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CD40 agonism improves anti-tumor T cell priming induced by the combination of radiation therapy plus CTLA4 inhibition and enhances tumor response

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ABSTRACT

Radiation therapy (RT) combined with CTLA4 blockers converts immunosuppressed (cold) mouse triple negative breast cancers (TNBCs) into immune infiltrated (hot) lesions. We have recently shown that targeting the myeloid compartment to improve dendritic cell activation is required for most TNBC-bearing mice to achieve superior therapeutic responses to RT plus CTLA4 inhibitors.

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Text

In the past decades, cancer immunotherapy has made tremendous progress by showing that therapeutic activation of T cells with immune checkpoint inhibitors (ICI) is effective at achieving durable tumor regression and improved survival in some patients. Since efficacy is limited to a small fraction of patients with preexisting anti-tumor T cells, the addition of other modalities is warranted. Adding hypo-fractionated radiation therapy can convert a tumor into an in-situ vaccine and enhance the response to CTLA4 inhibition (CTLA4i) in mouse models and in some patients.[1,2] Nevertheless, complete and durable responses are rarely observed. The tumor immune microenvironment (TIME) plays a crucial role in the response to cancer immunotherapy.[3] In addition to its direct cytotoxic effect on tumor cells, radiation has been shown to deeply remodel the TIME, shifting the balance between immunosuppressive and inflammatory signals in favor of the latter, but the degree to which this occurs is variable and may depend on the preexisting TIME.[4] Cross-presenting conventional dendritic cells type 1 (cDC1) play a central role in the generation of anti-tumor CD8 T cell responses. Several studies have shown that radiation leads to an increased recruitment to the tumor and activation of cDC1 that can prime CD8 T cells specific for tumor neoantigens, and while we have observed such neoantigen-specific CD8 T cell responses in a patient following radiation and ipilimumab,[2] this event remains a rare occurrence.

To investigate the mechanisms of resistance that prevent strong and durable responses to radiation and CTLA4i we used 4T1 and AT3, two murine syngeneic triple negative breast cancer tumors highly resistant to ICI.[5] Single-cell analysis coupled with T cell receptor (TCR) repertoire analysis was used to compare the phenotype of the T cells present in tumors that were untreated and treated with single-agent radiation (a non-ablative dose of three daily doses of 8 Gy), CTLA4i alone, or their combination. T cells were clustered according to gene expression patterns and the functional phenotype annotation was performed using the projecTILs computational pipeline.[6] T cells and myeloid cell present in the tumor and draining lymph nodes were also characterized by flow cytometry. The data generated about T cell functional subsets and their expression of checkpoint receptors were used to identify targets for additional immunotherapy to be tested for their ability to improve tumor responses to radiation and CTLA4i.

Specifically, radiation increased T cell clonality and tumor infiltration by exhausted CD8 T cells, whereas CTLA4i increased the ratio of CD4 helper to regulatory T cells but did not increase tumor infiltration by T cells. When used in combination, radiation and CTLA4i markedly enhanced T cell infiltration (Figure 1) and diversified the phenotype of the T cells present in the tumor. Among CD4, T-helper 1 cells dominated, while within CD8 compartment there was an increase in newly activated, effector memory and precursor exhausted T cells. In a multivariate analysis of two public data sets (METABRIC for TNBC and TCGA SKCM for melanoma, respectively) the gene signature encompassing these CD8 T cell phenotypes was associated with improved outcomes in patients, suggesting that this combination of CD8 T cell functional states is clinically relevant.

Given that in tumors treated with radiation and CTLA4i the majority of CD8 T cells were $PD1^+$ and the largest subset co-expressed LAG3, we tested if targeting these molecules could improve the response to radiation and CTLA4i. Surprisingly, there was no benefit. We then turned to the CD4 T cell compartment, where we found a significant fraction of regulatory T cells

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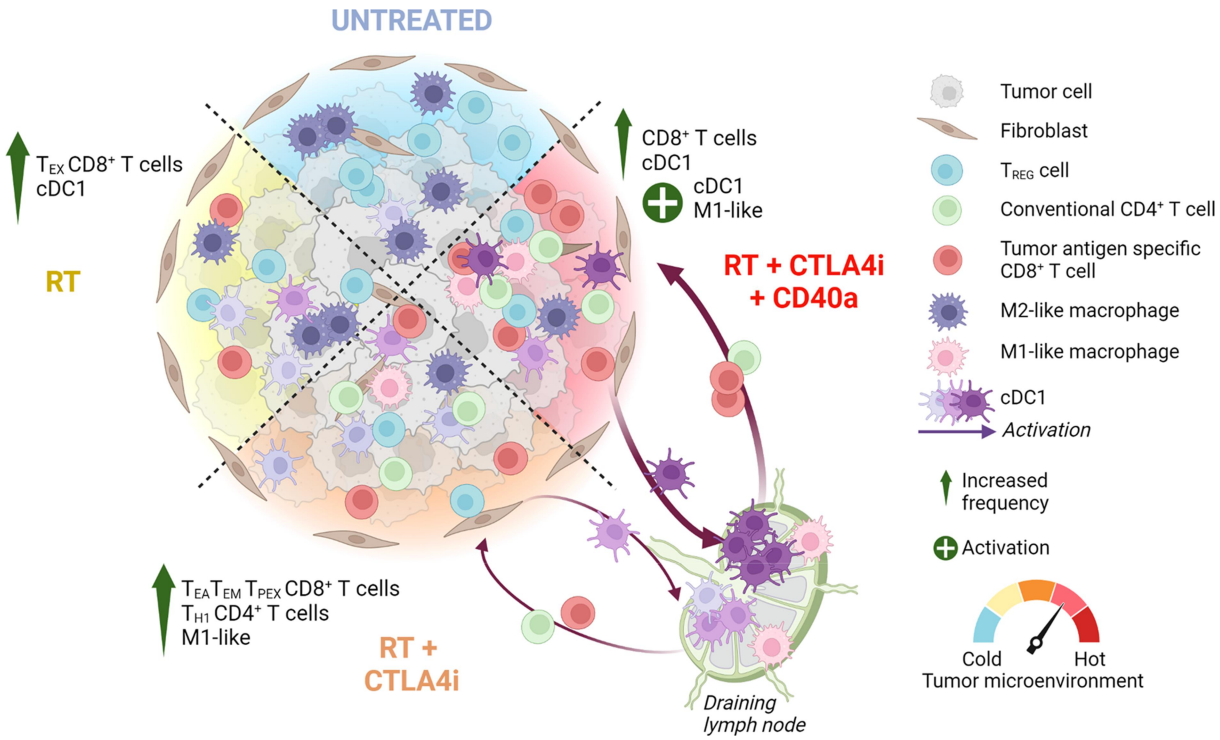

Figure 1. Dialing up the Heat in the Tumor. Radiation therapy (RT) increases infiltration by tumor-antigen specific $CD8^+$ T cells with an exhausted phenotype (T_{EX}). Addition of CTLA4 inhibitor (CTLA4i) increases $CD4^+$ T helper cells and $CD8^+$ early activation (T_{EA}), effector memory (T_{EM}), and precursor exhausted (T_{PEX}) T cells as well as type 1-like macrophages (M1-like). Further, activation of conventional dendritic cells type 1 (cDC1) by CD40 agonism (CD40a) improves priming of tumor-antigen specific $CD8^+$ T cells in the draining lymph node and their trafficking to the tumor, achieving the most profound tumor responses in mouse models of triple negative breast cancer. Created with BioRender.com.

expressing high levels of GITR and OX40. Antibodies targeting OX40 or GITR failed to improve the response to radiation and CTLA4i. Finally, flow cytometry analysis revealed that the expression of CD40-ligand was low in helper CD4 T cells. Since CD40 engagement on DC by CD40-ligand expressed by CD4 T cells is critical for DC activation, we hypothesized that radiation and CTLA4i may promote a sub-optimal helper function of CD4 T cells. Confirming this hypothesis, activation of dendritic cells by a CD40 agonist significantly increased priming of tumor antigen-specific CD8 T cells in the draining lymph node as well as their trafficking to the tumor, resulting in increased tumor responses in the majority of mice. A combination of three interventions, radiation, CTLA4i and CD40 agonism were required to achieve this effect.

These results emphasize the importance of considering combinations of therapies that target different immune cell compartments, rather than focusing only on re-invigorating T cells. This may be especially relevant when generating T cells is critical, as in the case of cold tumors. In this setting, CD40 agonism not only helps the dendritic cells to present the tumor antigens released by radiation but also decreases the myeloid-driven immunosuppression in the tumor.[7] The latter is a common feature in breast cancer, including in the majority of triple negative breast cancers that are not responsive to ICI.[8] Overall, our data suggest that for this and other cold tumor combinations of cytotoxic agents that induce an immunogenic cell death of the cancer cells with CTLA4i and CD40 agonism may be more effective than double checkpoint blockade (i.e., anti-PD1 plus anti-CTLA4) or other combinations targeting multiple T cell checkpoints without targeting antigen-presenting cells.

Recent clinical trial results testing the combination of anti-PD1 with CD40 agonism and chemotherapy in pancreatic cancer patients have been disappointing in that, whereas each double combination (anti-PD1 + chemotherapy and anti-CD40 + chemotherapy) showed activity, the triple combination of anti-PD1 plus CD40 agonism and chemotherapy did not show any additional benefits.[9] Given the critical role of CTLA-4i in enhancing T cell priming,[10] we provide the preclinical rationale[5] for testing CD40 agonism with CTLA4i and an inducer of immunogenic cell death such as radiation in the clinic.

Disclosure statement

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