Preliminary Data Summary

Project Title: The Interplay of Circulating MicroRNAs and Gut Microbiome Diversity in Predicting Early-Onset Type 2 Diabetes Mellitus Risk

Date: October 2025

1. Pilot Cohort Description

A pilot study was conducted on a small, retrospectively collected cohort () to assess the feasibility of simultaneous microRNA and microbiome analysis in our lab setting.

- **EO-T2DM ():** Mean age 35.5 years; mean HbA1c 8.1%.
- **High-Risk Pre-Diabetic ():** Mean age 38.2 years; mean HbA1c 6.1%.
- **Healthy Controls ():** Mean age 37.0 years; mean HbA1c 5.3%.

2. Key Preliminary Findings

2.1 MicroRNA Feasibility and Differentially Expressed microRNAs (DE-miRs)

- **Feasibility:** RNA isolation from of stored plasma yielded high-quality small RNA libraries suitable for sequencing.
- **Finding:** Comparing the EO-T2DM group to controls, three circulating microRNAs were found to be significantly dysregulated (FDR):
  - **miR-29a:** Downregulated by -fold in EO-T2DM. Known to target insulin signaling pathways.
  - **miR-143:** Upregulated by -fold in EO-T2DM. Associated with adipogenesis and inflammation.

2.2 Microbiome Diversity and Composition

- **Feasibility:** High-quality DNA was successfully extracted from all stored fecal samples.
- **Finding (Alpha Diversity):** The High-Risk group showed significantly reduced microbial -diversity (Shannon Index mean) compared to the Healthy Controls (Shannon Index mean). The EO-T2DM group had the lowest diversity (Shannon Index mean).
- **Finding (Taxa):** Significant depletion of the **genus *Faecalibacterium*** and enrichment of the **genus *Bacteroides*** was observed in both the EO-T2DM and High-Risk groups compared to Controls ().

3. Conclusion and Project Justification

These preliminary data demonstrate the technical feasibility of simultaneous molecular and microbial profiling and, more importantly, provide strong evidence for the dysregulation of key biomarkers (miR-29a, miR-143, *Faecalibacterium*) in the high-risk and EO-T2DM populations.

These findings strongly justify the proposed comprehensive, integrated analysis in a larger, prospectively recruited cohort.