Background Breast cancers have shown limited responses to current FDA approved forms of immunotherapy. Defective antigen presentation in breast cancers contributes to this deficient anti-tumor immunity. Flt3 ligand (Flt3L) is a growth factor that increases differentiation of cDC1 dendritic cells, critical mediators of antigen presentation. CD40 agonist (CD40a) activates all 3 classes of antigen presenting cells - dendritic cells, B cells, and macrophages. Synergy has been demonstrated between Flt3L and CD40a as well as between CD40a and chemotherapy in other cancers, however the combination of all three has not been studied in breast cancer.

Methods 6–8 week old C57BL/6 mice were injected with E0771 breast cancer cells. When tumors were 50 mm³, mice were first treated with pegylated liposomal doxorubicin (PLD) once by tail vein followed by Flt3L intraperitoneal (IP) daily for 5 days and then CD40a IP 1 week after. Tumor volumes were measured serially to assess pre-clinical activity of the drug combinations. To assess mechanism of action, tumors and lymph nodes were harvested from sacrificed mice for single cell RNA sequencing and immunohistochemistry analysis.

Results Treatment with PLD, Flt3L, and CD40a led to improved tumor control compared to control, PLD monotherapy, CD40a monotherapy, Flt3L monotherapy, PLD + Flt3L doublet, and PLD + CD40a doublet (p<0.0001). Immunohistochemistry revealed an increase in CD8+ T cell infiltration into the tumor microenvironment with addition of CD40a and Flt3L to PLD chemotherapy. Single cell RNA sequencing demonstrated Flt3L addition to PLD chemotherapy induced an increase in intra-tumoral cDC1 and other dendritic cell subsets with Flt3L treatment. CD40a addition to PLD instead led to a decrease in DC subsets in the tumor likely from activation and emigration, repolarization of intra-tumoral macrophages, and class switching of lymph node B cells. Addition of Flt3L and CD40a to PLD in triplet combination led to both sets of changes.

Conclusions Novel triplet combination with PLD, CD40a, and Flt3L leads to enhanced tumor control in the E0771 breast cancer mouse model. A clinical trial with this combination in metastatic triple negative breast cancer patients is open and recruiting (NCT05029999).

Ethics Approval The research was conducted in accordance with the ethical guidelines set forth by the Institutional Animal Care and Use Committee (IACUC).

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