Comparative Analysis: Responses to Immunotherapy, Standard-of-Care Treatment and Combined Therapy in MC38 Murine Colon Cancer

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Abstract

MC38 cells represent a widely recognized murine colon adenocarcinoma cell line extensively employed in preclinical investigations, notably within the area of immuno-oncology. Using a luciferase-tagged MC38 cell line that was developed at Aragen Bioscience, we successfully replicated the effects of anti-PD-1, anti-CTLA-4, and their combination in the subcutaneous MC38 tumor model. In addition, the standard-of-care chemotherapeutics irinotecan, oxaliplatin and fluorouracil inhibited tumor growth. Importantly, a trend of synergistic effect was observed upon combined treatment of irinotecan with either anti-PD-1 or anti-CTLA-4. Notably, irinotecan plus anti-PD-1 increased activated tumor-infiltrating CD4+ T and CD8+ T cells. For the first time, we demonstrated that anti-CTLA-4 alone and in combination with irinotecan prevented subcutaneous MC38 but not B16F10 tumor growth in re-challenged responders, suggesting a specific immunity toward MC38 tumor cells. We also showed that bioluminescent signals (BLI) highly correlated with caliper measurements, indicating this tagged cell line is a reliable option for imaging-based models.

Methodology

Subcutaneous MC38 tumor model:

Day 0 Implant tumors (n = 100) → *Day 7-28 • ICI IP Q3D x 3 • SOC IP Q7D x 3* → *Day 21 In-life blood collection* → *Day 28 TIL, histology of non-responders* → *Day 83 Rechallenge tumor-free survivors*

- MC38 (Kerafast) cells were tagged with luciferase in-house and implanted subcutan-eously in the lower right flank of C57BL/6 mice.
- Treatment commenced on day 7 post implantation (pi), and tumor progression was assessed by caliper and IVIS imaging.
- Anti-PD-1 and anti-CTLA-4 were administered every 3 days for 3 cycles, while irinotecan, oxaliplatin and fluorouracil (5-FU)

  were administered once weekly for 3 weeks.
- Day 23 pi: White blood cell count was analyzed from in-life bleed.
- Day 27 pi: Immunophenotyping and histological analysis of tumors were conducted from non-responders.
- Day 83 pi: Responders re-challenged with untagged MC38 cells in the lower left flank, while B16F10 cells (ATCC) were implanted into the lower right flank to assess tumor-specific immune respons.

Tumor-infiltrating lymphocytes:

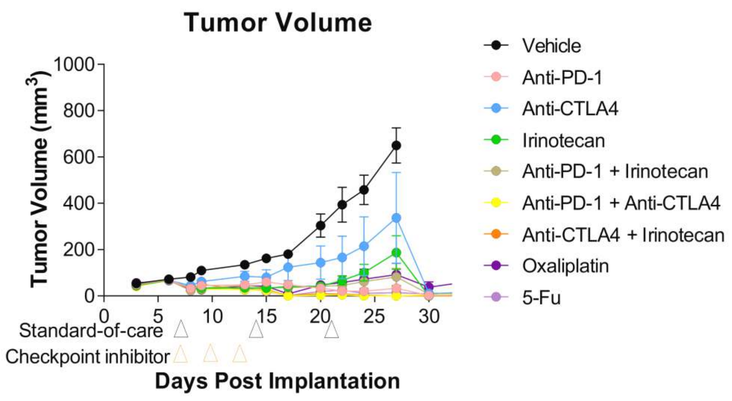
Tumors of non-responders were dissociated by enzyme digestion and analyzed by flow cytometry. Cell counts were normalized to tumor volume.

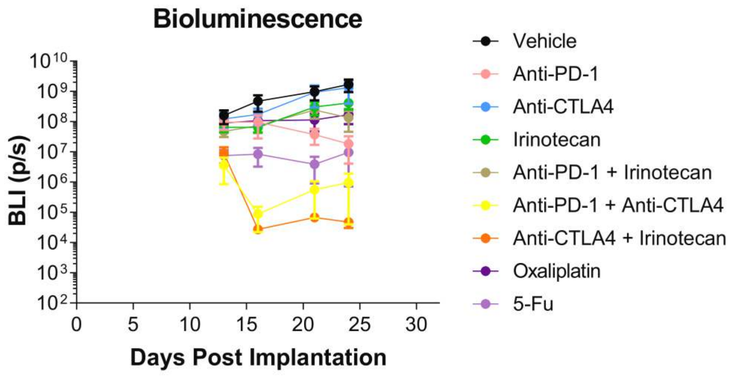
White blood cell counts:

Cells were quantified using the Procyte Dx hematology analyzer.

Results

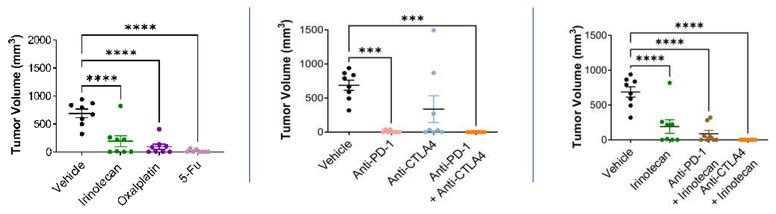
Comparative effects of immune checkpoint inhibitors and standard-of-care drugs on MC38 tumor growth



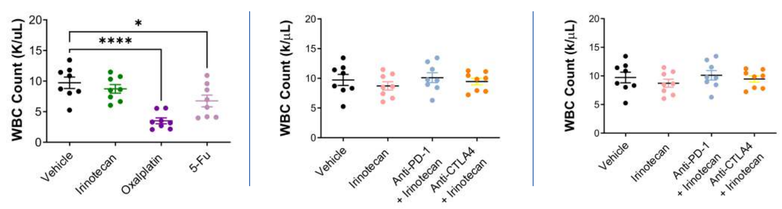


Results

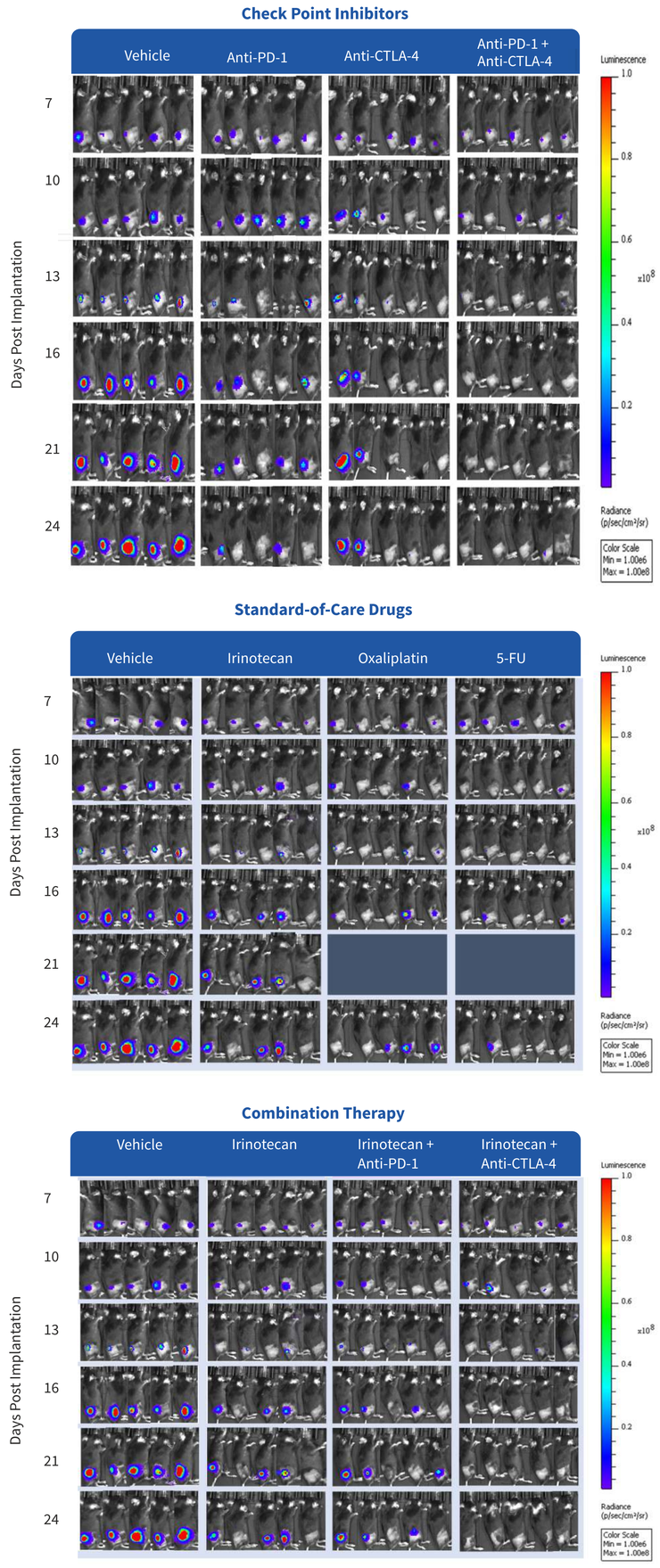
All treatment except anti-CTLA-4 significantly decreased tumor volume (day 27)

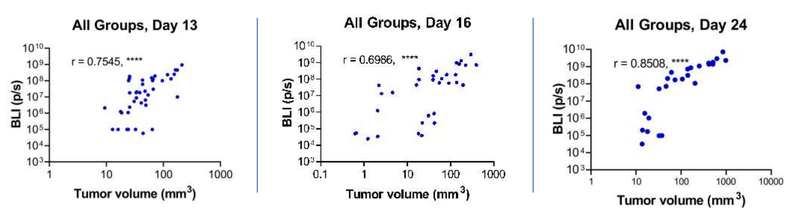


Oxaliplatin decreased white blood cell count (day 23)



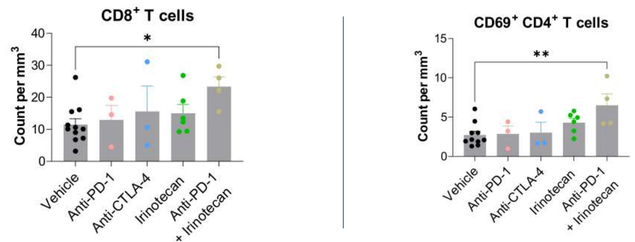
Bioluminescence imaging signals correlate with tumor volume measured by caliper



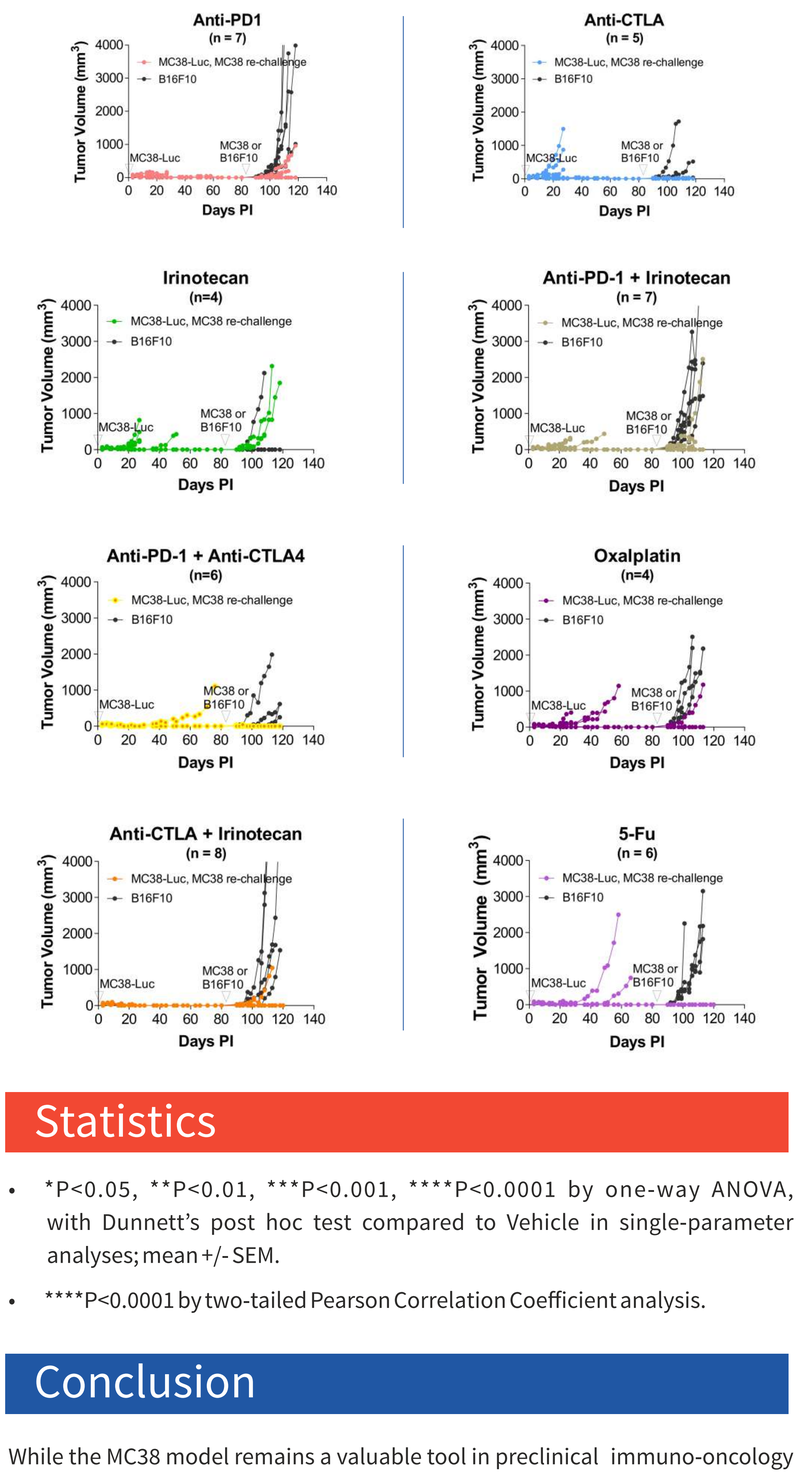


Results

Combination therapy of anti-PD-1 + irinotecan increased tumor-infiltrating activated CD4+ T and CD8+ T cells



Anti-CTLA-4 alone and in combination with irinotecan prevented tumor growth in re-challenged responders



Statistics

- *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 by one-way ANOVA, with Dunnett's post hoc test compared to Vehicle in single-parameter analyses; mean +/- SEM.
- ****P<0.0001 by two-tailed Pearson Correlation Coefficient analysis.

Conclusion

While the MC38 model remains a valuable tool in preclinical immuno-oncology research, a luciferase-tagged derivate cell line would enable the expanded use of the cancer cells in orthotopic or imaging-based studies. We developed such an MC38-Luc cell line and demonstrated its responsive to checkpoint inhibitors, small molecules and combination therapy, with BLI highly correlating with tumor volume measurement by caliper. Our study demonstrated that subcutaneous MC38-Luc tumor model is a robust model for testing multiple drug modalities including immunotherapy and chemotherapy.

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