Effectiveness of the dTAG System on Degradation of SOX9

HARVARD COLLEGE
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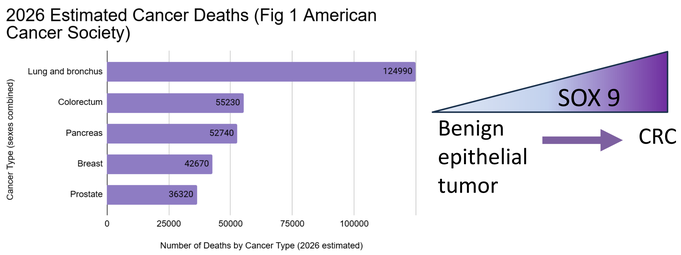
Introduction

Colorectal cancer (CRC) is the second leading cause of cancer death within the United States as of 2026. The SRY-box transcription factor 9 (SOX9) is a central regulator that promotes CRC by blocking intestinal stem cell differentiation (Liang et al., 2022). We used the degradation tag (dTAG) system for SOX9-specific protein degradation, where in the presence of the dTAG V1 small molecule degrader, the SOX9 protein is fused with an FKBP12 mutant and brought into close molecular proximity to the protein degradation machinery (Nabet et al., 2018)



In this project, we aimed to observe if the dTAG system could accomplish complete Sox9 knock out, given inconclusive results from another mouse model AOM/DSS Cdx2 Sox9 mouse model where knock-out was induced via tamoxifen injections.

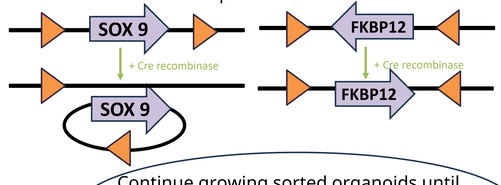
Methods

Bred mice for dTAG and $Sox9^{flox}$ genotypes

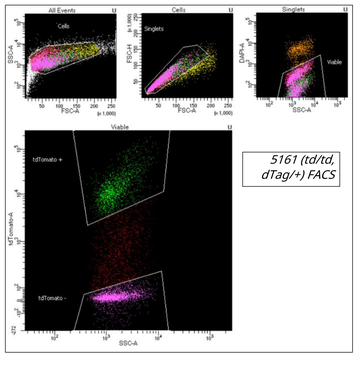
Isolated tissue and grew mouse colon cells into organoids

5161 (td/td, dTag/+) td positive 24 hours on DMSO vehicle control treatment

Transduced organoids with Adeno-Cre vector to excise Sox9 and flip inverted FKBP12 sequence



Performed FACS of transduced colon organoids using td+/- marker



5161 (td/td, dTag/+) FACS

Continue growing sorted organoids until treatment with dTAG V1 drug and DMSO vehicle control, imaging and sampling at 24 and 48 hours

Completed rPCR to ensure complete recombination (inverting of dTAG sequence and removal of floxed Sox9)

Performed western blots to measure level of protein expression

Acknowledgements

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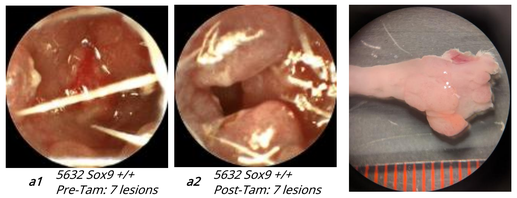
Results

First Model Azoxymethane/Dextran Sulfate Sodium (AOM/DSS) treatment with Tamoxifen injections in Cdx2 Sox9 mice

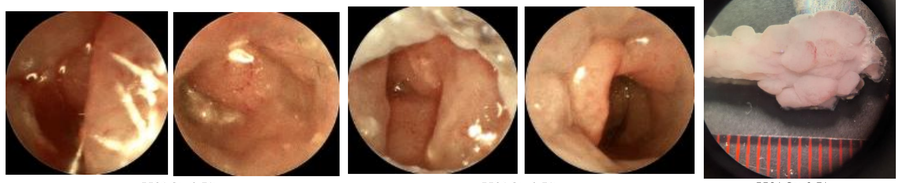


a1 5632 Sox9 +/+ Pre-Tam: 7 lesions

a2 5632 Sox9 +/+ Post-Tam: 7 lesions

a3 5632 Sox9 +/+ Sigmoid colon end

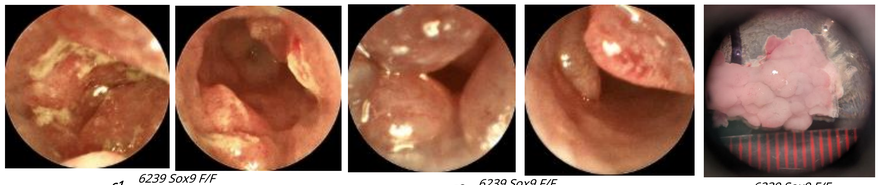
1. AOM injected to induce cancer process in colon
2. DSS given to mice in water to cause colitis, growing tumors
3. Tamoxifen injected based on mice's mass: drug used to delete SOX9 in $Sox9^{flox}$ mice.



b1 5581 Sox9 F/+ Pre-Tam: 11 lesions

b2 5581 Sox9 F/+ Post-Tam: 14 lesions

b3 5581 Sox9 F/+ Sigmoid colon end



c1 6239 Sox9 F/F Pre-Tam: 13 lesions

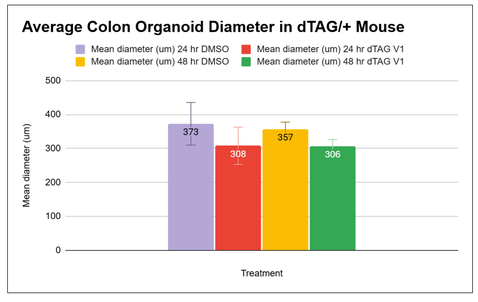
c2 6239 Sox9 F/F Post-Tam: 16 lesions

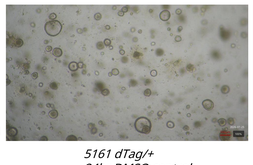
c3 6239 Sox9 F/F Sigmoid colon end

a. Baseline colonoscopy of tumor growth after AOM/DSS (figures a1, b1, c1) compared to post-Tamoxifen colonoscopies (figures a2, b2, c2) and dissections (figures a3, b3, c3)
b. Since induced recombination did not demonstrate significant effects or cause a decrease on established tumors after 8 doses of Tamoxifen, we consider the second model.

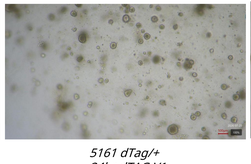
Second Model Degradation tag (dTAG) system

a. Comparison of organoid size after 24 hours of treatment with dTAG V1 demonstrates association that knocking out Sox9 leads to a decrease in stemness evidenced by, on average, smaller and darker organoids.

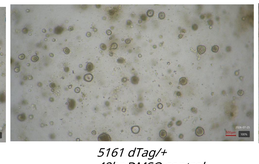




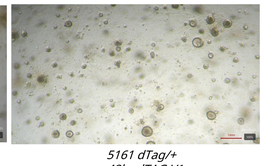
5161 dTag/+ 24hr DMSO control



5161 dTag/+ 24hr dTAG V1



5161 dTag/+ 48hr DMSO control



5161 dTag/+ 48hr dTAG V1

References

American Cancer Society. (2025). American Cancer Society | Cancer Facts & Statistics. In *American Cancer Society | Cancer Facts & Statistics*. https://cancerstatisticscenter.cancer.org/

Li, C., Lau, H. C., Zhang, X., & Yu, J. (2022). Mouse Models for Application in Colorectal Cancer: Understanding the Pathogenesis and Relevance to the Human Condition. *Biomedicines*, *10*(7), 1710. https://doi.org/10.3390/biomedicines10071710

Liang, X., Duronio, G. N., Yang, Y., Bala, P., Hebbar, P., Spisak, S., Sahgal, P., Singh, H., Zhang, Y., Xie, Y., Cejas, P., Long, H. W., Bass, A. J., & Sethi, N. S. (2022). An Enhancer-Driven Stem Cell-Like Program Mediated by SOX9 Blocks Intestinal Differentiation in Colorectal Cancer. *Gastroenterology*, *162*(1), 209–222. https://doi.org/10.1053/j.gastro.2021.09.044

Nabet, B., Roberts, J.M., Buckley, D.L. *et al.* The dTAG system for immediate and target-specific protein degradation. *Nat Chem Biol* **14**, 431–441 (2018). https://doi.org/10.1038/s41589-018-0021-8

SciDraw. (2025). SciDraw | Scientific Drawings. In *Scidraw.io*. https://scidraw.io/category/mouse

Conclusions

- AOM/DSS and tamoxifen model is thus far unideal for observing Sox9 knock-out effects.
- Continued observation is needed in organoids until we can achieve rapid, complete, and reversible Sox9 degradation.
- Testing whether Sox9 degradation can induce regression of established CRC in vivo will serve as a proof of concept that supports the development of Sox9 degrader as a novel therapeutic in humans.

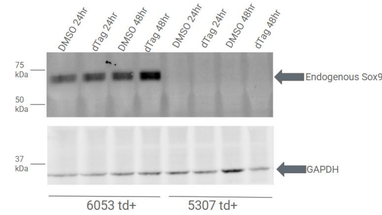
Next Steps

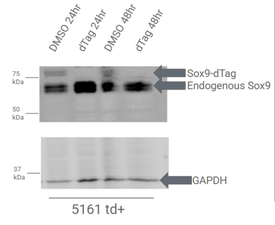
Having seen the effects of dTAG V1 treatment within mouse colon organoids, we will conduct more experiments to ensure its efficacy *in vitro* before the natural next step of *in vivo* work in determining if the degradation model is still viable then for Sox9 elimination.

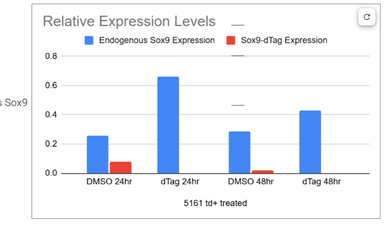
Our findings will open up more pathways to more efficiently, effectively, and reversibly study the effect of the protein's loss for CRC therapeutic targeting.

Second Model Cont.

b. Western blot







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