Development of a Synthetic Biotic for the Treatment of Enteric Hyperoxaluria

Mark Charbonneau
Synlogic, Inc.
Synthetic Biotic Therapeutics: A New Class of Medicines

Bacteria and Humans Co-Evolved and Co-Exist

We Rationally Design Bacteria to Provide Clinical Benefit

The Result Is Therapeutic Bacteria With Programmable Therapeutic Mechanisms
Library of Parts To Generate Prototypes

Synthetic Biology Library Rapidly Generates Drug Candidates

**Component**

**Bacterial Chassis**

Probiotic *E. coli* Nissle 1917: Decades of human use & safety data

**Effectors**

Produce therapeutic benefit and generate biomarkers

**Switch**

Inducer-promoter pair: Controls gene expression *in vivo*

**Biocontainment**

Auxotrophies: Prevent growth within or external to the body

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**Benefit**

Toxin Biomarker 1

- PheP: High-affinity uptake

- Metabolic conversions

Effector 1

- Biomarker 1

Inducer

- Effector 2

- Biomarker 2
Synlogic Internal GMP Manufacturing Capabilities
In-house Process Development and Clinical Manufacturing for Early & Mid-Stage Trials

**Discovery**
- Strain Engineering

**Lead Optimization**
- Candidate Selection & Process Dev.
- Testing (in vitro and in vivo)
- Microbioreactors
  - High throughput strain screening & process development

**Pre-IND**
- Process development and lab scale production

**Phase 1**
- Optimized Process Scale-up

**Mid-Stage Trials**
- Biotherapeutic Manufacturing
  - Fermentation
  - Harvest/Formulation
  - Lyophilization, milling, & capsule fill

**Analytical Methods Development and Validation**

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Building a Diverse Portfolio of Synthetic Biotic Medicines
Platform for Clinical Benefit Across Multiple Disease States

Internal Focus: Metabolic Diseases

Consumption of toxic metabolites from the GI tract

External & Collaboration Focus: Immunomodulation

Immunology and oncology: Leveraging the ability of bacteria to interact with the immune system
Why Metabolic Diseases For Synthetic Biotic Medicines?

Validated Biology
Diseases with known pathophysiology. Dietary intervention provides support for GI-based approach
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Unmet Medical Need
Across both inherited and acquired metabolic diseases
Why **Metabolic Diseases** For Synthetic Biotic Medicines?

**Validated Biology**
Diseases with known pathophysiology. Dietary intervention provides support for GI-based approach

**Unmet Medical Need**
Across both inherited and acquired metabolic diseases

**Unique Advantage of SYNB**
Bacteria act catalytically, can contain multiple enzyme pathways and are protected from digestion within the GI tract.
Why Metabolic Diseases For Synthetic Biotic Medicines?

Validated Biology
Diseases with known pathophysiology. Dietary intervention provides support for GI-based approach

Platform Proof of Mechanism
PKU program demonstrated we can consume toxic metabolites in the GI tract. Subsequent programs build on experience.

Unmet Medical Need
Across both inherited and acquired metabolic diseases

Unique Advantage of SYNB
Bacteria act catalytically, can contain multiple enzyme pathways and are protected from digestion within the GI tract.
### Robust Pipeline

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**Key**
- Metabolic Diseases
- Immunomodulation
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**Key**

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# Enteric Hyperoxaluria

## Pathology

Pathogenic hyperabsorption of dietary oxalate, often accompanies bowel disease or bariatric surgery.

## Urinary Oxalate Levels

45 – 130 mg / 24 hrs (up to 3x normal)

## Onset

Adult

## Clinical Mgmt

Limited nutrition options; treatment of kidney stones as they occur; nephrocalcinosis; dialysis

## U.S. Epidemiology

200,000 – 250,000

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**Dietary Sources of Oxalate**

- Oxalate
- Hyperoxaluria
- Kidney stones

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**Hyperoxaluria**

- Dietary sources of oxalate
- Pathogenic hyperabsorption of dietary oxalate
- Often accompanies bowel disease or bariatric surgery
- Urinary oxalate levels: 45 – 130 mg / 24 hrs (up to 3x normal)
- Onset: Adult
- Clinical Mgmt: Limited nutrition options, treatment of kidney stones as they occur, nephrocalcinosis, dialysis
- U.S. Epidemiology: 200,000 – 250,000

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**Kidney stones**

- Dietary sources of oxalate
- Pathogenic hyperabsorption of dietary oxalate
- Often accompanies bowel disease or bariatric surgery
- Urinary oxalate levels: 45 – 130 mg / 24 hrs (up to 3x normal)
- Onset: Adult
- Clinical Mgmt: Limited nutrition options, treatment of kidney stones as they occur, nephrocalcinosis, dialysis
- U.S. Epidemiology: 200,000 – 250,000
Kidney Stone Disease and Gut Microbiota
Importance of GI Tract Microbiota in Oxalate Degradation

**Oxalobacter formigenes**, a gut commensal bacterium

- Gram negative rod bacterium
- Obligate anaerobe
- Colonizes 38-77% of healthy human gut
- Dependent on oxalate for growth and survival
- **Degrades oxalate to produce formate and CO₂**

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**Oxalobacter colonization associated with reduced stone formation**

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**Oxalobacter formigenes** provides a pathway to degrade dietary oxalate
Considerations for GI based therapies for EH

- Pathogenic hyperabsorption of dietary oxalate
- Dietary oxalate absorbed throughout GI tract
- Opportunity to degrade oxalate throughout GI tract, esp. in colon

Intestinal Degradation of Oxalate Throughout GI Tract Could Enhance Oxalate Lowering
# Hyperoxaluria strain SYN8802

Engineered to convert oxalate to formate

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<th>Approach</th>
<th>Benefit</th>
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<td>Bacterial Chassis</td>
<td><em>E. coli</em> Nissle</td>
<td>Decades of human use</td>
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<td>Switch</td>
<td>FNR promoter</td>
<td>Gene expression in low oxygen environment of gut</td>
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<tr>
<td>Pump</td>
<td><em>OxlT</em></td>
<td>Pumps oxalate in &amp; formate out</td>
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<td>Effectors</td>
<td><em>OxdC, ScaaE3, Frc</em></td>
<td>Catalyze conversion of oxalate to formate</td>
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<td>Safety Features</td>
<td>Δ<em>thyA</em></td>
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**Component Approach Benefit**

**SYN-HOX** Designed with pathways to degrade oxalate in the GI tract
SYNB8802 Activity in vitro and Bioavailability

*In vitro* activity of SYNB8802 in Simulated Intestinal Fluids

SYNB8802 Has Potential To Operate Throughout The GI Tract To Lower Absorption Of Oxalate Into The Blood
SYNB8802 Activity \textit{in vitro} and Bioavailability

\textit{In vitro} activity of SYNB8802 in Simulated Intestinal Fluids

![Graph showing in vitro activity of SYNB8802 in Simulated Intestinal Fluids]

- Oxalate Consumed [mM]
- Simulated Compartment: Stomach, Colon

Viable SYNB8802 Recovered in Feces after Oral Dose (NHP)

![Graph showing recovery of SYNB8802 in feces]

- Time post administration (hrs)
- SYN-HOX fecal recovery (CFU/g feces)

SYNB8802 Has Potential To Operate Throughout The GI Tract To Lower Absorption Of Oxalate Into The Blood
SYNB8802 Attenuates Urinary Oxalate Increase in Healthy Non-Human Primates

**Dietary Intervention Increases Urinary Oxalate**

400 mg oxalate elevates urinary oxalate

**SYN-HOX Consumes Oral Load of Oxalate in Non-Human Primates**
SYNB8802 Attenuates Urinary Oxalate Increase in Healthy Non-Human Primates

Dietary Intervention Increases Urinary Oxalate

400 mg oxalate elevates urinary oxalate

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SYNB8802 Attenuates Urinary Oxalate Increase

High Oxalate Diet

400 mg oxalate elevates urinary oxalate

SYNHOX (CFU)

* p < 0.05 , ** p < 0.01 versus vehicle

SYN-HOX Consumes Oral Load of Oxalate in Non-Human Primates

* p < 0.05 , ** p < 0.01 versus vehicle
In Silico Simulations (ISS) of SYNB8802 Activity in Humans

How do we build confidence that a candidate strain is ready for clinical studies?

Key Question

Is SYNB8802 activity **sufficient** to deliver a clinical benefit to enteric hyperoxaluria patients?
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The Challenge

Strain function *in vivo* is complex and dynamic
- Strain activity is **not** constant
- Gut conditions are **not** constant
- Strain *competes* with host for metabolites
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Our Approach

Leverage in vitro data and knowledge of disease biology to perform In Silico Simulations (ISS) of strain activity in human GI tract
ISS Models of Strain Activity in Humans

Strain Function \textit{in vivo} is Complex and Dynamic

Our Approach

Leverage \textit{in vitro} data and knowledge of disease biology to perform \textit{In Silico Simulations (ISS)} of strain activity in human GI tract

\textit{In vitro} Assay Data

Literature Evidence

\textbf{Inputs}

\textbl{ISS Model}

Ordinary Differential Equations

Parameter Estimates

\textbf{Outputs}

Biomarker Production

Serum/Urine Metabolite Concentrations
Validated predictions of strain activity in humans

Example: ISS Model for PKU Program (SYNB1618)
SYNB8802 ISS Model

Simulated Strain Activity in GI impacts Urinary Oxalate
SYNB8802 ISS Model

Simulated Strain Activity in GI impacts Urinary Oxalate

Meal

Endogenous Production

Gut

Blood

Urine

Fecal excretion

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SYNB8802 ISS Model
Simulated Strain Activity in GI impacts Urinary Oxalate

Modeling Suggests SYNB8802 may Achieve >20% Urinary Oxalate Lowering at Target Dose Range

3x daily dosing with meals
Enteric Hyperoxaluria: Phase 1 Design Provides PoC Opportunity

Phase 1a
Healthy Volunteers

Multiple Ascending Dose
• High oxalate & low calcium diet run-in
• Primary: Safety & tolerability
• Secondary: Microbial kinetics of strain
• Exploratory: Change in plasma and urine biomarkers

Phase 1b
Enteric Hyperoxaluria Patients

Cross-over
• 3x daily dosing
• N = 20 patients (Roux-en Y gastric bypass)
• UOx >70 mg/day

Roux-en-Y Gastric Bypass Population Provides Opportunity to Demonstrate Urinary Oxalate Lowering in Disease State
SYNB8802 Conclusions

Enteric Hyperoxaluria results in significant kidney damage with limited treatment options.

SYNB8802 has the potential to meaningfully lower urinary oxalate levels.

SYNB8802 Phase 1 clinical study initiated ahead of schedule.