Oral bexarotene improves tumor response to conventional breast cancer chemotherapy possibly through modification of the tumor immune environment in mouse mammary tumor models

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Introduction:

- Increased Th1 immune activating immune infiltrate improves survival and predicts pathologic complete response in breast cancer (1,2).
- Chemotherapy modulates the tumor immune environment, in breast cancer, doxorubicin increases CD8 and paclitaxel decreases CD4 T regulatory cells in the tumor (3).
- Bexarotene, an oral retinoic acid receptor X agonist, *in vitro* decreases CD8 T cell apoptosis and increases IL2 expression (a potent Th1 cytokine) and to increase intratumoral CD8 T cell infiltrate (4,5).
- As monotherapy bexarotene has a 20% clinical response in metastatic breast cancer (6)
- TgMMTV-neu mice are an ideal model for this study because they are immunologically competent and genetically similar to human luminal B breast cancer (7).
- **The goal of this study is to demonstrate whether the well tolerated oral agent bexarotene can enhance the anti-tumor response of conventional chemotherapy through increasing intratumoral CD8$^+$ T cells in TgMMTV-neu transgenic mice.**

Methods:

Maintenance the TgMMTV-neu mice. TgMMTV-neu mice were obtained from Jackson Laboratories and maintained under strict inbreeding conditions. The TgMMTV-neu mice were tested for expression of the neu transgene. All mice were bred and maintained under specific pathogen free conditions.

Tumor evaluation in the TgMMTV-neu mice: TgMMTV-neu mice were enrolled into the study at 6-8 weeks. Therapy was started when the mice had 100 mm^3 tumors and was continued for four weeks unless the tumors were ulcerated or > 1000 mm^3. Mice were examined for tumor development three times per week from six weeks of age until sacrifice. Tumor volumes were calculated from raw measurements by [(length) x (width) x (depth) x (π/6)]. Multiple tumors were tracked independently. Tumor growth rates were calculated by determining the change in volume between subsequent measurements.

Dosing and delivery of bexarotene and chemotherapy. The chemotherapies were given intravenously through the tail vein and were given weekly. Doxorubicin was dosed at 5 mg/kg, cyclophosphamide was dosed at 100 mg/kg, and paclitaxel was dosed at 10 mg/kg and control mice had PBS infused weekly. Bexarotene was given 5 days a week as an oral gavage dissolved in 100 µL sesame oil and control mice were given 100 µL of sesame oil 5 days per week. The dose of 50 mg/kg bexarotene was determined by a dose response study measuring the anti-tumor activity of bexarotene at 100 mg/kg, 50 mg/kg, and 25 mg/kg and the lowest effective anti-tumor dose was selected for all further experiments (Figure 4).

Evaluation of the tumor immune infiltrates. At sacrifice, immune cells were isolated from the spleen through a 70µm cell strainer and the tumor cells were further enriched through FICOL gradient. Fresh cells were stained with PE CD3, PE Cy-5 CD4, and PE Cy-7 CD8. These cells were analyzed by flow cytometry on a FACSCanto flow cytometer and analyzed with FlowJo software. Results are reported as total percentage of a cell population or ratio of cell quantities as indicated.

Statistical analysis Graphs, t-tests, and ANOVA comparisons were completed using GraphPad Prism v5.03 software. A One-Way ANOVA with Tukey's post-test was used for comparisons of tumor growth differences and a two-Way ANOVA with Bonferroni's post-test was used for grouped comparisons. Significance was considered at p<0.05 for all statistical tests.

Bexarotene enhances chemotherapy tumor inhibition in TgMMTV-neu mice

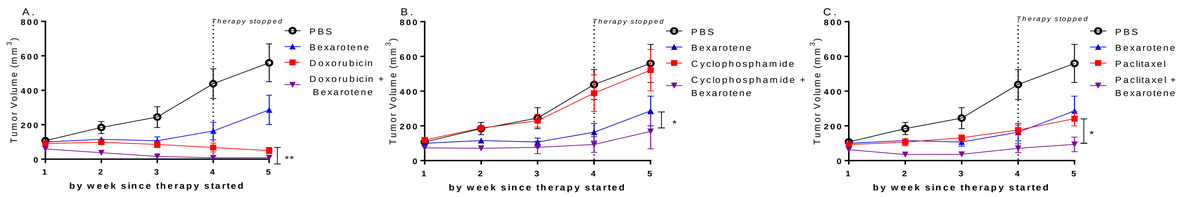

Figure 1. The combination of bexarotene and with each chemotherapy decreased tumor growth more than either the chemotherapy or bexarotene alone. TgMMTV-neu mice (n=5/group) with ~ 100 mm^3 tumors were treated with TgMMTV-neu mice (n=5/group) with ~ 100 mm^3 tumors were treated with (A) bexarotene and doxorubicin (B) bexarotene and cyclophosphamide and (C) bexarotene and paclitaxel. PBS (black circles), bexarotene alone (blue triangles), chemotherapy alone (red boxes), or chemotherapy plus bexarotene (purple triangles). Average tumor growth is shown by week until 1 week after discontinuation of therapy. Bexarotene enhanced tumor inhibition in (A) doxorubicin (B) cyclophosphamide and (C) paclitaxel over these chemotherapies alone. ** p< 0.01 *p <0.05

Bexarotene and chemotherapy increase tumor infiltrating CD8 T cells

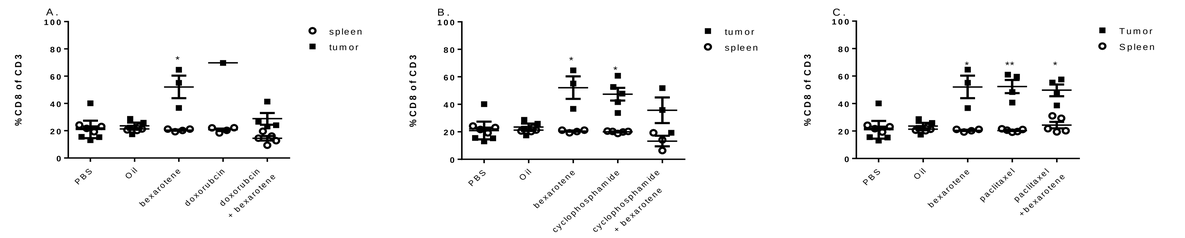

Figure 2. Bexarotene, cyclophosphamide, and paclitaxel increase intratumoral CD8 infiltrate but addition of bexarotene to chemotherapy does not further increase CD8 infiltrate . TgMMTV-neu mice (n=5/group but FACS only able to be performed if adequate numbers of cells were isolated from the tumor). (A)-(C) are % CD8 of CD3. Black squares are tumor and open circles are spleen. ** p<0.01 * p<0.05.

Bexarotene and chemotherapy decreases tumor infiltrating CD4 T cells, increasing the CD8:CD4 ratio

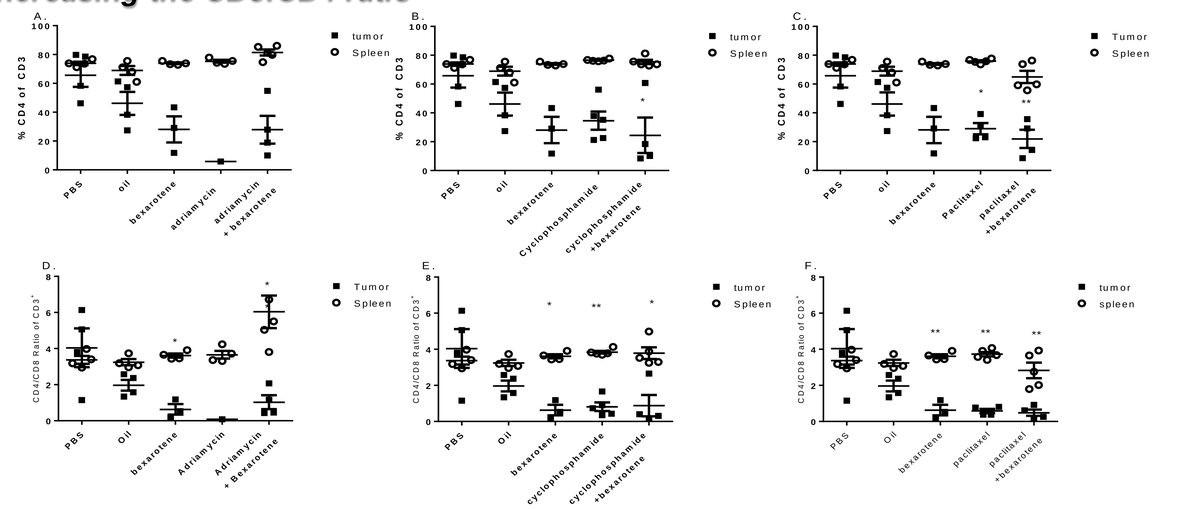

Figure 3. Bexarotene, doxorubicin, cyclophosphamide, and paclitaxel decrease CD4 tumor infiltrate therefore decreasing CD8:CD4 ratio. TgMMTV-neu mice (n=5/group but FACS only able to be performed if adequate numbers of cells were isolated from the tumor). (A)-(C) are % CD4 of CD3, (D)-(F) ratio of CD8:CD4. Black squares are tumor and open circles are spleen.
** p<0.01 * p<0.05.

Conclusions:

- As monotherapy, all agents but cyclophosphamide can inhibit tumor growth in the TgMMTV-neu mouse
- Bexarotene in combination with doxorubicin, cyclophosphamide, or paclitaxel augments the anti-tumor function further than either the chemotherapy or bexarotene monotherapy alone
- Both bexarotene and the intravenous chemotherapies alter the tumor immune environment, increasing the CD8 T cells and decreasing the CD4 T cells
- When added to chemotherapy, bexarotene decreases the intratumoral CD4 infiltrate decreasing the CD4:CD8 ratio.
- Bexarotene may be a well-tolerated oral immunomodulatory agent which can be used to improve response to neoadjuvant chemotherapy.

References:

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