

Evaluating the Efficacy of Immunotherapy in Triple Negative Breast Cancer

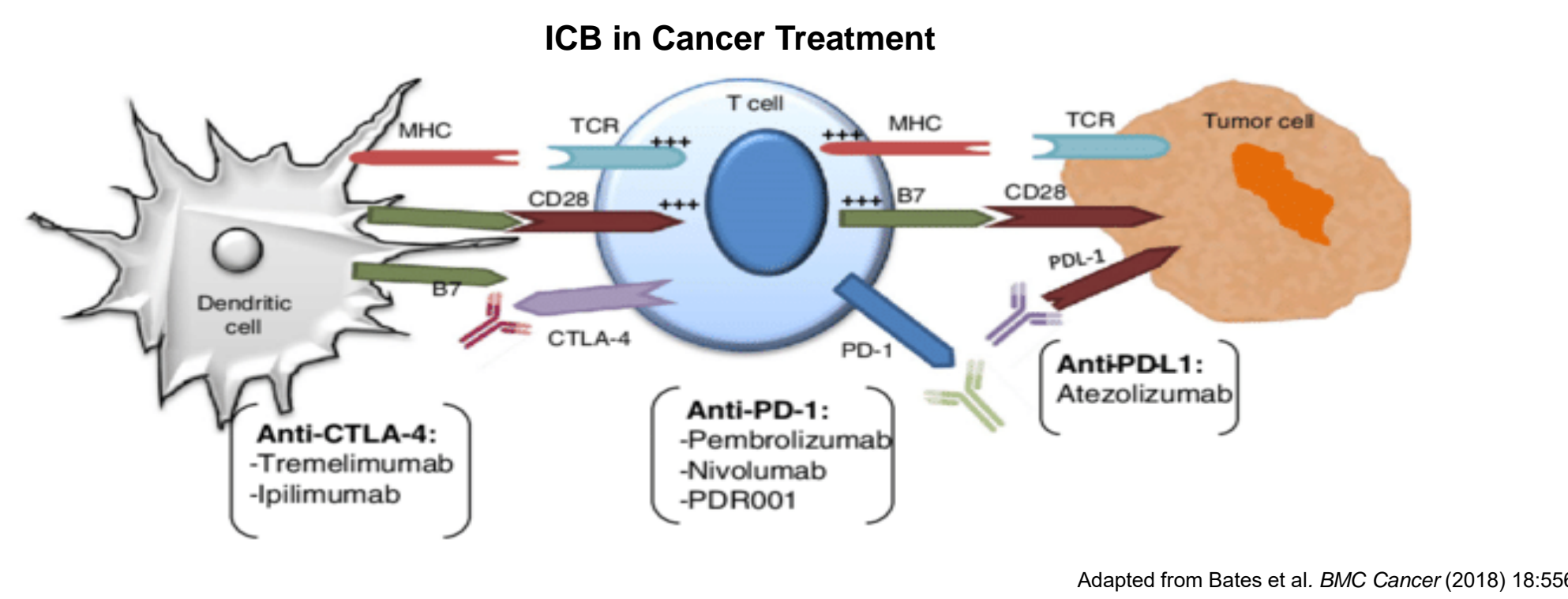
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Introduction

- Tumor-infiltrating lymphocytes (TILs) in the breast tumor microenvironment (TME) dictate tumor response to therapy through immune activating or suppressive mechanisms.
- Using immune checkpoint blockade (ICB) to target and activate TILs has garnered clinical success in treating several tumor types, however yielded limited results in breast cancer.
- Recent clinical trials have demonstrated that combining ICB with standard-of-care chemotherapy increases progression-free survival (IMpassion130), specifically PD-L1 positive tumors, and pathologic complete response (KEYNOTE-522) in triple-negative breast cancer patients.
- While promising, TNBC clinical trials lack an experimental ICB-only group and patients receive several other treatments prior to ICB, therefore it is necessary to determine the therapeutic benefit of immunotherapy alone or as first-line treatment.**



Objectives

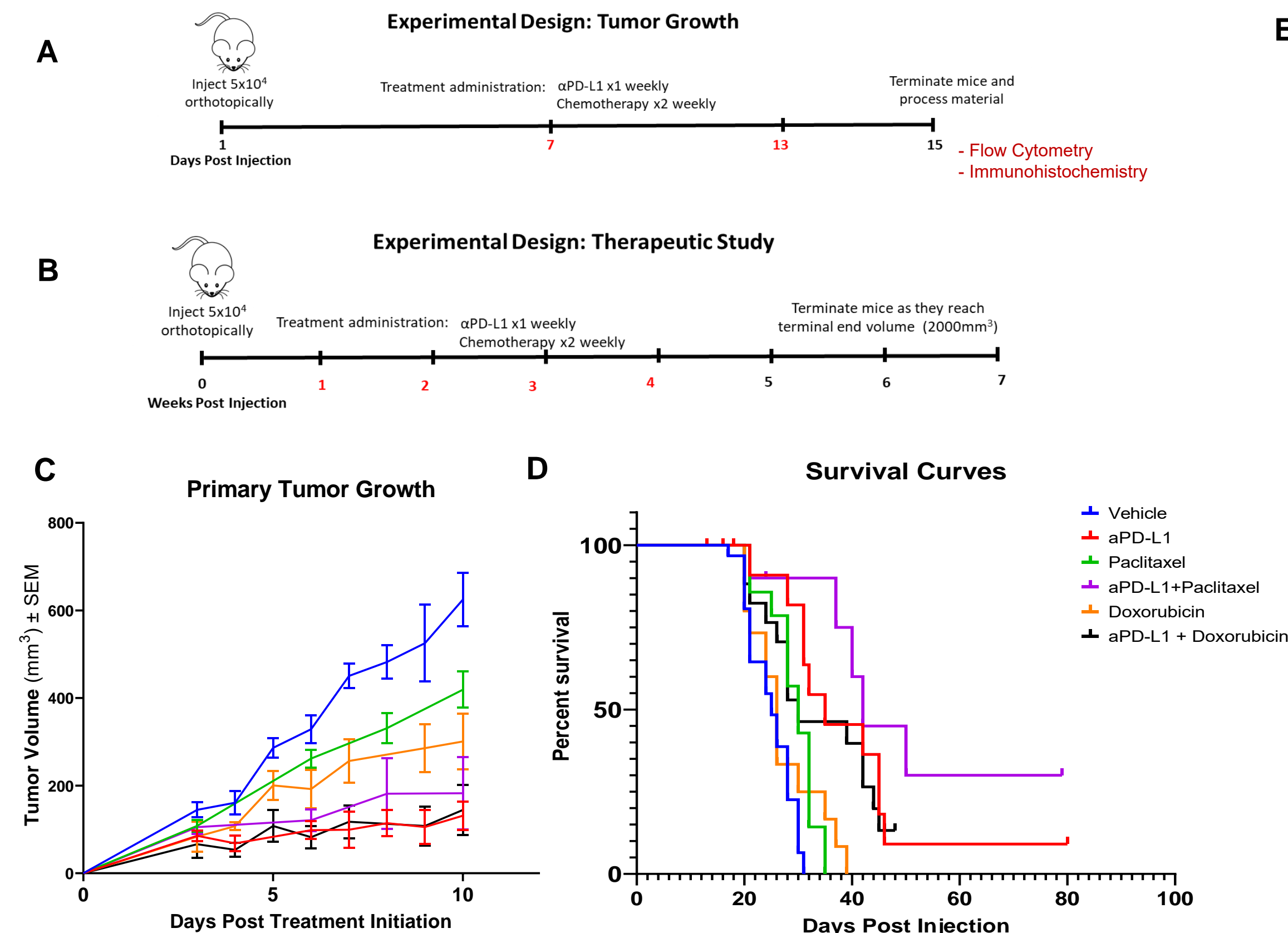
- Evaluate the synergistic therapeutic benefits of different combinatorial strategies that increase tumor cell immunogenicity and sensitize tumors to immunotherapy in TNBC.
- Identify the most efficacious therapeutic combinations for eliciting the most robust immunogenic responses and eradicating TNBC tumor progression.

Methods

Animals: 5 x 10⁴ EMT6 cells were injected into the inguinal mammary fat pad of five-week-old female BALB/c mice. Mice were intraperitoneally injected with aPD-L1 (Bio X Cell Therapeutics), Paclitaxel (Hospira, Inc), Doxorubicin (Pfizer, Inc.), or saline as a vehicle control. Tumor progression was documented by palpation and tumor volumes assessed by caliper measurements 3x weekly.

Flow Cytometry: Tumors were dissociated in a collagenase and protease enzyme cocktail using gentleMACS Dissociator (Miltenyi Biotec.). Cells were FC blocked with anti-mouse CD16/32, then stained with viability dye. Cells were stained with conjugated antibody cocktail specific to cell type (BioLegend) as follows: Dendritic Cells: CD45 and CD11c, Myeloid Cells: CD45, CD11b, LY-6G, and LY-6C, B Cells: CD45 and CD19, and Natural Killer Cells: CD45 and NKp46. Samples were analyzed using a Nxt Attune Analyzer and files analyzed with FlowJo software.

ICB blunts tumor growth and promotes survival in TNBC

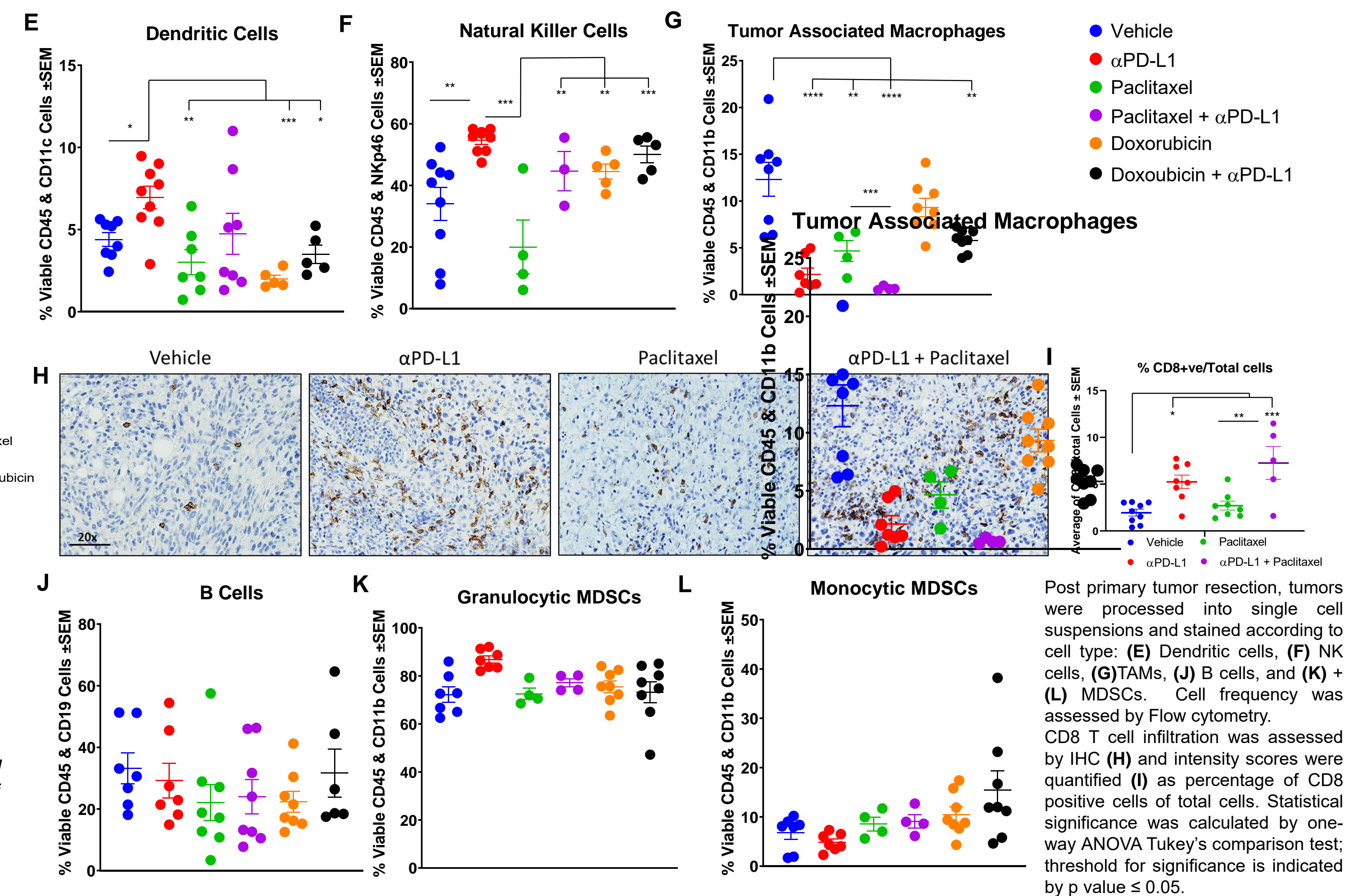


Experimental schematic for modeling TNBC *in vivo*. Mice were injected with EMT6 orthotopically and treatments were started when tumor volumes were 100-200mm³ and terminated after 1 or 4 weeks of treatment for tumor growth/flow cytometry (A) and survival studies (B), respectively. Systemic administration of single-agent aPD-L1 or in combination with chemotherapy reduces the growth rate of primary tumor (C) and enhances *in vivo* survival (D).

Conclusions

- Single-agent αPD-L1 is similarly efficacious as combined with chemotherapy in controlling primary tumor growth rate and extending a survival benefit (even post treatment cessation) in mammary tumor-bearing mice.
- Single-agent αPD-L1 and in combination with chemotherapy significantly enhances the infiltration of antigen presenting cells and cytotoxic immune cells into primary mammary tumors with little impact on B cells and myeloid-derived suppressor cells.

ICB increases the infiltration of APCs and cytotoxic immune cells and attenuates immune-suppressive populations in TNBC tumors



Future Directions

- Perform single cell RNA sequencing to characterize the genetic signatures of TILs within TME.
- Perform T cell receptor sequencing of TIL subsets to identify immunogenic tumor antigens.
- Deplete candidate immune populations (Natural Killer cells, macrophages) to determine the underlying mechanism of action for immunotherapy response in TNBC.