A Probiotic Gene Cassette for Ulcerative Colitis Treatment

Jason M. Crawford, Ph.D.  (Emilee Shine)
&
Richard A. Flavell, Ph.D., F.R.S.  (Abigail Jarret)

GutReactions
> 1.4 million Americans and rising are afflicted with IBD

Patients are generally treated with mesalamine/sulfasalazine

Estimated $10 billion in world drug revenue for IBD in 2017

35% of patients report no improvement and nearly 20% experience adverse events

2/3 of patients eventually have surgical resection of the bowel

**Goal: Bugs as Drugs**

Develop probiotic for UC with higher efficacy and lower toxicity

**Develop a probiotic for ulcerative colitis (UC)**
Products for ulcerative colitis (UC) and colorectal cancer

Upper Respiratory Tract

Esophagus

Liver

Abdominal aorta

Gall bladder

Stomach

Duodenum

Spleen

Colon

Pancreas

Small intestine

Appendix

Rectum

Asacol (mesalamine) delayed-release tablets

400 mg per tablet

Asacol HD (mesalamine) delayed-release tablets

800 mg per tablet

Nicorette

MINT

STOP SMOKING AID

108 PIECES, 2mg EACH
Inflammatory bowel diseases vs. Cancer

**“Mutualist”**

- Known to be efficacious in clinical trials
- Pathway is found in a variety of *E. coli* (99% identical)
- We have identified pathway products with probiotic activities
- These products represent commercially-relevant deliverables

**“Pathogen”**

- Genes in colibactin pathway are required for probiotic efficacy
- *E. coli* probiotics contribute to deleterious Enterobacteriaceae load
- Mature colibactin causes colorectal cancer
Inflammatory bowel diseases vs. Cancer

Known to be efficacious in clinical trials
Pathway is found in a variety of *E. coli* (99% identical)
We have identified pathway products with probiotic activities
These products represent commercially-relevant deliverables

Genes in colibactin pathway are required for probiotic efficacy
*E. coli* probiotics contribute to deleterious Enterobacteriaceae load

Mature colibactin causes colorectal cancer
Establish commercial viability of novel products for UC

“Mutualist”
Inflammatory bowel diseases vs. Cancer
Known to be efficacious in clinical trials
Pathway is found in a variety of E. coli (99% identical)
We have identified pathway products with probiotic activities
These products represent commercially-relevant deliverables

Genes in colibactin pathway are required for probiotic efficacy
E. coli probiotics contribute to deleterious Enterobacteriaceae load.

“Pathogen”
Mature colibactin causes colorectal cancer

mutaflor.ru
cancer.gov

“Mutualist”
“Pathogen”
Colibactin polypharmacology regulates probiotic activity

**Probiotic**

- L-Asn (ClbN)
- L-Ala (ClbB)
- malonyl-CoA (ClbC)
- SAM (ClbD)

**Carcinogenic**

- L-Cys (ClbE)
- Gly (ClbF)
- L-Cys (ClbG)

**Pro-drug cleavage moiety**

- Bithiazole tail
- DNA binding element

**Electrophilic warhead**

- ClbA, pantetheinyltransferase
- ClbB, 3-hydroxybutyryl-CoA dehydrogenase
- ClbE, freestanding thiolation (T) domain
- ClbF, acyl-CoA dehydrogenase
- ClbG, freestanding acyltransferase (AT)
- ClbL, amidase
- ClbM, inner membrane transporter
- ClbP, inner membrane peptidase
- ClbQ, editing thioesterase
- ClbR, transcriptional regulator
- ClbS, resistance protein

**Module skipping**

Crawford lab: 6 years and 10 publications
Cassette products 1 & 2 regulate catecholamine pathways

- Cassette 1 produces a 5-HT\textsubscript{7} GPCR antagonist, a phenotype that ameliorates colitis in mice
- Cassette 2 produces a 5-HT\textsubscript{7} GPCR antagonist and functional alkyl amine analogs
- Cassettes lack genotoxicity
- Probiotics have been engineered in our lab
**Programmed probiotics**

**Step 1:** 6 months: Engineered Nissle evaluation and optimal cassette selection

**Step 2:** 12 months: Generation of *Lactobacillus* strains containing the optimal cassette

18 months: Evaluate engineered *Lactobacillus* as probiotic lead in mouse model

Colitis scoring, toxicity, weight loss & death

16S microbiome profiling

Inflammatory cytokine signaling
IL-10, IL-5, IL-1β, IL-18

Metabolomics

---

**WT Nissle 1917**

**Mutant Nissle 1917**

**Cassette Selection**

**Adoptive T-cell Transfer**

**CD4+CD45RB**

**Microbiome**

**Metabolism**

**Cytokine Secretion**
Step 1: Evaluate efficacy of engineered probiotics in hand versus Mutaflor

Genesis Biotech Group: $235,000 0-6 month period; Go, No Go

Step 2: Transfer optimal cassette into *Lactobacillus acidophilus*

Compare *L. acidophilus* deliverable to Mutaflor, mesalamine, and Humira

Genesis Biotech Group: $157,100 6-15 month period; Go, No Go

Aim for human studies within 2 years
Wildtype colibactin pathway in Mutaflor is toxic to mice
Genesis Notes: Quote will be in 3 parts

1. “Protocol Transfer”
2. Step 1
3. Step 2