**Name of Award**: METAvivor Research Award

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**Descriptive title of project:** "*In situ* radio-immunotherapy to maximize the engagement of Batf3 dendritic cells to treat metastatic breast cancer"

Fumito Ito, MD, PhD **"*In situ* radio-immunotherapy to maximize the engagement of Batf3 dendritic cells to treat metastatic breast cancer"**

**SIGNIFICANCE:** This project is significant because *1) in situ* radio-immunotherapy (ISRI) may be a useful treatment for patients with unresectable and metastatic breast cancer; *2)* ISRI may generate tumor-specific T cells without the need for identification of patient- and tumor-specific antigens such as neoantigens, or isolation, modification, or *ex vivo* expansion of patient- and tumor-specific T cells (e.g. chimeric antigen receptor (CAR) T cells or neoantigen vaccine therapy); *3)* we will significantly improve our understanding of Batf3 DC biology; and *4)* this research will broaden our knowledge about systemic immunity elicited by radiation therapy (RT)

**IMPACT:** Despite significant advances in chemotherapy and targeted therapy, metastatic breast cancer remains challenging to treat. Revolutionary immunotherapy that unleashes patients' T lymphocytes to attack cancer can cause long-lasting immune responses in significant number of cancer patients (1-5). One major limitation of immunotherapy for breast cancer patients; however, is lack of T lymphocytes in the tumor for immunotherapy to be effective (6-9). Our new strategy is to convert tumors into cancer vaccine manufacturing factories, and educate T lymphocytes to attack widely metastatic cancers. Successful completion of the proposed study should provide a solid foundation for the future development of clinical trial with large cohorts of patients, and for the generation of more effective personalized cancer treatment targeting unresectable and metastatic breast cancer.

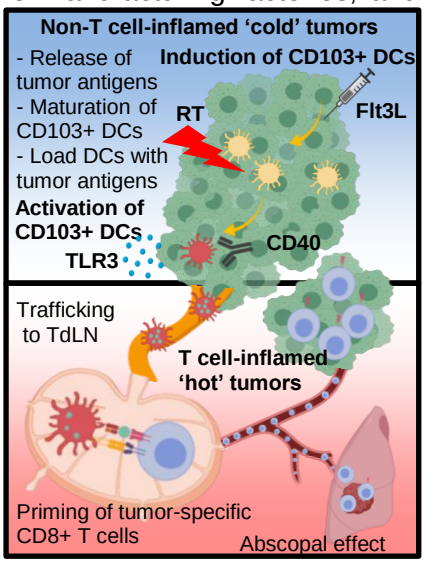


**Fig. 1: Graphical working model.**

**BACKGROUND:** Batf3$^{+}$ dendritic cells (Batf3 DCs) display enhanced abilities to phagocytose dead cells and prime tumor-specific T cells among various DC subsets (10-16). We hypothesized that the induction and activation of tumor-residing Batf3 DCs would facilitate the priming, expansion, and infiltration of tumor-specific T cells into the non-T cell-inflamed tumors. To test this hypothesis, we developed a novel combinatorial *in situ* radio-immunotherapy (**ISRI**) regimen (**Fig. 1**) comprised of intratumoral administration of *1)* Fms-like tyrosine kinase 3 receptor ligand (Flt3L) to mobilize Batf3 DCs to the TME; *2)* RT to promote immunogenic death of cancer cells and maturation of DCs; and *3)* dual TLR3/CD40 stimulation to activate Batf3 DCs for priming and expansion of CD8 T cells. Excitingly, our research has revealed that this regimen can trigger priming of tumor-specific T cells, increase T cells in the tumor, mediate rapid and robust regression not only of primary, but also untreated distant tumors, render non-T cell-inflamed breast tumors responsive to anti-PD-L1 therapy, eradicate large tumors, and develop tumor-specific systemic immunological memory (data not shown). Furthermore, this strategy significantly decreases distant metastatic tumor burden, and improves survival (**Fig. 2**).

**APPROACH:** In **Aim 1** and **2**, we will determine the critical immunological mechanisms generating abscopal effects by ISRI, and potential synergy with anti-PD-1 therapy in orthotopic mouse models of spontaneous and established breast cancer lung/brain metastasis. In **Aim 3**, we would like to propose a first in human phase I clinical study to evaluate safety and treatment activity of ISRI. We will treat unresectable and metastatic stage IV breast cancer patients with four cycles of in ISRI: intratumoral injection of Flt3L (CDX-301) for 5 days (D1-5), RT (9 Gy x 1) on day 8, and administration of TLR3 (Poly-ICLC)/CD40 agonist (CDX-1140) on day 9. [A similar combination without CDX-1140 was tested for lymphoma recently (17).] To validate conversion of non-T cell-inflamed tumors to T-cell inflamed tumors, we will obtain serial tumor biopsies, and examine alteration of tumor-infiltrating immune cells in the non-T cell-inflamed TME by high-dimensional single-cell RNA sequencing, imaging mass cytometry, and TCR sequencing.

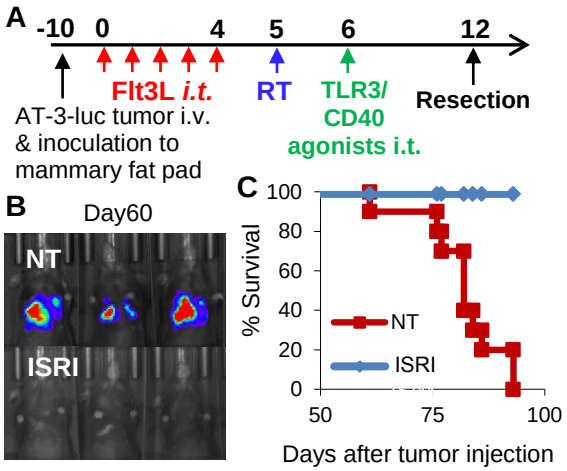


**Fig. 2** (**A**) AT-3 expressing luciferase (AT-3-luc) cells were orthotopically implanted to mammary fat pad, and injected i.v. to establish primary tumors and lung metastases. Primary tumors were untreated (NT) or treated with intratumoral (i.t.) injection of Flt3L, radiotherapy (RT) (9Gy) and i.t. TLR3/CD40 agonists (ISRI), followed by resection to evaluate growth of lung metastases. (**B**) Representative bioluminescent imaging on day 60. (**C**) Survival curves in AT3-luc tumor bearing mice treated with NT or ISRI (n = 9-10 per group).

**REFERENCES:**


1. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012;366(26):2443-54.
2. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366(26):2455-65.
3. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. N Engl J Med 2015;373(17):1627-39.
4. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 2014;515(7528):558-62.
5. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2016;375(19):1823-33.
6. Dirix LY, Takacs I, Jerusalem G, Nikolinakos P, Arkenau HT, Forero-Torres A, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. Breast Cancer Res Treat 2017.
7. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med 2018;379(22):2108-21.
8. Emens LA, Cruz C, Eder JP, Braiteh F, Chung C, Tolaney SM, et al. Long-term Clinical Outcomes and Biomarker Analyses of Atezolizumab Therapy for Patients With Metastatic Triple-Negative Breast Cancer: A Phase 1 Study. JAMA oncology 2019;5(1):74-82.
9. Emens LA. Breast Cancer Immunotherapy: Facts and Hopes. Clin Cancer Res 2018;24(3):511-20.
10. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. Cancer Cell 2014;26(5):638-52.
11. Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, et al. Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. Cancer Cell 2016;30(2):324-36.
12. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 2015;523(7559):231-5.
13. Spranger S, Luke JJ, Bao R, Zha Y, Hernandez KM, Li Y, et al. Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. Proc Natl Acad Sci U S A 2016;113(48):E7759-e68.
14. Fuertes MB, Kacha AK, Kline J, Woo SR, Kranz DM, Murphy KM, et al. Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. The Journal of experimental medicine 2011;208(10):2005-16.
15. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. Science 2008;322(5904):1097-100.
16. Shortman K, Heath WR. The CD8+ dendritic cell subset. Immunol Rev 2010;234(1):18-31.
17. Hammerich L, Marron TU, Upadhyay R, Svensson-Arvelund J, Dhainaut M, Hussein S, et al. Systemic clinical tumor regressions and potentiation of PD1 blockade with in situ vaccination. Nat Med 2019.