A Novel Human IL-22-Secreting Synthetic Biotic Medicine for the Treatment of Inflammatory Bowel Disease

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All authors were Synlogic employees and shareholders at the time the work was performed.
Intestinal Homeostasis is Disrupted in IBD

Disease pathophysiology in IBD linked to compromised barrier function and increased inflammation

Mucosal healing is the next therapeutic goal to achieve stable remission in IBD patients

Zhang 2017, Neurath 2014, Bourma and Strober 2003
IL-22 as a Therapeutic Effector
Promotes barrier function and mucosal healing

- IL-22 promotes epithelial barrier functions and mucosal healing.
- Extensive pre-clinical evidence supports a critical role for IL-22 in IBD.
- Many IL-22 associated molecules are encoded by IBD susceptibility genes and patients with ulcerative colitis have evidence of IL-22 dysfunction.
- Systemically administered hIL-22-Fc results in pharmacodynamic responses but is associated with dose-dependent skin adverse effects.

Can local delivery of IL-22 in the gut promote epithelial barrier healing in IBD patients without systemic toxicity?
Synthetic Biotics: A Novel Approach to Immune Diseases
Exploiting the Interaction Between Bacteria and the Immune System

Bacteria and Immune System are Intimately Linked
- Immune system has evolved to recognize bacteria
- Bacteria have evolved mechanisms to control the immune response

Synthetic Biotics Enable Unique Therapeutic Opportunities
- Bacteria has evolved to survive the gastrointestinal tract
- Local delivery to the site of disease

Synthetic Biotics Enable Multiple Therapeutic Modalities
- Ability to deploy multiple pathways
- Broad expression of bacterial and mammalian effectors
# Engineering *E. coli* Nissle to Secrete hIL-22

## Component

<table>
<thead>
<tr>
<th>Component</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>e. coli</em> Nissle</td>
<td>Probiotic: Decades of human use &amp; safety data</td>
</tr>
<tr>
<td>hIL-22</td>
<td>Improvement of epithelial barrier health</td>
</tr>
<tr>
<td>FNR promoter</td>
<td>Induced by lack of oxygen in the gut</td>
</tr>
<tr>
<td>Secretion system</td>
<td>Diffusible outer membrane (DOM) to enable protein secretion</td>
</tr>
</tbody>
</table>

### Diagram

- **IL-22 secretion**
- **Inducer (hypoxia in the gut)**
- **DOM modification**
- **FNR**
- **hIL-22**
Engineered EcN Produces IL-22 with Comparable Bioactivity to rhIL-22

In vitro Characterization of EcN-hIL-22

**hIL-22 Production**

- **hIL-22 Production**
  - CTRL
  - EcN
  - hIL-22

**hIL-22 Bioactivity**

- **STAT3 Activation** (OD 450-570 nm)
  - EcN-hIL-22
  - rhIL-22
  - CTRL EcN

IL-22 concentration (ng/mL)
EcN-hIL-22 is Viable in the Gut of DSS-treated mice

*In vivo* kinetics of a single dose, $1 \times 10^{10}$ CFUs/ mouse

- **Small intestine**
- **Cecum**
- **Colon**
- **Feces**

- **Dose:**
  - $1 \times 10^{10}$
  - $1 \times 10^{9}$
  - $1 \times 10^{8}$
  - $1 \times 10^{7}$
  - $1 \times 10^{6}$

- **CFU/organ:**
  - $10^{11}$
  - $10^{10}$
  - $10^{9}$
  - $10^{8}$
  - $10^{7}$

- **Time Post-Gavage:**
  - 1h
  - 3h
  - 6h
  - 24h
  - 48h

- **LOD:**
  - $10^{3}$

- **cN-hIL-22**
EcN-hIL-22 is Active in the Colon of DSS-treated Mice

*In vivo* cytokine production 6 hours after a single dose, 1e10 CFUs/mouse

### Fecal CFU

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>EcN</th>
<th>EcN-hIL-22</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFUs/g of feces</td>
<td>10^{10}</td>
<td>10^{11}</td>
<td>10^{10}</td>
</tr>
</tbody>
</table>

### Fecal hIL-22

<table>
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<th>EcN</th>
<th>EcN-hIL-22</th>
</tr>
</thead>
<tbody>
<tr>
<td>hIL-22, pg/g of feces</td>
<td>10,000</td>
<td>50,000</td>
<td>40,000</td>
</tr>
</tbody>
</table>

### Colon hIL-22

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>EcN</th>
<th>EcN-hIL-22</th>
</tr>
</thead>
<tbody>
<tr>
<td>hIL-22, pg/g of tissue</td>
<td>2,000</td>
<td>3,000</td>
<td>2,000</td>
</tr>
</tbody>
</table>

**** indicates statistical significance.
Strain Delivers Biologically Active hIL-22 to the Inflamed Gut

*In vivo* target engagement after prolonged bacterial exposure (up to 24h)

**Colon Reg3β mRNA**

**Colon Birc5 mRNA**

**Antimicrobial**

**Anti-apoptotic**
Summary and Conclusions
Engineered probiotic E.coli Nissle strain produces bioactive human IL-22

**Summary**

- Engineered EcN secretes high levels of bioactive hIL-22 *in vitro*.

- EcN-hIL-22 is viable in different compartments of the mouse inflamed gut.

- EcN-hIL-22 secretes high levels of hIL-22 in inflamed colon:
  - hIL-22 detected in feces and colon.

- Bacterially produced hIL-22 is bioactive in the gut:
  - Target engagement in inflamed colonic tissue.

**Conclusions**

- Engineered EcN has the potential to deliver hIL-22 locally in the gut to improve mucosal healing and achieve remission in IBD patients while preventing the adverse effects associated with systemic delivery of the protein.

- These data support the development of Synthetic Biotic medicines as a novel approach to treat intestinal immune-mediated diseases.