Effective anti-neu initiated anti-tumor responses require the complex role of CD4⁺ T cells

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Supplemental Figure 1: The percentage of tumor infiltrating CD4⁺ cells greater than CD8⁺ cells. Tumor bearing mice were treated with 200 μg of anti-neu (clone 7.16.4) on day 11 and 18; 3–4 days later, tumors were resected and analyzed for the presence of CD4⁺ and CD8⁺ T cells via flow cytometry. Data are representative of three independent experiments.
Supplemental Figure 2: Anti-CD4 depletion effectively removes CD4⁺ cells. WT BALB/c mice (n=5/group) were inoculated s.c. with 4 x 10⁵ TUBO cells and treated i.p. with 150 μg of anti-neu (clone 7.16.4) on day 11 and 18, and 200 μg of a CD4-depleting antibody (clone GK1.5) or control antibody (rIgG) was administered i.p. on day 10 and 15. Peripheral blood was acquired on day 20. Because the clone of the CD4-depleting antibody is the same clone used for most CD4-labeling antibodies available for flow cytometry, depletion of CD4 cells was analyzed by gating on CD3⁺ cells and then examining the reducing of the CD3⁺CD8⁻ population.
Supplemental Figure 3: B cells are not required for anti-neu therapy. WT BALB/c mice (n=5/group) were inoculated s.c with $4 \times 10^5$ TUBO cells and treated i.p with 200 $\mu$g of anti-neu (clone 7.16.4) or control (mlG) antibody on day 11 and 16 (black triangles), with or without the administration of 250 $\mu$g of a CD20-depleting antibody (clone 18B12) on day 0, 7, and 14 (grey triangles). Statistical analysis was performed using a repeated-measurement Two-Way ANOVA on time points after anti-neu therapy. Data are representative of three independent experiments.