ENGINEERING AND MANUFACTURING OF PROBIOTIC *E. COLI* TO TREAT METABOLIC DISORDER

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The fields of synthetic biology and microbiome research developed greatly over the last decade. The convergence of those two disciplines is now enabling the development of new therapeutic strategies, using engineered microbes that operate from within the gut as living medicines. Inborn errors of metabolism represent candidate diseases for these therapeutics, particularly those disorders where a toxic metabolite causing a syndrome is also present in the intestinal lumen. Phenylketonuria (PKU), a rare inherited disease caused by a defect in phenylalanine hydroxylase (PAH) activity, is one such disease and is characterized by the accumulation of systemic phenylalanine (Phe) that can lead to severe neurological deficits unless patients are placed on a strict low-Phe diet. As an alternative treatment, *Escherichia coli* Nissle (EcN), a well-characterized probiotic, was genetically modified to efficiently import and degrade Phe (SYNB1618). The coupled expression of a Phe transporter with a Phe ammonia lyase (PAL) allows rapid conversion of Phe into trans-cinnamic acid (TCA) in vitro, which is then further metabolized by the host to hippuric acid (HA) and excreted in the urine. Experiments conducted in the *enu2-/-* PKU mouse model showed that the oral administration of SYNB1618 is able to significantly reduce blood Phe levels triggered by subcutaneous Phe injection. Decreases in circulating Phe levels were associated with proportional increases in urinary HA, confirming that Phe metabolism was caused by the engineered pathway in SYNB1618. Subsequent studies have shown that SYNB1618 is similarly operative in a non-human primate model, providing a translational link to inform future human clinical studies. Consistent with preclinical studies, recent Phase 1/2a clinical data demonstrate that oral administration of SYNB1618 resulted in significant dose-dependent production of biomarkers specifically associated with SYNB1618 activity, demonstrating proof-of-mechanism of this cell therapy.