#3719. Systemic and Subcutaneous Modeling of A20 Murine B Cell Lymphoma in Mice: A Comparative Assessment

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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.
- Orthotopic modeling is becoming increasingly employed in preclinical development as it is posited to have improved clinical translatability compared to subcutaneous implantation.
- We developed a luciferase enabled A20 murine B cell lymphoma cell line (A20-luc) to better understand the utility of subcutaneous and orthotopic modeling of A20 in preclinical oncology drug development.
- A panel of checkpoint inhibitors and costimulatory agonists were assessed for anti-tumor response both in the subcutaneous and systemic disease settings.
- Investigation of infiltration of lymphoid and myeloid cell subsets in subcutaneous A20 tumors were evaluated. Knowledge of this immune composition can help guide rational monotherapy and combination strategies.

Materials and Methods

- To establish subcutaneous disease, A20 cells were implanted into the right axilla of female Balb/c mice. Dosing was initiated when tumors were established, and tumor progression was monitored by caliper measurements. Antibodies were dosed for two weeks (Bio X Cell, West Lebanon, NH).
- To establish systemic disease, A20 cells were stably transfected to express luciferase (A20-luc) and were implanted via injection of the lateral tail vein of female Balb/c mice. Dosing was initiated when systemic disease was established, and tumor progression was monitored by *in vivo* bioluminescence imaging (BLI). BLI was performed with an IVIS Spectrum (PerkinElmer, Waltham, MA) to quantify whole-body disease burden. Total burden was calculated using proprietary BLIZZARD™ software from a fixed volume ROI covering the whole mouse. Antibodies were dosed for two weeks (Bio X Cell, West Lebanon, NH).
- For immunophenotyping, naïve subcutaneous tumors were harvested, dissociated (Miltenyi, Germany) and labeled for flow cytometry using the MI-CompT™ and MI-CompMyeloid™ antibody panels. Data was acquired on an Attune™ NxT flow cytometer (Thermo Fisher Scientific) and analyzed with FlowJo software (FlowJo, LLC, Ashland, OR). In this analysis, CD45+ A20 tumor cells have been excluded.
- Animal care and use was conducted in alignment with animal welfare regulatory requirements in an AAALAC-accredited facility.

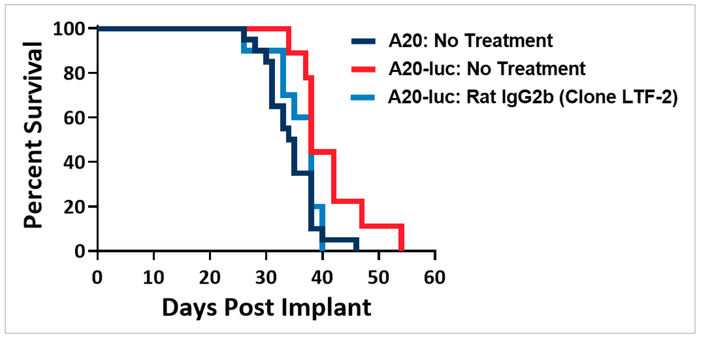

Figure 1. Similar systemic progression of A20 parental and A20-luc models. Survival following intravenous implant of A20 or A20-luc cells. A20 and A20-luc data represent independent studies combined for comparison purposes.

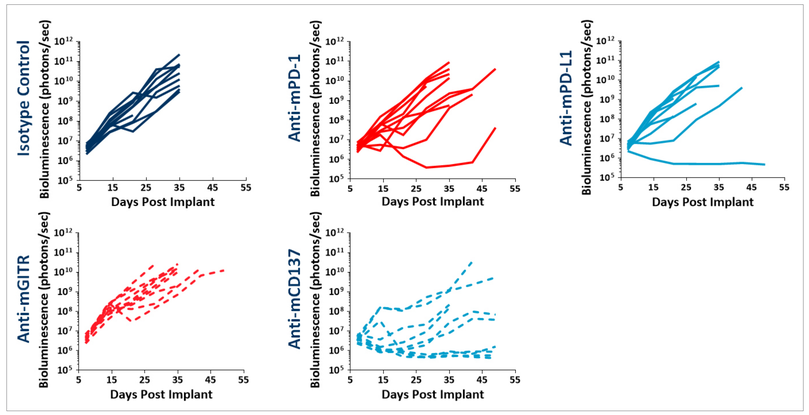

Figure 2. Systemic A20-luc is moderately responsive to checkpoint blockade and costimulatory agonists. Individual tumor progression of A20-luc tumor-bearing mice following intravenous implant and treated with immunotherapy. Treatments were administered on Days 7, 10, 14 and 17 post-implantation.

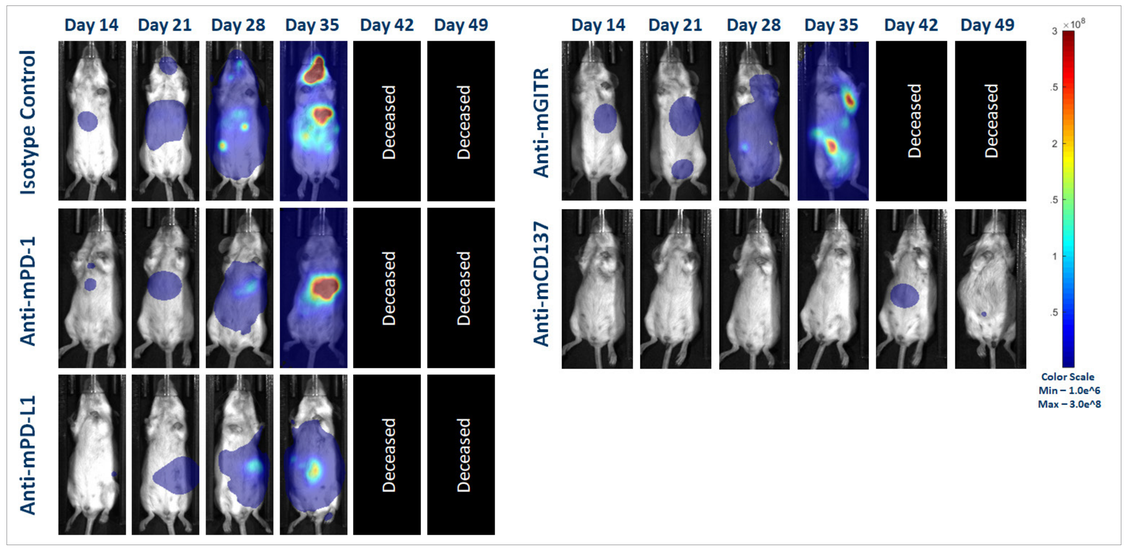

Figure 3. Bioluminescence imaging of systemic A20-luc consistent with liver metastasis. Representative bioluminescence images of A20-luc tumor-bearing mice treated with immunotherapy. Treatments were administered on Days 7, 10, 14 and 17. By necropsy, areas of high signal in abdomen is suggestive of metastatic foci in liver and potentially lymph node.

Results and Conclusions

- Survival kinetics of systemic A20 and A20-luc murine B cell lymphoma are similar, with A20-luc having a slightly slower disease progression. The A20-luc model offers an avenue to explore murine B cell lymphoma as a systemic disease, including the treatment of liver metastasis.
- Based on bioluminescence imaging, A20-luc signal in late-stage disease is focused in the area of the liver. This observation is supported by > 80% presentation of liver nodules upon necropsy (data not shown).
- With only moderate activity in response to immunotherapy, the high infiltrate of T cells seen by flow cytometry are hypothesized to either lack signals required for activation or are reactive to non-tumor-associated antigens.
- There is little to no difference in immunotherapy responses to established systemic or subcutaneous A20 lymphoma, so model translatability is equivalent for the immunotherapies tested. Labcorp is actively engaged in orthotopic modeling of other histotypes to confirm trends in anti-tumor response of cognate orthotopic and subcutaneous models.

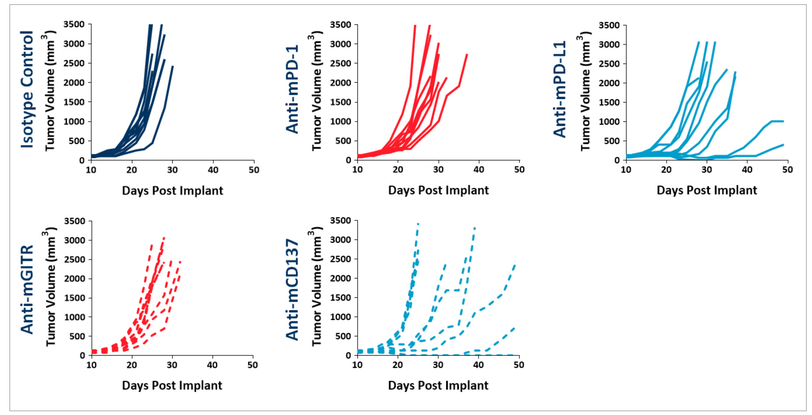

Figure 4. Established subcutaneous A20 tumors are moderately responsive to immunotherapy. Individual tumor progression of A20 tumor-bearing mice following subcutaneous implant and treated with immunotherapy. Animals were randomized on Day 10 post-implantation and treatments were administered on Days 11, 14, 18 and 21.

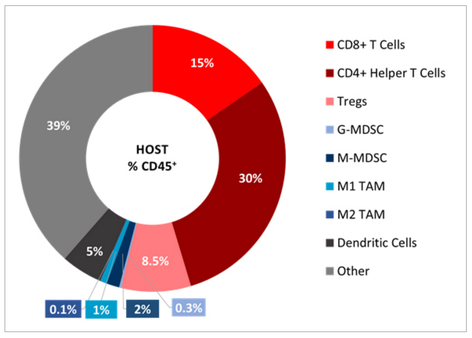

Figure 5. A20 subcutaneous tumors have a high T cell infiltrate. Subcutaneous tumors were collected on Day 19 post-implant following a single dose of isotype control antibody on Day 10. Mean tumor volume at sampling was 293 mm^3 (n=5). A20 tumor cells are CD45+, but are excluded from analysis. Subsets considered "other" include undefined macrophage cells, host derived B cells, NK and NKT cells. Further studies are needed to fully define this compartment.

Presented at the AACR Annual Meeting 2019

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