Immune effects of spatially fractionated radiation therapy in triple negative breast cancer

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Objectives: Triple negative breast cancer (TNBC) is a heterogenous disease with a high incidence of primary and acquired resistance to immune checkpoint inhibitors (ICIs), due to mechanisms such as decreased CD8+ T cell infiltration in tumors and resistance from the presence of T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. Spatially fractionated radiation therapy (SFRT) is hypothesized to enhance immune system activation through high dose peaks that contribute to antigen presentation as well as sparing of immune cells and vascular access in low dose volumes. Further understanding of SFRT-induced immune activation and suppression is essential to strategically combine SFRT with ICIs to improve local control, reduce toxicity, and augment abscopal responses in metastatic TNBC.

Methods: 4T1 murine breast carcinoma cells were injected subcutaneously into bilateral hindlimbs of adult BALB/c mice. Mice were randomized into groups of 11 and ipsilateral hindlimb syngeneic tumors were irradiated using an SFRT GRID collimator of thickness 3 mm (peak to valley dose ratio (PVDR) 3.3), SFRT GRID collimator of thickness 5 mm (PVDR 3.5), or whole-tumor open field with a dose of 22 Gy compared to a control unirradiated group. The dose of 22 Gy was selected based on clinical SFRT practice and previous preclinical 4T1 data from our group suggesting a dose threshold. The contralateral hindlimb tumors were not irradiated but used for observing distal bystander effects. The GRID collimators made of brass plates were drilled with 5 holes of 2 mm diameter equally spaced in a cross pattern with center-to-center distance of 3 mm, a hole in the center, and a lead shield outside of the irradiation field. Radiation was delivered when serum amyloid A was estimated to be high, to minimize potential confounding immune oscillation effects. The mice were randomized to tumor growth and survival or cytokine measurements and flow cytometry. Bioluminescence imaging was also performed.

Results: The tumor growth curves showed no significant difference between groups, with the whole tumor and GRID treated groups showing an initial growth plateau followed by subsequent rapid growth. Whole tumor treated mice showed higher survival of 5 days, but with side effects of sensitive skin, ruffled fur, and diarrhea compared to the GRID treated and control mice. The GRID treated tumors showed development of necrosis, while the control mice had an increase in tumor volume without necrosis. Mice in the 5 mm GRID cohort had elevated IFN gamma and IL10. Flow cytometry showed a significant increase in exhausted T cells ($PD1^+$, $CTLA4^+$) as well as significantly increased Dendritic Cells ($CD155^+$) within the 5 mm GRID treated tumors and whole tumor treated groups. All groups including the controls showed a high number of M2 macrophages, M-MDSC indicative of an anti-inflammatory tumor microenvironment.

Conclusion(s): Our findings suggest that SFRT may promote antitumor immunity through high T cell infiltration in both treated and untreated tumors, as well as upregulate CTLA-4 and TIGIT checkpoints,

and may therefore synergize the effect of ICIs. However, since SFRT also shows high levels of MDSCs, macrophages, and dendritic cells in both the treated and untreated tumors, this may have prevented any meaningful reduction in tumor growth past an initial plateau once the T cells became exhausted. SFRT was better tolerated than whole-tumor irradiation. A combination of ICIs, SFRT, and drug(s) to minimize MDSCs is a strategy that warrants further evaluation to optimize immune activation and reduce immune suppression to improve checkpoint inhibitor resistance in TNBC.