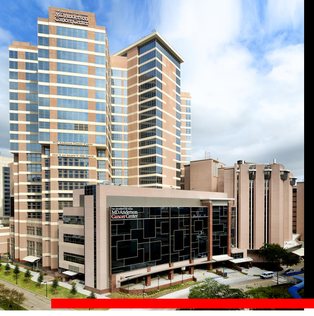

# Enhancing Anti-Tumor Immunity through High-LET Radiation and Immunotherapy

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## Introduction

While there have been many strides made in immunotherapy, many cancers are still resistant to immunotherapy. Radiotherapy generates damaged DNA, which then activates immunostimulatory signals and potentially elicits an anti-tumor immune response. We hypothesized that damaged DNA resulting from high linear energy transfer (LET) radiotherapy will enhance immunostimulatory mechanisms, leading to decreased tumor growth.

## High LET Particles

LET is energy transferred per unit distance. Higher LET induces more DNA lesions than lower LET, allowing for higher cell kill. Alpha particles have higher LET compared to other forms of conventional radiotherapy. Alpha particles are ideal high LET agents because as they deposit energy, clustered DNA lesions are created. As a component of DNA damage, micronuclei, small pockets of nucleic material in the cytoplasm, can occur, stimulating downstream immune pathways.

## Diffusing Alpha-emitters Radiation Therapy (DaRT)

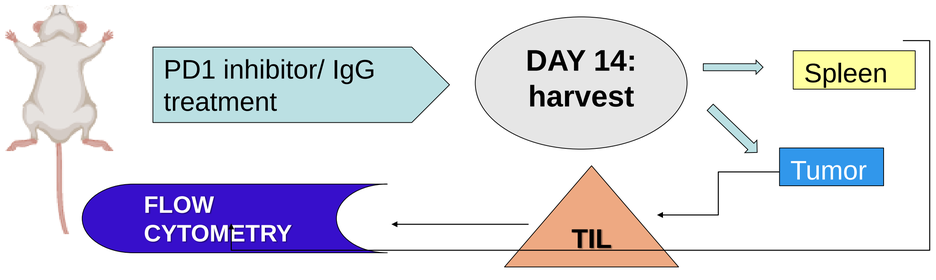
Diffusing Alpha-emitters Radiation Therapy (DaRT) is a technology that efficiently delivers alpha particles to tumor sites. DaRT technology overcomes the short range of alpha particles by implanting a radium-224-affixed metal seed in a tumor. As radium decays, radon gas forms and emits alpha particles. The diffusing radon gas perfuses across the tumor volume with subsequent alpha decay, allowing for the dose of alpha particles to cover 2-3 millimeters instead of 80 or less microns .

## Immune Checkpoint Blockade: PD1

Programmed cell death protein 1, PD1, is an immune checkpoint that downregulates the immune system to prevent autoimmunity. PD1's interaction with programmed death-ligand 1, PD-L1, is what gives healthy cells the ability to tolerate cytotoxic T cells. Cancer cells exhibit high levels of PD1 to bypass immune checkpoints and acquire immune resistance. By administering a PD1 inhibitor to tumor sites, we expected to see PD1's interaction with PD-L1 to be blocked, allowing for T cells to attack cancer cells. In order to test our hypothesis, we evaluated tumor growth delay by using a high LET DaRT with PD1 inhibitor treatment.


**Figure 1.** Radium Decay Chain

## Methods




- 4T1 cancer cell line injected in the left leg of 40 balb/c mice 12 days before DaRT seed implantation (day 0)
- Mice were divided into 4 experimental groups; received treatment every 3 days
  a. GROUP 1: {HOT} DaRT radioactive seed and PD1 inhibitor
  b. GROUP 2: {HOT} DaRT radioactive seed and IgG
  b. GROUP 3: {INERT} seed and PD1 inhibitor
  c. GROUP 4: {INERT} seed and IgG
  ***IgG treatment administered as control
- Tumor measurements taken every 2 days using calipers
- Mice were euthanized on Day 14; spleen and tumor harvested
- Tumors were processed and tumor infiltrating lymphocytes extracted
- Lymphocytes were stained for immune markers:
  - CD4+ (helper T cells)
  - CD8+ (killer T cells)
  - Interferon gamma, IFNg+ (gives anti-tumor inflammation properties to killer cells)
  - T-cell immunoglobulin, TIM3 (exhausts T cells and suppresses immunity)

## Results

After DaRT implantation on day 0, tumor growth increased steadily in groups 3 and 4, while tumor growth delay was seen in groups 1 and 2. The normalized tumor volume of group 1 was significantly smaller than both INERT groups' tumor volume on Day 8 ($p<0.0024$ group 3, $p<0.0001$ group 4) and day 14 ($p<0.009$ group 3, $p<0.0001$ group 4). INERT groups' CD4+ and CD8+ levels were significantly higher than group 1's levels ($p<0.05$). TIM3 levels were significantly higher in group 1 compared to all other groups ( $p<0.0059$ group 2, $p<0.0001$ group 3, $p<0.0001$ group 4). Group 1 exhibited significantly higher IFNg+ levels ($p<0.0228$) compared to group 4.

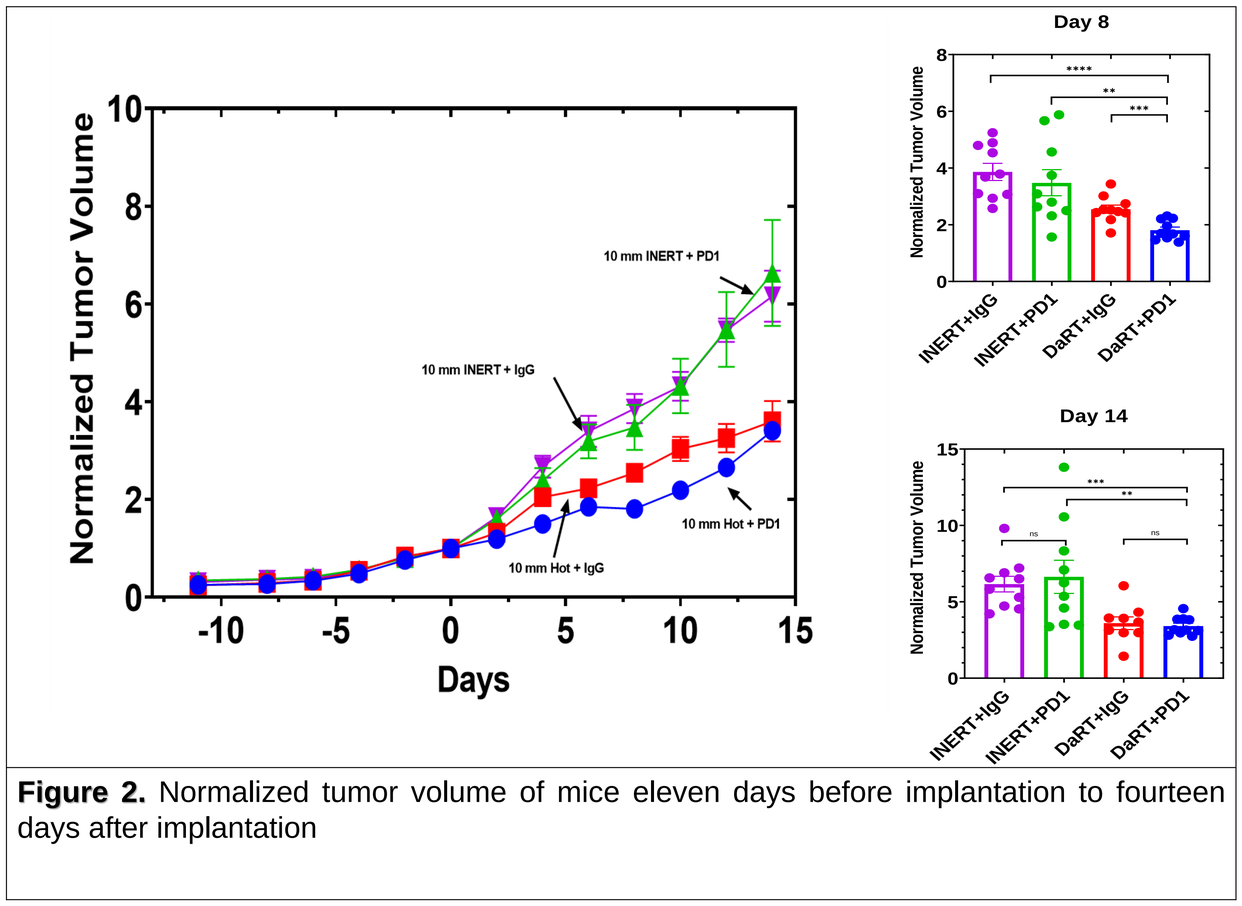



**Figure 2.** Normalized tumor volume of mice eleven days before implantation to fourteen days after implantation

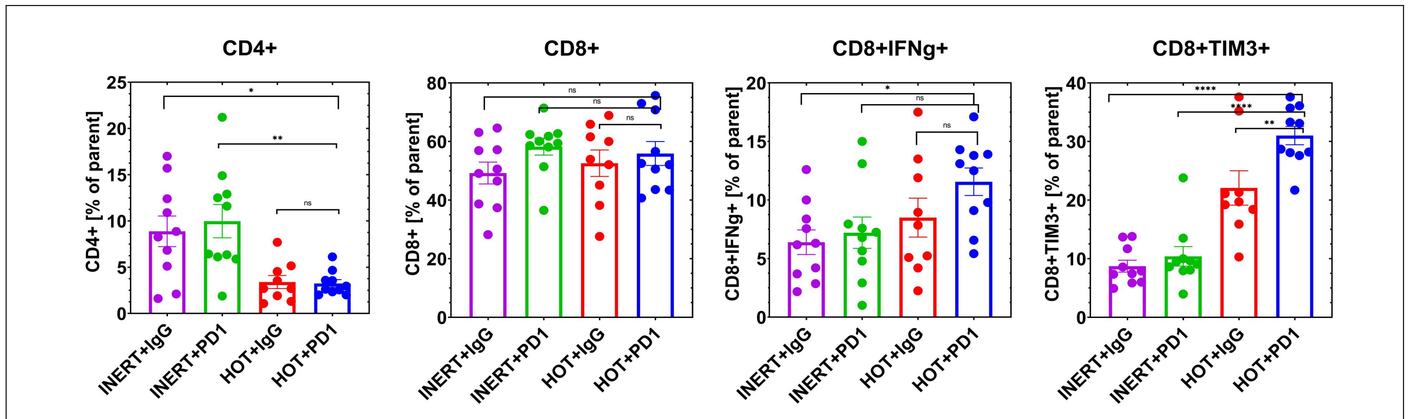



**Figure 3.** Flow cytometry results for immune markers.

## Conclusion

Our results showed that combining high LET particles with immunotherapy facilitated slower tumor growth. While combining DaRT with a PD1 inhibitor did enhance immune response, immune system suppression was still seen with the upregulation of suppressive immune markers and downregulation of activating immune markers.

## References

1. Bonaventura P, Shekarian T, Alcazer V, Valladeau-Guilemond J, Valsesia-Wittmann S, Amigorena S, Caux C, Depil S. Cold Tumors: A Therapeutic Challenge for Immunotherapy [published online ahead of print 2019/02/26]. Front Immunol. 2019;10:168.

2. Scheithauer H, Belka C, Lauber K, Gaipl US. Immunological aspects of radiotherapy. Radiat Oncol. 2014;9:185.

3. Filatenkov A, Baker J, Mueller AM, Kenkel J, Ahn GO, Dutt S, Zhang N, Kohrt H, Jensen K, Dejbakhsh-Jones S, Shizuru JA, Negrin RN, Engleman EG, Strober S. Ablative Tumor Radiation Can Change the Tumor Immune Cell Microenvironment to Induce Durable Complete Remissions. Clin Cancer Res. 2015;21(16):3727-3739.

4. Tang C, Welsh JW, de Groot P, Massarelli E, Chang JY, Hess KR, Basu S, Curran MA, Cabanillas ME, Subbiah V, Fu S, Tsimberidou AM, Karp D, Gomez DR, Diab A, Komaki R, Heymach JV, Sharma P, Naing A, Hong DS. Ipilimumab with Stereotactic Ablative Radiation Therapy: Phase I Results and Immunologic Correlates from Peripheral T Cells [published online ahead of print 2016/11/01]. Clin Cancer Res. 2017;23(6):1388-1396.