metastases

Conclusions
These preliminary results suggest that the combination of αTEA and ionizing radiation may be a viable therapy for the treatment of metastatic breast carcinoma. This therapeutic combination may provide more durable response to radiotherapy while simultaneously reducing further metastatic spread.

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