Tumor Microbiome in Murine 4T1 Triple Negative Breast Cancer

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Abstract

The presence of certain microorganisms has been known to have a strong association with the prognosis and development of human cancers. Recent findings show that the gut microbiome can have a significant role in activating antitumor innate immune responses. In addition, the microbiome in the tumor and its compartment has been found to impact progression and immunotherapy outcomes in patients.

Herein, we utilized 16S deep sequencing platform to profiled 4T1 tumor microbiome of mice treated with immune checkpoint therapy (anti-PD-1 + anti-CTLA4) and found that: 1) 4T1 tumors are mostly colonized by aerobic bacteria, 2) tumor progression may be affected by gut microbiota composition, and 3) immune checkpoint therapy may affects microbiome profile and the biodiversity. Furthermore, preliminary qPCR evidence indicates that 4T1 tumors may feature a low microbial load, compared with pancreatic, liver, and lung tumors.

Future applications of probiotics as an anti-tumor drug vectors remain open for future exploration.

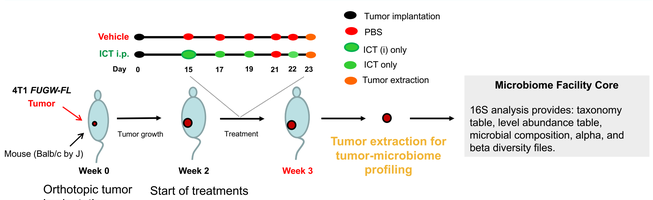
Key Questions

1. Are 4T1 tumors **colonized by bacteria?**
2. Does the microbiome affect **tumor progression?**
3. Does **ICT treatment** affect the tumor microbiome?

This is important because it can…

- Help **understand factors** that determine ***failure*** or ***success*** of treatment.
- Open a **new drug delivery** route using **oral intake of probiotics**.

Methods



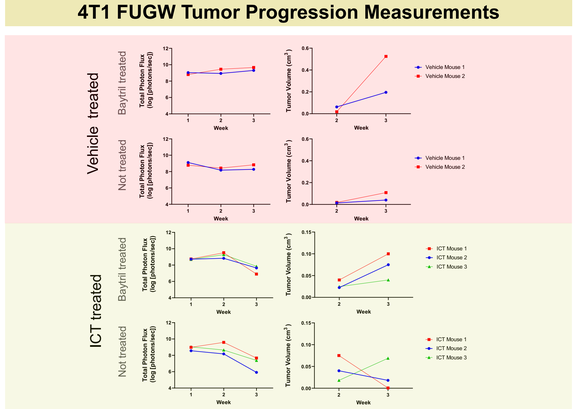
Microbiome Facility Core

16S analysis provides: taxonomy table, level abundance table, microbial composition, alpha, and beta diversity files.

Baytril Treated Arm	Not treated Arm
Vehicle n =2	Vehicle n =2
ICT (α-PD-1 + α-CTLA-4) n = 3	ICT (α-PD-1 + α-CTLA-4) n = 3

Results/Observations

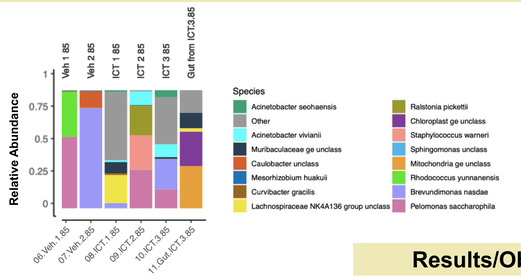
4T1 FUGW Tumor Progression Measurements



Question 1

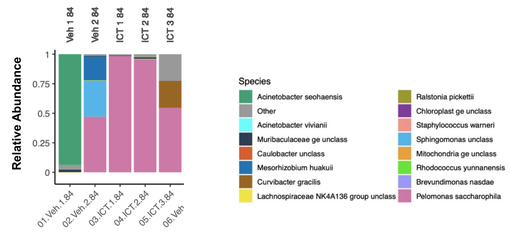
Are 4T1 Tumors colonized by bacteria?

4T1 FUGW Tumor Microbiome Composition in Untreated Mice



Does gut microbiome affect tumor microbiome?

4T1 FUGW Tumor Microbiome Composition in Baytril Mice



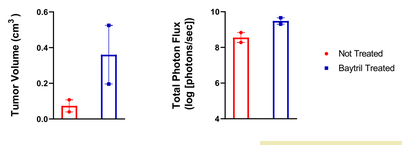
Results/Observations

1. Bacteria colonizes 4T1
2. Tumor microbiome affected by oral antibiotic treatment

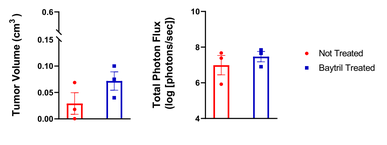
Question 2

Does the 4T1 tumor microbiome affect tumor progression?

Vehicle Control



ICT Treated



Results/Observations

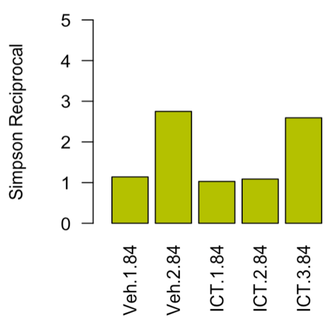
1. Untreated mice have lower tumor volume and BLI than Baytril group, indicating relationship between microbiome and tumor progression

Question 3

Does Immune Checkpoint Therapy (ICT) affect the tumor microbiome?

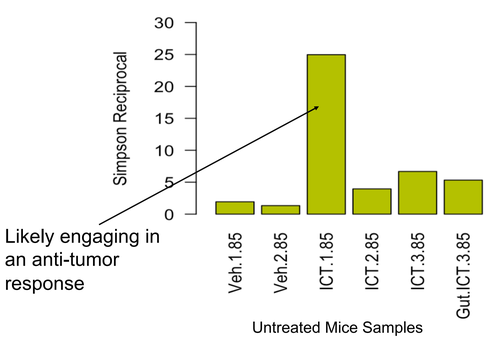
Simpson Reciprocal Values in 4T1 FUGW Baytril Antibiotic Mice Tumors

The Simpson's reciprocal index quantifies biodiversity by taking into account species richness and evenness. High species diversity suggests a greater number of successful species and a more stable ecosystem

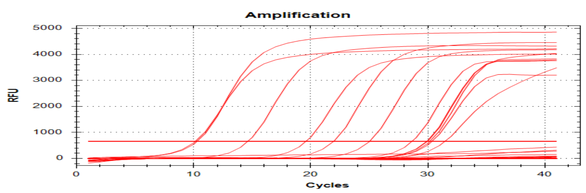


Simpson Reciprocal Values in 4T1 FUGW Untreated Mice Tumors

Likely engaging in an anti-tumor response



qPCR Standard Curve



Bacterial load is below detectable levels for qPCR, indicating that 4T1 tumors have lower bacterial load than pancreatic, liver, and lung tumors.

Results/Observations

1. ICT Treatment may result in an increase in biodiversity of species

Preliminary Conclusions

Initial Questions	This Project's Findings
•Are 4T1 tumors colonized by gut bacteria?	•Yes
•How does tumor microbiome affect tumor progression?	•Increased tumor volume in vehicle control mice treated orally with antibiotic points at an antitumor role for the tumor microbiota
•Does ICT treatment affect the tumor microbiome?	•May increase diversity of tumor microbiota •Unknown if it increases/decreases number of bacteria •Changes in tumor microbiome affecting treatment is to be determined

Future Work

1. Identify bacterial candidates
   - Previously known to colonized human tumors
   - Biosafety Level 1
   - Commercially available
   - Culturable
   - Genome sequenced
2. Culture bacteria – for lab repository and experimental purposes
3. Experimental Opportunities
   - *In vivo* real time imaging of gut bacteria colonization of tumors – engineer Lux operon into bacteria
   - Bacteria as drug-delivery vector

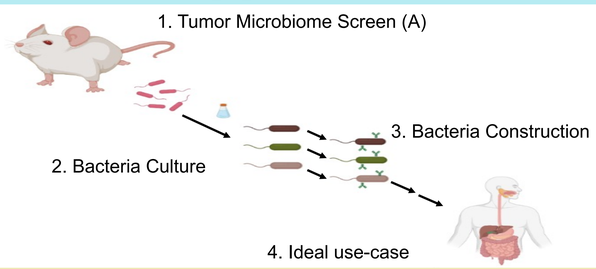
Candidate Microorganisms

Pelomonas saccharophilia
- ~ 60% relative abundance in vehicle control mice 1, genome sequenced
- Aerobic, gram negative, Biosafety level 1, commercially available

Brevundimonas nasdae
- ~ 80% relative abundance in vehicle control mice 2, genome sequenced
- Aerobic, gram negative, Biosafety level 1, commercially available

Lachnospiraceae NK4A136
- ~ 20% relative abundance in ICT mouse 1, genome sequenced
- Immunogenic, indirectly associated with immune therapy response1.
- Anaerobic, gram negative, Biosafety level 1, commercially available

Application



Acknowledgements

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