Novel Broad-Spectrum $\beta$-Lactamase Therapy to Protect the Gut Microbiome from Antibiotics

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Importance of Intestinal Health Has Long Been Recognized

“ALL DISEASE BEGINS IN THE GUT!”
-Hippocrates
400 B.C.

Gut Microbiome Involved in:
- Digestion
- Nutrient absorption
- Vitamin synthesis
- Bile salt metabolism
- Stimulation of immune system

Disrupted by:
- Antibiotic use

Synthetic Biologics is developing therapies to protect the gut microbiome from the damage caused by antibiotic use
β-Lactamases: From Enemies to Therapies

**Strategy:** β-lactamase enzyme is intended to degrade residual antibiotics in the GI tract to protect the microbiome

**Product:** Capsule with enteric-coated enzyme

**Outcome:** Prevention of *Clostridium difficile* infection and antibiotic-associated diarrhea

Orally-delivered β-lactamases intended to degrade residual antibiotics in the GI tract to protect the gut microbiome without affecting antibiotic efficacy.
β-Lactamase Clinical Efficacy: Degradation of Intestinal Penicillins

P1A
• Clinical isolate from *Bacillus licheniformis*
• Class A serine β-lactamase
• Degrades penicillins

Clinical results

<table>
<thead>
<tr>
<th>Similarity Index</th>
<th>Amp-Resistant Bac</th>
<th>GI Tract</th>
<th>Systemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1A Alone</td>
<td>Amp Alone</td>
<td>Amp+P1A</td>
<td>Amp+P1A</td>
</tr>
<tr>
<td>Amp+P1A</td>
<td>Amp+P1A</td>
<td>Amp+P1A</td>
<td>Amp+P1A</td>
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</tbody>
</table>

Stop Amp


P1A does not degrade cephalosporins, a major risk factor for *Clostridium difficile* infection
Ceftriaxone and Pip/Tazo are the Most Frequently Used IV β-Lactams

### IV β-Lactam Use

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>EU</th>
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<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Days on Therapy</td>
</tr>
<tr>
<td>Total IV Abx</td>
<td>23 million</td>
<td>170 million</td>
</tr>
<tr>
<td>IV β-lactams</td>
<td>17 million</td>
<td>73 million</td>
</tr>
<tr>
<td>% of Total</td>
<td>72%</td>
<td>43%</td>
</tr>
</tbody>
</table>

### Individual β-Lactam Antibiotics: Days on Therapy

**US**
- Ceftriaxone: 30%
- Pip and Pip/Tazo: 27%
- Cefazolin: 23%
- Other: 9%
- Amp and Amp/Sulbactam: 11%

**EU**
- Ceftriaxone: 32%
- Pip and Pip/Tazo: 20%
- Cefazolin: 8%
- Other: 5%
- Cefotaxime: 14%
- Cefuroxime: 13%
- Amp and Amp/Sulbactam: 14%

Arlington Medical Resources (AMR), a Decision Resources Group Company 2014 audits of acute care hospital antibiotic utilization
SYN-004 Degrades Cephalosporins

- SYN-004 was engineered from P1A
- Contains one amino acid substitution: D276N

E. coli growth microtiter plate assay

SYN-004 efficiently degrades cephalosporins, including ceftriaxone, cefuroxime, cefoperazone, ceftazidime, and cefotaxime
SYN-004 Oral Formulation is Stable in Human Chyme

SYN-004 Enteric-Coated Pellets

Enteric-coated SYN-004 pellets remain intact at low pH and released enzyme retains biological activity for at least 6 hours in human intestinal contents.
SYN-004 is in Phase 2 Clinical Trials

**Preclinical Results**
- Safe in two GLP toxicity studies in dogs
- Well tolerated with a NOAEL of 57 mg/kg/day, highest dose tested
- Not detected systemically
- Did not affect ceftriaxone blood levels

**Clinical Results**
- Phase 1 clinical studies demonstrated SYN-004 safety and tolerability with a single dose of up to 750 mg and multiple doses of 300 mg 4X a day for 7 days
- SYN-004 was neither systemically bioavailable nor immunogenic
- Phase 2a clinical studies were initiated in 1H 2015
- A Phase 2b clinical study is on track to be initiated in 3Q 2015

SYN-004 efficiently degrades penicillins and cephalosporins but does not degrade carbapenems
P2A, NDM, and KPC are Broad-Spectrum Carbapenemases

**P2A**
- Clinical isolate from *Bacillus cereus*
- Class B metallo-β-lactamase
- Requires Zn$^{2+}$ for activity
- Resistant to β-lactamase inhibitors

**NDM**
- New Delhi metallo-β-lactamase
- Class B metallo-β-lactamase
- Requires Zn$^{2+}$ for activity
- Resistant to β-lactamase inhibitors

**KPC**
- *Klebsiella pneumoniae* carbapenemase
- Class A serine β-lactamase
Expression of P2A, NDM, and KPC in *E. coli*

- Over 100 *E. coli* strains were generated
- P2A, NDM, and KPC scaled to 5L bioreactor fermentation

Carbapenemases were efficiently produced in *E. coli* and retained biological activity following purification.
Antibiotic Degradation Profile of Selected Carbapenemases

P2A, NDM, KPC were compared to SYN-004

*E. coli* growth microtiter plate assay

P2A and NDM display the broadest antibiotic degradation profiles including penicillins, cephalosporins, and carbapenems and are resistant to \(\beta\)-lactamase inhibitors
P2A is Stable in Human Chyme

Purified P2A was incubated in human chyme and activity assessed using the CENTA assay

- P2A displayed sustained biological activity in human chyme in the presence of Zn$^{2+}$
- P2A was sensitive to pH as increasing the pH of Chyme 3 improved P2A stability
P2A Degrades Meropenem in Dog GI Tract

- Fistulated dogs (n=6) received IV meropenem (30 mg/kg)
- P2A (liquid formulation) was delivered orally (1 mg/kg) following antibiotic injection
- Levels of meropenem and P2A in the jejunal contents and serum were measured

<table>
<thead>
<tr>
<th>Treatment (n=3)</th>
<th>Dog</th>
<th>P2A (U/g)</th>
<th>Meropenem (ug/g)</th>
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</thead>
<tbody>
<tr>
<td>Meropenem Alone</td>
<td>1</td>
<td>NA</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>NA</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>NA</td>
<td>3.0</td>
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<tr>
<td>Meropenem + P2A</td>
<td>4</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.2</td>
<td>2.0</td>
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P2A degraded the meropenem in the dog GI tract and did not affect meropenem serum levels
Conclusions

- SYN-004 is intended as an orally-delivered β-lactamase to protect the gut microbiome from IV penicillins and cephalosporins to prevent *Clostridium difficile* infection

- Clinical validation was achieved with the SYN-004 precursor, P1A

- SYN-004 is progressing though Phase 2 clinical trials

- SYN-004 is a broadly acting cephalosporinase that does not degrade carbapenems

- P2A, NDM, and KPC were evaluated as pipeline candidates

- P2A was chosen based on broad antibiotic degradation and stability in human chyme

- P2A formulation and evaluation in a pig microbiome model is in progress
# Acknowledgements

**Synthetic Biologics, Inc.**

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<tr>
<th>Research</th>
<th>Development</th>
<th>Clinical and Nonclinical</th>
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