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Evaluating drug delivery methods and αPD-1 combination therapies in a murine colorectal cancer model

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Introduction

- Colorectal cancer (CRC) is the third most diagnosed cancer and the third leading cause of cancer-related deaths in men and women in the US[1].
- CRC can be treated with immunotherapy, specifically with antibodies that target immune checkpoint pathways, which regulate T cell activation[2-3] [Figure 1].

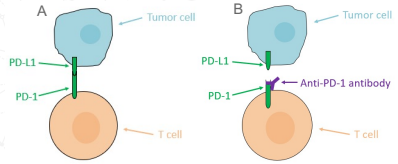

Figure 1. T cell regulation via the immune checkpoint pathway programmed death-1 (PD-1). 1A shows the activation of the pathway versus the inhibition by αPD-1 antibody shown in 1B.

- Proteins PD-L1 and PD-1 engage with each other and activate the checkpoint pathway. Pathway activation downregulates overall T cell activity[3].
- When an immune checkpoint inhibitor (ICI) such as αPD-1 is introduced, the antibody binds to PD-1 to block activation. The resulting inhibition allows continued T cell activity[3].
- CRC tumors tend to be "cold" but can be turned "hot" with various therapeutic strategies such as chemokine modulation (CKM)[4] [Figure 2].

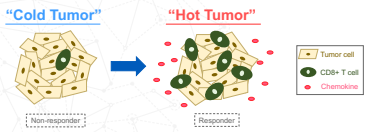

Figure 2. A method for turning "cold" tumors "hot".

- Cold tumors consist of few immune cells, making it less likely to respond to an ICI[4].
- Hot tumors consist of many immune cells, making it more likely to respond to an ICI[4].
- CKM can turn cold tumors into hot tumors by regulating intratumoral chemokine expression to favor recruitment of immune cells[5].

- COX-2 is an overexpressed enzyme in the tumor microenvironment[6].
- Higher levels of COX-2 expression are correlated with shorter survival times and larger tumors in cancer patients[6].
- Celecoxib, a COX-2 inhibitor, can block COX-2[7].
- Increasing celecoxib doses in CRC mouse models can slow tumor growth and extend life span[8].
- A limitation to studying celecoxib in CRC mouse models is that it is injected twice daily, leading to:
  - Pain and discomfort[7]
  - Additional stress[7]
- Pain and stress can have physiological or biological impacts, resulting in experimental bias and difficulty interpreting data in animal models.

Goals

- To evaluate an alternative delivery method of celecoxib to avoid repeated injections.
- This experiment compares two methods of delivering celecoxib:
  1. Intraperitoneal (IP) injections
  2. Oral delivery via chow

Hypothesis

- Delivery of celecoxib via oral administration will have comparable effectiveness as IP injections.

Methods

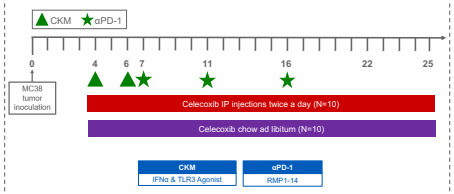

Figure 3. Experimental design. On day 0, twenty C57BL/6 mice were subcutaneously injected with 5 X 10^5 MC38 cells to induce CRC tumors. All 20 mice received CKM on days 4 and 6 via IP injection and all 20 mice received αPD-1 on days 7, 11, and 16 via IP injection. Celecoxib was delivered by IP injection or ad libitum in the chow beginning on day 4 and continued until day 25. Readouts included survival, tumor incidence, tumor growth, and change in weight.

Results

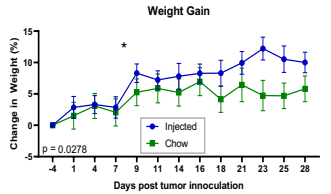

Figure 4. While mice receiving celecoxib via IP injection and chow both gained weight following tumor inoculation, celecoxib-fed mice gained more weight. N = 10/group; p values determined 2-way ANOVA.

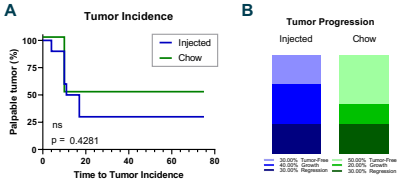

Figure 5. Tumor incidence and regression are similar regardless of delivery method. A. 30% of the injected group and 50% of the chow group never exhibited a palpable tumor. N = 10/group; p values determined by log rank test. **B.** Among mice with palpable tumors, 3/7 (43%) and 3/5 (60%) experienced tumor regressions with injected and chow celecoxib, respectively. N=10/group

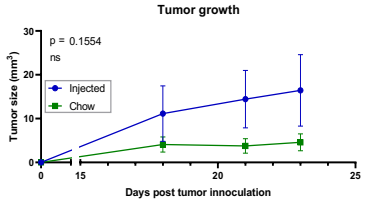

Figure 6. Mice administered celecoxib via IP injection demonstrated a trend towards faster tumor growth compared to those with ad libitum celecoxib chow. When comparing early-stage tumors (within 3 weeks of inoculation), the injected group had slightly larger tumors than the chow group. N = 10/group; p values determined by 2-way ANOVA.

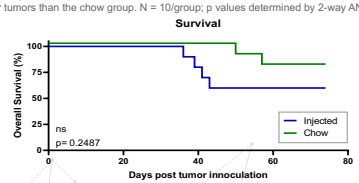

Figure 7. Overall survival is similar regardless of delivery method. Two of the mice in the chow group have died as compared to 4 mice in the injected group. N= 10/group; p values determined by log rank test.

Conclusions

- Celecoxib chow has a significant impact on body weight as compared to injected celecoxib.
  - Using celecoxib in chow may add experimental variability based on weight gain.
- Celecoxib delivery methods do not have a significant impact on tumor incidence, progression, growth rate or survival.
- Modifying experimental protocols to include celecoxib delivery via chow would be feasible for future experiments.
  - This may alleviate some variability caused by excessive handling of experimental mice.

Future Questions

- Does celecoxib improve the efficacy of CKM + αPD-1?
  - Explore additional treatment groups: control, CKM, αPD-1, celecoxib, CKM + apd-1, CKM + celecoxib, αPD-1 + celecoxib, CKM + αPD-1 + celecoxib.
- How is the immune response affected?
- Is this experiment strain and/or site specific?
  - Investigate a BALB/c model and additional cell lines modeling different cancer types.

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