# Machine Learning Modeling of Gut Microbiome-Metabolome Profile Links Alcohol & HIV-Associated Dysbiosis to Immune Senescence

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## Introduction

- People with HIV experience precious aging and elevated prevalence of aging-related diseases. Precious aging in people with HIV is thought to be mediated by chronic inflammation and immune system remodeling.
- In this study, we investigate the relationship between gut microbiome and CD8 T cell senescence in participants in the New Orleans Alcohol and HIV cohort. A summary of the cohort is listed below.
- We will utilize an ensemble neural network to predict participant's CD8 T cell senescence category using microbiome and metabolomics data
- We hypothesize that gut microbial and metabolome remodeling mediates immune aging and intestinal leak.

## Methods

- Fecal Samples were collected from HIV+ participants in the New Orleans Alcohol and HIV cohort. DNA was isolated from fecal samples, and PCR was performed to amplify the V4 region of the 16s gene., sequenced using an Illuminia Miseq V2.
- Metabolomics analysis was preformed on fecal samples using an UHP-LC/MS. Mass spectrometry results were mapped to a reference database of 1000s of metabolites.
- Flow cytometry was performed on participant PBMCs to quantify CD8 T cell populations and ELISA assays were performed to measure fecal and serum alpha-1-antitrypsin.
- Keras and Tensorflow were used to develop and train neural networks. Features were selected by initially modeling with xgBoost and subset based on importance. Mint Tea was used to generate networks of microbiome and metabolome features predictive of high or low sCD14, A1AT gradient, and Senescent CD38+ CD8 T cells

## Cohort Demographics

| Variable | Female n= 80 | Male n=188 | p-value |
|---|---|---|---|
| Age | 47.5 ± 10.5 | 49.1 ± 10.7 | 0.251 |
| Body Mass Index | 27.9 ± 7.4 | 27.2 ± 7.0 | 0.449 |
| African American Race, % | 86.2 | 80.3 | **0.001*** |
| **HIV** | | | |
| CD4 > 350 cells/uL, % | 87.5 | 85.1 | 0.143 |
| Viral Load <20 copies/mL, % | 82.5 | 86.7 | 0.332 |
| ART % | 95.0 | 98.9 | 0.572 |
| **Alcohol** | | | |
| Years Drinking | 27.47± 11.6 | 27.34 ± 13.6 | 0.810 |
| Avg. drinks/day | 4.5 ± 6.9 | 4.7 ± 5.9 | 0.193 |
| PEth Positive, % | 38.4 | 67 | **0.001*** |
| **Tobacco** | | | |
| Years Smoking | 23.0 ± 15.7 | 23.1 ± 16.4 | 0.935 |
| Pack/years | 15.1 ± 16 | 14.2 ± 16.7 | 0.537 |

**Table 1. NOAH Cohort Demographics Summary**

## Ensemble Neural Network Architecture

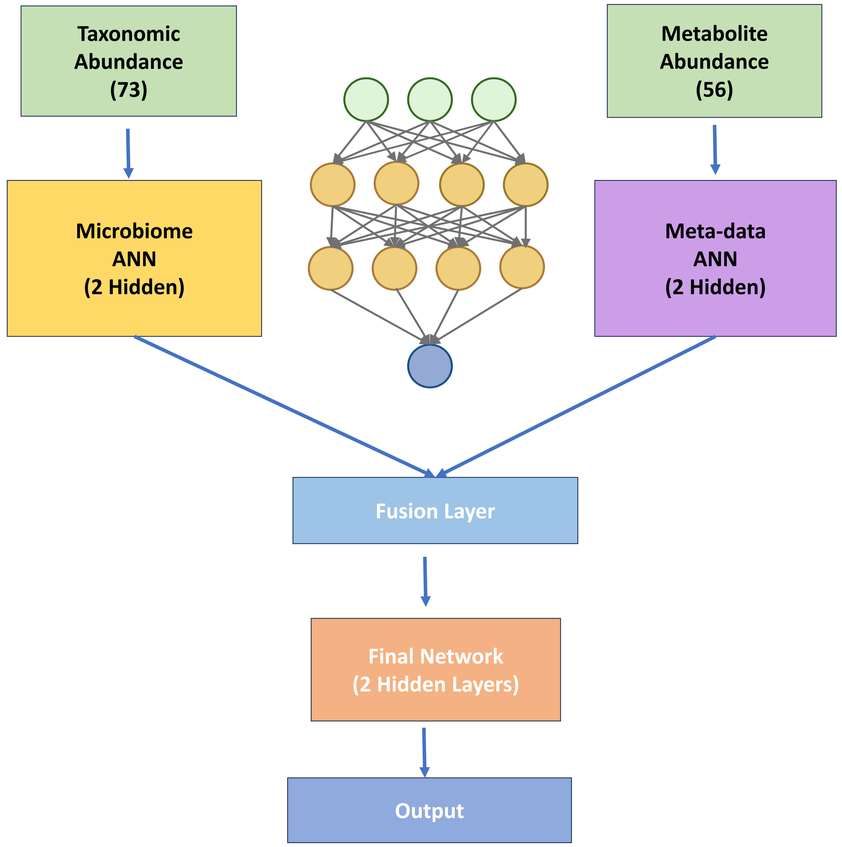


**Figure 1. Ensemble Neural Network Architecture**

## Modeling Results

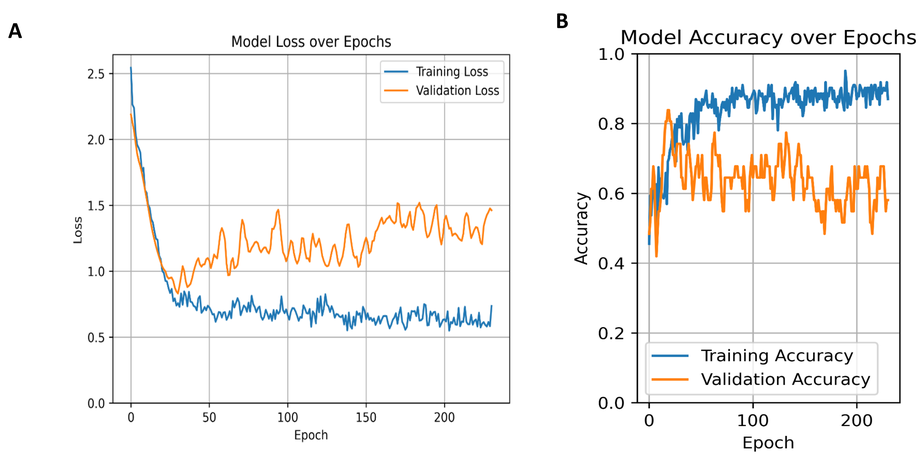


**Figure 2. Model Predicting CD8 38+ Senescent T cell category** (A) Training and Validation Loss Curve (B) Training and Validation Accuracy Curve

## Feature Importance

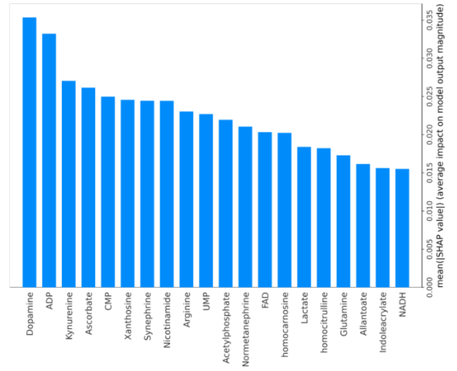


**Figure 3. Preliminary SHaP Feature Importance of Neural Network Predicting Senescent T cell category.**

## Inter-Omic Networks

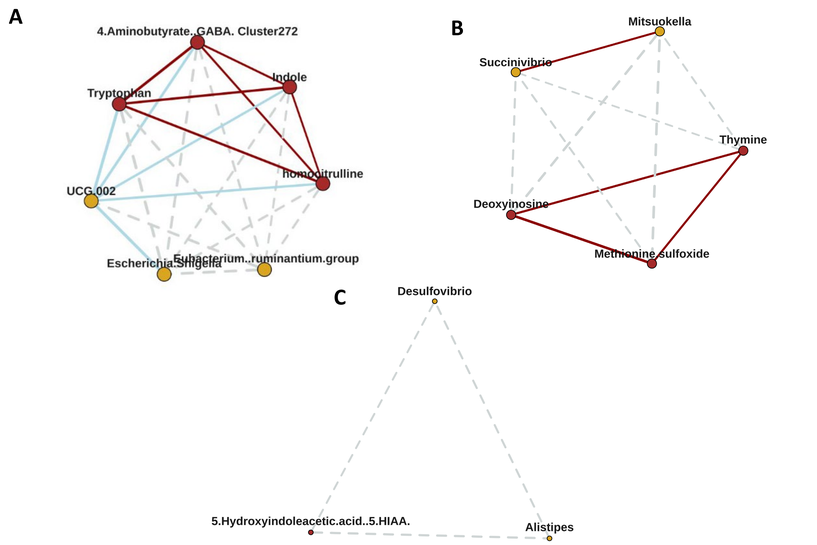


**Figure 4. Networks Generated from Mint Tea Analysis Predictive of (A) sCD14, (B) A1AT Gradient, and (C) Senescent CD38+ T cells**

## Conclusions

- ANNs modeling gut metabolome and microbiome accurately classified senescent CD8 T cell populations. Features found to be important included metabolites involved in the indole tryptophan pathway.
- Mint Tea analysis identified networks of microbes and metabolites predictive of leak measure abundance and senescent CD8 T cell populations
- Both findings reflect a relationship between gut microbiome-metabolome, leak and senescence.

## Citations

1. Welsh DA, Ferguson T, Theall KP, Simon L, Amedee A, Siggins RW, Nelson S, Brashear M, Mercante D, Molina PE. The New Orleans Alcohol Use in HIV [NOAH] Study: Launching a translational investigation of the interaction of alcohol use with biological and socioenvironmental risk factors for multi-morbidity in people living with HIV. *Alcohol Clin Exp Res*. 2019;43(4):704-709. doi:10.1111/acer.13980.
2. Maffei VJ, Siggins RW, Luo M, Brashear MM, Mercante DE, Taylor CM, Molina P, Welsh DA. Alcohol Use Is Associated With Intestinal Dysbiosis and Dysfunctional CD8+ T-Cell Phenotypes in Persons With Human Immunodeficiency Virus. J Infect Dis. 2021 Mar 29;223(6):1029-1039. doi: 10.1093/infdis/jiaa461. PMID: 32725203; PMCID: PMC8006423.

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