Targeting dendritic cells with CD40 agonist-based triplet immunotherapy induces IL12B-driven tumor control in triple-negative breast cancers

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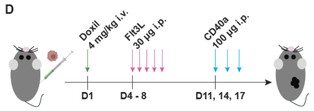
Background

- There is a need for improved immunotherapy strategies beyond current FDA approved treatments for breast cancer.
- Defective antigen presentation has been shown to play a contributory role in deficient anti-tumor immunity in breast cancers.
- Flt3 ligand (Flt3L) is a growth factor that increases differentiation into DC1 dendritic cells. CD40 agonist (CD40a) activates all 3 classes of antigen presenting cells – conventional dendritic cells (cDCs), B cells, and macrophages. Synergy with chemotherapy and CD40a has been observed in other cancers.

Methods

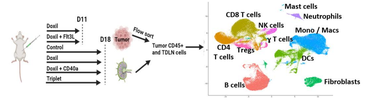
- 50-100 mm^3 breast tumor bearing animals received treatment with Doxil + Flt3L + CD40a therapy as per Fig. 1 vs IgG control, monotherapy, or doublet combinations and tumor growth measured.

Figure 1: Triplet therapy treatment schema



- Tumors were characterized using scRNAseq of CD45+ immune cells, flow cytometry, IHC, and TCRseq.
- Mice were sacrificed and tumor and draining lymph nodes were isolated from E0771 tumor bearing mice D11 in tumors treated with Doxil or Doxil + Flt3L to study microenvironment before treatment with CD40a, and D18 in tumors treated with isotype control, Doxil + isotype, Doxil + CD40a, and triplet therapy (Fig. 2).

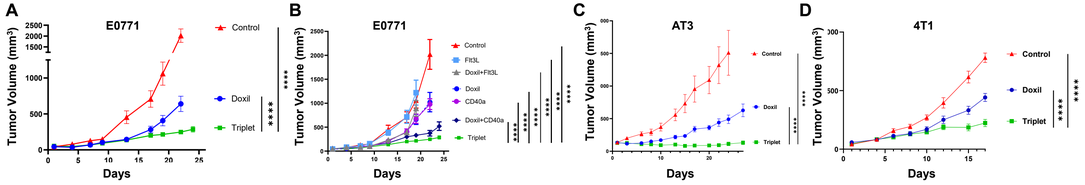
Figure 2: Immunophenotyping schema



- To identify immune cell subsets responsible for tumor control after triplet therapy, we depleted immune cell subsets (CD8, CD4, Batf3-/-, CSF1R macrophages, B cells), select chemokines / cytokines (CXCL9, IL-12b) with antibodies and genetic depletion models. We also blocked lymph node egress with FTY720.

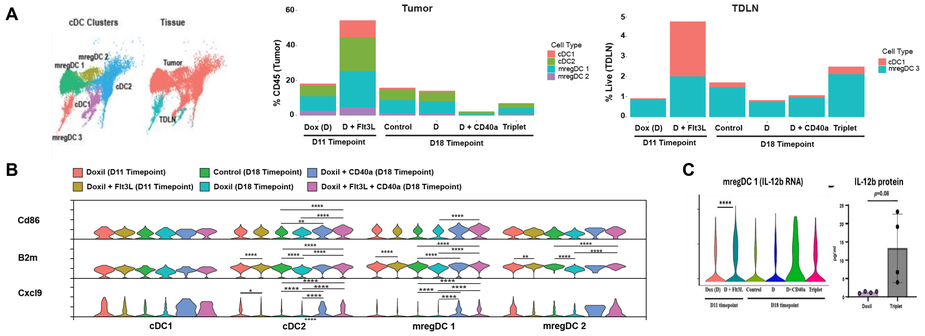
Results

Figure 3: Triplet therapy Doxil+ Flt3L + CD40 agonist therapy leads to enhanced tumor control and survival in TNBC mouse models.



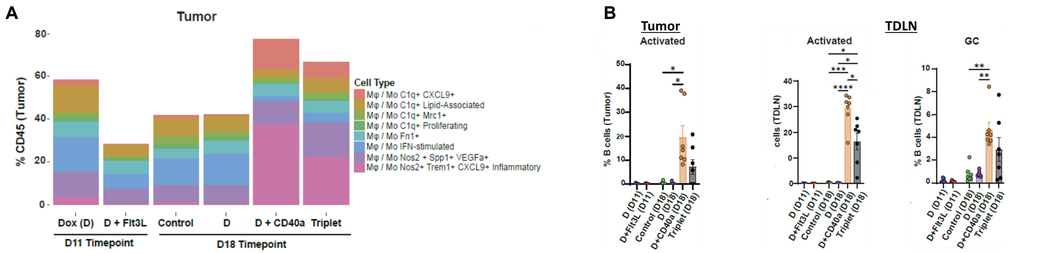
(A) E0771 tumor bearing mice were treated with Doxil + Flt3L + CD40a and demonstrated significant improvement in tumor volume compared to chemotherapy alone and control mice. (B) Superiority of triplet therapy was then compared to other monotherapy and doublet controls. Efficacy was also demonstrated in AT3 and 4T1 tumor models (C-D).

Figure 4: Flt3L increases all cDC subsets in tumor and TDLN, whereas CD40a primarily reduces intra-tumoral cDC infiltration and promotes inflammatory CXCL9 and IL-12b expressing cDC subsets.



(A) Flt3L increases all cDC subsets whereas CD40a decreases cDCs in the tumor due to potential migration to TDLN. (B) Treatment induces elevated Cd86, B2m, Cxcl9 across cDC subsets and (C) IL12b in mregDC population. Total IL-12b quantification by ELISA in tumor lysate.

Figure 5: CD40a activates the other 2 main antigen presenting cells – tumor associated macrophages and B cells.



(A) CD40a component of therapy profoundly expands Cxcl9 expressing inflammatory macrophages in the tumor and (B) activates B cells in the tumor and TDLN including promoting germinal center B cells.

Figure 6: Triplet therapy increases intra-tumoral CD8 T cell infiltration.

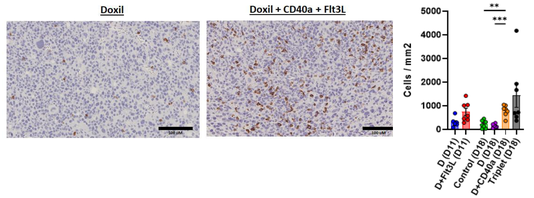
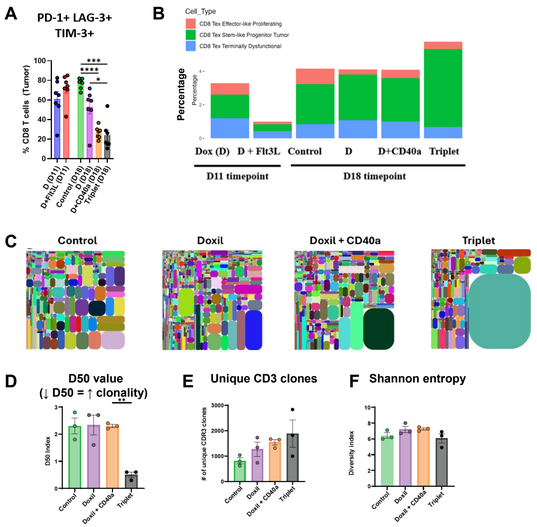
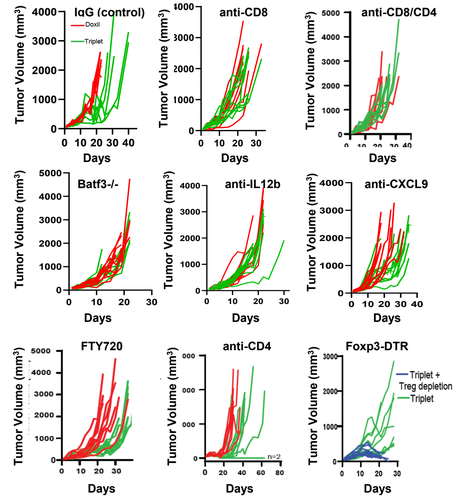


Figure 7: Triplet therapy increases stem-like, less exhausted, clonally expanded T cells compared to Doxil.



(A-B) Triplet therapy reduces exhausted CD8 T cells by flow cytometry while scRNAseq shows an increase in stem-like subsets and decrease in terminally dysfunctional exhausted T cells. (C) Tree maps illustrating TCR clonal expansion of intratumoral T cells induced by triplet therapy, where each color-coded shape represents a distinct DNA clonotype. (D) Quantitative diversity analyses demonstrated triplet therapy reduces D50 scores, defined as the percent of dominant and unique clones accounting for 50% of total number of CDR3 sequences, (E) trend towards increase in unique CDR3 sequences, and (F) and do not change Shannon entropy with triplet therapy.

Figure 8: Treatment efficacy is dependent on cDC1, IL12b, and both CD8 / CD4 T cells. Efficacy is enhanced by depleting Tregs.



Treatment efficacy was completely abrogated with CD8 / CD4 co-depletion, lack of cDC1 cells (Batf3-/- model), and IL-12b blockade. Efficacy not impacted with anti-CSF1R macrophage depletion or B cell depletion (data not shown). Efficacy was maintained in absence of CXCL9 and with blockade of lymph node egress (FTY720) suggesting importance of pre-existing T cells for response. Treatment was further enhanced with Treg blockade, consistent with tumor free mice on CD4 depletion alone.

Conclusions

- Treatment with triplet therapy (Doxil+Flt3L+CD40a) improves tumor control compared to standard chemotherapy in TNBC.
- Treatment efficacy is dependent on cDC1 cells, both CD8 and CD4 T cells, and IL-12b cytokine secretion.
- Lymph node egress experiments suggest importance of pre-existing intra-tumoral T cells over infiltration of new clones.
- Treg depletion enhances response to triplet therapy.
- We are leading a clinical trial testing this novel triplet combination of Doxil + Flt3L CDX-301 and CD40 agonist CDX-1140 in patients with metastatic HER2 negative breast cancer (NCT05029999).

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