Identification of Specific Bacteria Involved in Lung Tumor Progression & Immune Cell Infiltration

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Background

- The **lung** is exposed to and is colonized by a **diverse bacterial community** in many pathological conditions[1]
- **Commensal microbiota** are important **biomarkers** and **regulators of oncogenesis** and **therapeutic responses**[2]
- A potential mechanism through which microbiota may influence tumor growth is via **modulating the tumor-associated immune response**
- We observed that **depletion of local microbiota** in addition to anti-PD1 treatment **reduced lung tumor burden** (Figure 1)
- We also identified that the **Type I Interferon response in cDC1** (a subset of dendritic cells called convention type 1 dendritic cells) was **involved in microbiota mediated cDC1 inhibition**, which impaired anti-tumor T cell immunity (Figure 1)

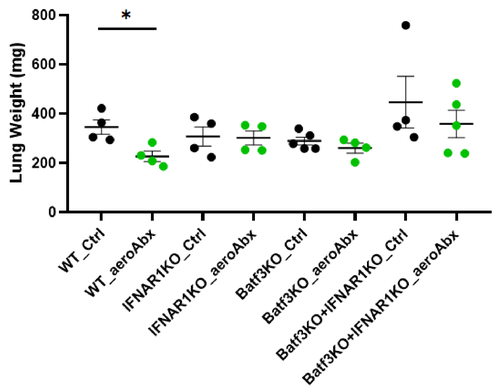
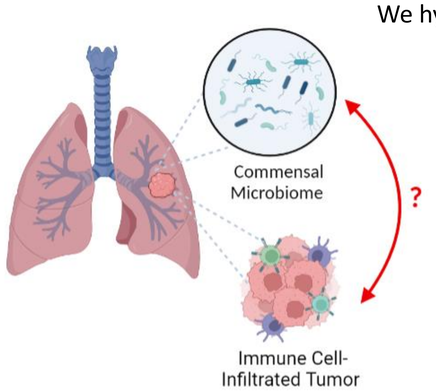


Figure 1. Whole Lung Weight of Mice With Knockout of IFN-I, cDC1s, or Both Treated With Anti-PD1 and Either Aerosolized Antibiotics or Water

Hypotheses



We hypothesize

1) presence of **specific bacteria** that would be **enriched within cancerous human lung tissue** samples compared to healthy ones

and

2) these cancer-associated bacteria would mechanistically **affect the production and proliferation of specific immune cell types**

Methods

Screening & Specimen Collection

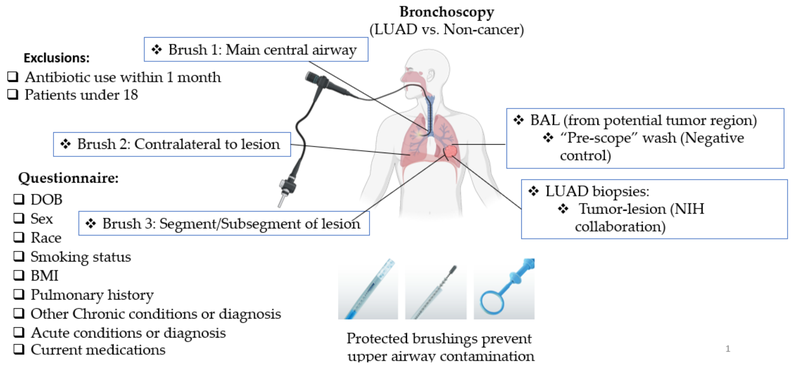


Figure 2. Collection Process of Human Lung Samples From Individuals Identified with Lung Cancer. BAL and three different types of brushing from the lung are taken and stored in a 25% glycerol solution for preservation.

Extraction & Sequencing of 16S (Bacterial) DNA

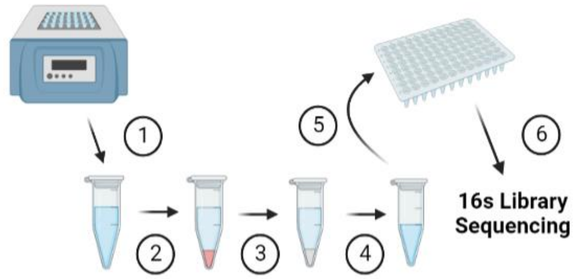


Figure 3. Process for Extracting & Sequencing 16s DNA.
(1) Heat inactivation of potential disease-causing bacteria
(2) Spin down to create cell pellet
(3) Purification of sample with phenol chloroform & other buffers
(4) Extraction of DNA
(5) Aliquot into plate for sequencing
(6) Send for 16s Library sequencing

Sequencing Data Analysis

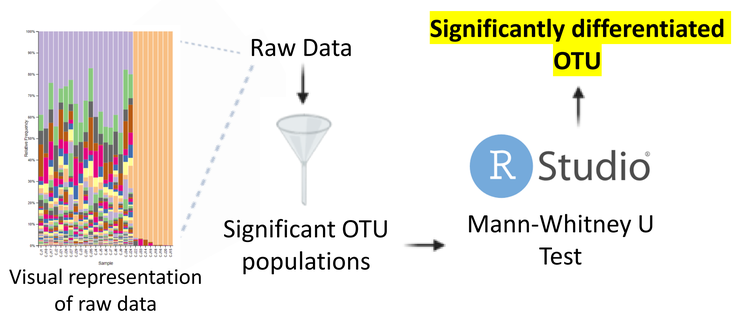
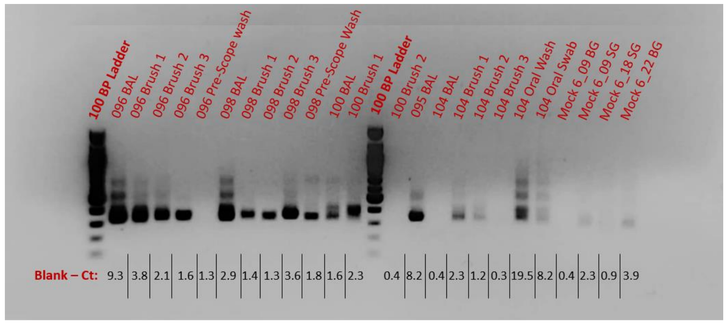


Figure 4. Flow Diagram To Determine Significantly Differentiated OTU From Sequencing Data Results.

Results



Pt 095 = Squamous Cell
Pt 096 = Adenocarcinoma
Pt 098 = Lung Abscess
Pt 100 = SCLC
Pt 104 = Adenocarcinoma

Figure 5. Gel Imaging of Extracted Bacterial DNA of Various Human Sample Lung Specimens with Controls. Lung human samples are low-biomass, so it is important to verify that the DNA extracted from these samples are bacterial DNA, which are around 350 BP.

- Currently have collected samples from **18 patients** confirmed to have lung cancer
- **Sent extracted bacterial DNA** from bacteria grown in an enriched culture for **library genome sequencing**

Future Directions

- **Analyze sequencing results** and **identify specific bacteria** that are **enriched in lung cancer patients** compared to healthy individuals
- **Confirm** similar findings in the rest of the collected samples
- **Inoculate mouse model** with this specific bacteria strain to **identify changes in the immune cell landscape** within the tumor microenvironment

References

1) Helmink, B.A., et al., *The microbiome, cancer, and cancer therapy.* Nat Med, 2019. **25**(3): p. 377-388. Dickson, R.P., et al., The Microbiome and the Respiratory Tract. Annu Rev Physiol, 2016. 78: p. 481-504.
2) Lloyd, C.M. and B.J. Marsland, Lung Homeostasis: Influence of Age, Microbes, and the Immune System. Immunity, 2017. 46(4): p. 549-561.

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