#6679. Immunophenotypic and Transcriptome Analyses of CT26 and 4T1-Luc Tumor Models Following Anti-mCTLA-4 Treatment

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Abstract
- Informed selection of syngeneic models for use in preclinical drug development is critical for success.
- CT26 colorectal and 4T1-Luc breast tumors were treated with anti-mCTLA-4 to address how the tumor microenvironment influences therapeutic outcome.
- The effects of anti-mCTLA-4 treatment had on immune subset infiltration into the tumor as well as lymphocyte activation and exhaustion biomarkers were assessed by flow cytometry.
- Transcriptome analyses was used to explore differences in induction of immune-related gene expression between the CT26 and 4T1-luc tumor models following anti-mCTLA-4 therapy.

Methods
- CT26 or 4T1-Luc cells were implanted into the right axilla or MFP #4, respectively, of female Balb/c mice. Animal care and use was performed in conformance with the Guide for the Care and Use of Laboratory Animals in an AAALAC-accredited facility.
- Dosing was initiated with established disease, and tumor progression was monitored by caliper measurements. Anti-CTLA-4 (9D9) or isotype control (MPC-11) were dosed twice per week for two weeks (Bio X Cell, West Lebanon, NH).
- Subcutaneous tumors were harvested, dissociated (Miltenyi, Germany), and stained for flow cytometry. Data was analyzed with FlowJo software (Floors, LUC, Ashland, OR).
- mRNA was extracted from paraffin embedded samples and mRNA expression was determined.

Conclusions
- Baseline immune profiles and anti-mCTLA-4 induced immune modulation in CT26 and 4T1-Luc tumors are indicative of immunoresponsive (warm) and immunosuppressive (cold) phenotypes respectively.
- Changes in immune infiltrate of CT26 tumors following treatment with anti-mCTLA-4 suggest activation of an immune response. 4T1-luc tumors do not have demonstrable alterations to immune cell subset distribution, further supporting an immunosuppressive phenotype.
- Gene expression signatures related to immune activation are upregulated in CT26 but not 4T1-Luc, and support findings observed from the flow cytometry data.
- Immune cell subset distribution in the tumor microenvironment and relative gene expression are two potential biomarkers to predict anti-tumor response.

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