Conclusions Combined radio-immunotherapy can enhance specific T cell responses to the NY-ESO-1 antigen that correlates with beneficial clinical outcome of the patient.

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Trial Registration NOT applicable

Ethics Approval The study was approved by the Institutional Review board committee of Hamad Medical Corporation, Doha, Qatar.

Consent The patient signed an informed consent form to carry out the study and to publish the data.

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Abstract 463 Figure 2 Enzyme-linked ImmunoSpot assay for IFN-γ production by T cells from patient’s peripheral blood mononuclear cells

Abstract 463 Figure 3 Phenotyping and functional characterization of patient’s T cells from peripheral blood

Abstract 463 Figure 4 Differential expression of cytokines/chemokines in patient’s plasma

Conclusions Combined radio-immunotherapy can enhance specific T cell responses to the NY-ESO-1 antigen that correlates with beneficial clinical outcome of the patient.

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CLONAL REPLACEMENT OF TUMOR-INFILTRATING CD8+ T CELLS BY INDUCTION AND ACTIVATION OF TUMOR-RESIDING BATF3-DEPENDENT DENDRITIC CELLS

1Takaaki Oba*, 1Mark Long, 2Tibor Keler, 2Henry Marsh, 1Hans Minderman, 1Scott Abrams, 1Song Liu, 1Fumito Ito. 1Roswell Park Comprehensive Cancer Center, Williamsville, NY, USA; 2Celldex Therapeutics, Inc., Hampton, NJ, USA

Background The ability of cancer cells to ensure T-cell exclusion from the tumor microenvironment (TME) is a significant mechanism of resistance to anti-PD-1/PD-L1 therapy. Evidence indicates crucial roles of Batf3-dependent conventional type 1 dendritic cells (cDC1s) for inducing antitumor T-cell immunity. However, strategies to maximize the engagement of cDC1s into such ‘immune cold tumors’ remain elusive. Using multiple syngeneic orthotopic mouse models of tumors resistant to anti-PD-L1-therapy, we hypothesized that in situ induction and activation of tumor-residing cDC1s overcomes poor T-cell infiltration.

Methods We utilized three mouse non-T cell-inflamed tumor models that are refractory to anti-PD-L1 therapy (AT-3, B16 and 4T1), and evaluated the efficacy of the combinatorial therapeutic regimen, in situ immunomodulation (ISIM) comprised of intratumoral administration of Fms-like tyrosine kinase 3 receptor ligand (Flt3L) to mobilize cDC1s to the TME, local radiotherapy (RT) to promote immunogenic death of cancer cells and maturation of DCs, and peritumoral CD40/toll-like receptor 3 (TLR3) agonists administration to activate antigen-loaded cDC1s for priming and expansion of tumorspecific CD8+ T cells.

Results Intratumoral administration of Flt3L increased the number of CD103+ DCs in the TME, and RT induced upregulation of CD40 and CD86 in the tumor-residing CD103+ DCs. In situ CD40/TLR3 stimulation facilitated trafficking of CD103+ DCs carrying tumor-associated antigens (TAA) to the tumor draining LN (TdlN), and generation of tumor-specific CD8+ T cells in TdlNs, indicating cross-presentation of TAA. Consequently, ISIM triggered infiltration of tumor-specific stem-like Tcf1+/CD8+ T cells into the TME, mediated rapid regression of untreated distant and primary tumors, and rendered poorly T cell-infiltrated tumors responsive to PD-L1 blockade in multiple mouse tumor models. Moreover, T-cell receptor (TCR) sequencing of TILs revealed that ISIM
facilitated the infiltration of novel clones in the TME. Importantly, serial ISIM further reshaped the TCR repertoires in the TME which had been destined to become resistant to anti-PD-L1 therapy, and rendered tumors continuously responsive to anti-PD-L1 therapy, resulting in durable complete responses and establishment of tumor-specific immunological memory.

**Conclusions** Taken together, ISIM not only increased CD8+ T-cell infiltration but also reshaped the intratumoral TCR repertoires. These findings provide insights into the utility of an in situ combinatorial immunotherapeutic regimen for overcoming resistance to anti-PD-L1 therapy due to tumor-mediated mechanisms of immune cell exclusion.

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465 **RADIOTHERAPY AND CTLA-4 BLOCKADE EXPAND ANTI-TUMOR T CELLS DIFFERENTIATION STATES AND COOPERATE WITH CD40 AGONIST TO INDUCE TUMOR REJECTION**

1Nils Rudqvist*, 1Claire Luullier, 1Maud Charpentier, 1Erik Wernertberg, 1Sheila Spada, 1Caroline Sheridan, X1 Kathy Zhou, X1 Tao Zhang, *Jennifer Sims, 1Alicia Alonso, 1Sandra Demaria. 1Weill Cornell Medicine, Houston, TX, USA; 2Memorial Sloan Kettering Cancer Center, Tarrytown, NY, USA

**Background** Radiotherapy (RT) in combination with CTLA-4 inhibition (CTLA4i) can expand and activate T-cells to reject tumors in both mice and some patients with tumors unresponsive to CTLA4i alone. However, only a subset of patients achieves long-term control of metastatic disease. Similar responses to RT+CTLA4i are seen in the 4T1 mouse model of triple negative breast cancer (TNBC), making it an ideal model to interrogate the interaction between RT and CTLA4i, and identify barriers to its effectiveness.

**Methods** Mice were inoculated in one or both flanks with 4T1 cells. In some experiments one tumor was removed for analysis before start of treatment with RT (3 × 8 Gy) and/or anti-CTLA-4 antibody (9H10, 3 × 200 μg i.p.). The intratumoral T cell response was assessed using bulk and single cell RNA/TCR sequencing. The METABRIC dataset was used to associate gene expression signatures with patient survival. In some experiments, RT+CTLA4i was combined with PD-1, LAG-3, or CD40 Abs.

**Results** RT, alone and with CTLA4i, increased the TCR repertoire clonality and activated T cell density in the tumors (figure 1A-G). In untreated tumors, GzmB+Prf1+Lag3+Pdl1+ and Cd8 + T cells (cluster 0) were most common. CTLA4i ‘unlocked’ Ifng+Cd40lg+ Cd4+ T cells (cluster 2) while RT favored expansion/persistence of Cd8+ T cell clusters. In tumors of mice treated with RT+CTLA4i activated Treg cells (cluster 1) were decreased and Ifng+Cd40lg+Cd4+ T cells (cluster 2) increased. Relatively among CD8+ T cells, Ifng+Tnf+Cd8+ (cluster 4) was expanded at the expense of cluster 0 (figure 2A-F). Gene signatures defining clusters 0, 2, and 4 were associated with improved survival in the METABRIC TNBC patient cohort using a multivariate model (figure 2G-H). In mice, AH1-tumor antigen-specific CD8+ T cells occupied different transcriptional states, with a shift to cluster 4 in mice treated with RT+CTLA4i (figure 2I), suggesting that multiple functional T cell states are required for tumor rejection. Based on the T cell phenotypes expanded by RT+CTLA4i, antibodies to PD-1, LAG-3, and CD40 were tested for the ability to enhance RT+CTLA4i therapy. Only CD40-agonist improved significantly tumor control (figure 3A-B).

**Conclusions** Altogether, these results revealed that RT and CTLA4i have complementary effects and besides driving T cells into tumors shape CD4 and CD8 T cell functional differentiation towards subsets that are associated with improved