#1683. A Comparative Evaluation of Immunotherapy Responses in Murine Colon Carcinoma

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Introduction and Background

- With the drive in oncology to develop molecules targeting the immune system, there is a need for reliable and well-characterized preclinical models.
- The focus of immuno-oncology drug development has shifted to combination strategies with approved immunotherapies, immunometabolism targets and costimulatory molecules.
- To better understand the utility of these agents in preclinical development, we examined the response of CT26 to combinations with anti-mPD-1, a panel of costimulatory molecules, and epacadostat, an IDO inhibitor, using in vivo tumor growth delay studies.
- Knowledge of immune composition and localization can help guide rational monotherapy and combination strategies. Investigation into these parameters are also contained in this body of work.

Materials and Methods

- CT26 cells were implanted subcutaneously (SC) into the right axila of Balb/c mice. Dosing was initiated when tumors were established, and tumor progression was monitored by caliper measurements. Epacadostat (MedChem Express) and fluorouracil (Fresenius Kabi) were administered for three weeks. Antibodies were dosed for two weeks (Bio X Cell, West Lebanon, NH). Focal radiation was delivered as a single bolus using image-guided techniques (SARRP; Xstrahl Inc., Suwanee, GA).
- For immunophenotyping, naïve tumors were harvested, dissociated (Miltenyi, Germany), and labeled with directly conjugated fluorescent antibodies. Data was acquired on an Attune NxT Flow Cytometer (Thermo Fisher Scientific) and analyzed with FlowJo software (Tree Star, Inc., Ashland, Oregon).
- For immunohistochemistry, tumors were harvested, fixed in 10% NBF and embedded in paraffin for sectioning. Tissue sections were then processed and labeled using direct (CD4 or CD8) or indirect (CD45) methods with chromogen substrate on the Bond RXm (Leica Biosystems). Images were obtained on the Aperio VERSA (Leica Biosystems).

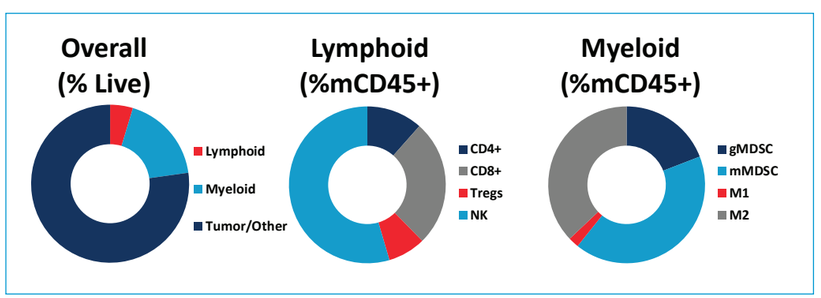

Figure 1.Tumor infiltrate in CT26 tumors (% live cells).

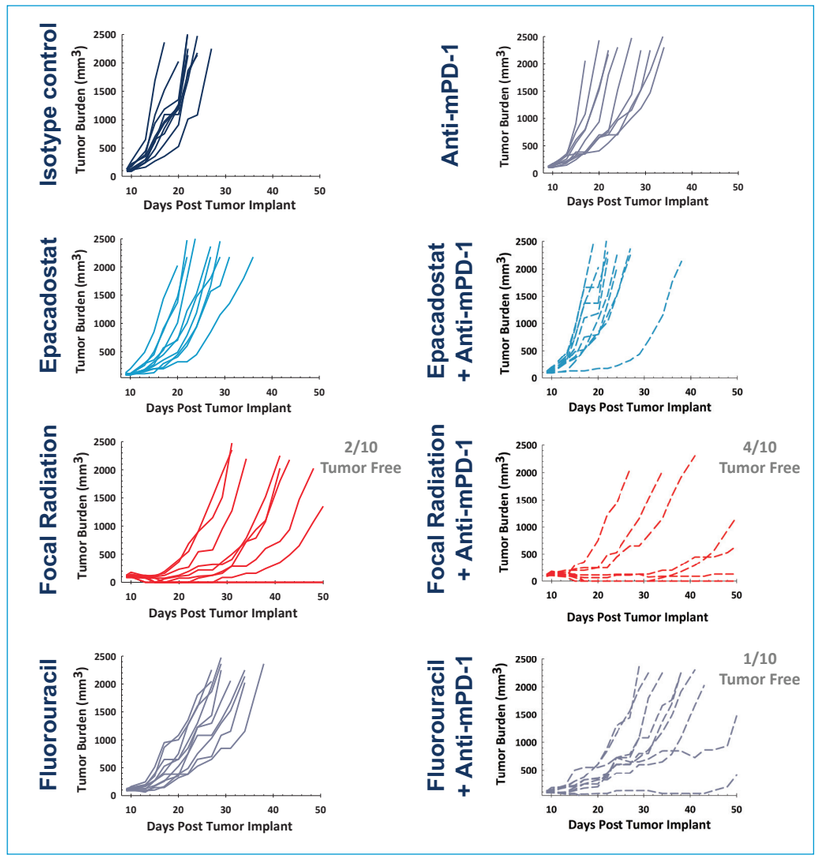

Figure 2. Efficacy evaluation of anti-mPD-1 combinations against CT26.

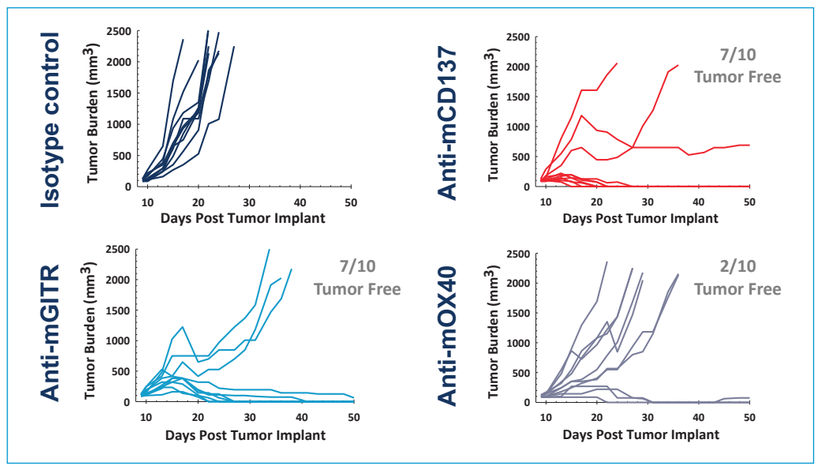

Figure 3. Efficacy evaluation of costimulatory agents against CT26.

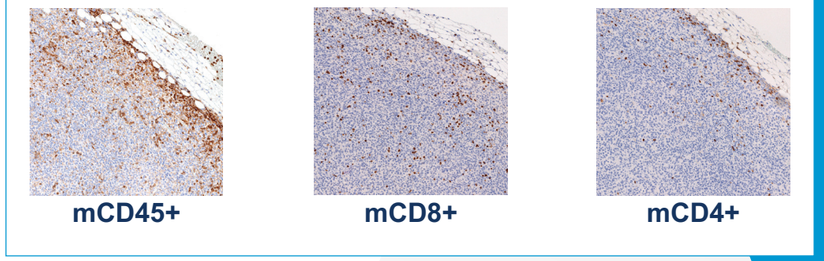

Figure 4. Spatial localization of immune cells in CT26 iumors.

Results and Conclusions

- Focal radiation and fluorouracil monotherapies demonstrate efficacy in the CT26 model and is further enhanced with the addition of anti-PD-1, likely through reported alterations in the tumor microenvironment.
- Epacadostat as monotherapy or in combination with anti-mPD-1 did not impact overall CT26 tumor progression.
- Anti-mGITR, anti-mCD137 and anti-mOX40 monotherapies have strong activity against CT26.
- CT26 tumors are well infiltrated, with CD8+ T cells, NK cells, mMDSCs, and M2 macrophages being most prevalent. The immune cell infiltrate observed by flow cytometry is reflected in the spatial localization which also indicates that a subset of immune cells are excluded from naive tumors.

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