Development of β-Lactamase Therapies to Protect the Gut Microbiome from Antibiotics

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Importance of Intestinal Health Has Long Been Recognized

Gut Microflora 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 microflora from the damage caused by antibiotic use

“ALL DISEASE BEGINS IN THE GUT!”
- Hippocrates
400 B.C.
**Strategy:** Orally administered β-lactamase to degrade residual antibiotics in the GI tract without affecting systemic antibiotic efficacy

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

**Outcome:** Prevention of *Clostridium difficile* infection and antibiotic-associated diarrhea
Issues to Consider in Developing $\beta$-Lactamases as Therapeutics

- Choice of $\beta$-Lactamase
- Can it be manufactured
- Does it have a suitable degradation profile
- Is it stable in chyme
- Is it compatible with enteric coating
- Is it efficacious and safe in animal models

Advance to human clinical trials
Isolated from *Bacillus licheniformis* | Class A serine β–lactamase | Degrades penicillins

**GI Tract** | **Systemic** | **Similarity Index** | **Amp-Resistant Bac**

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Pip/Tazo Alone | Pip/Tazo Alone | Amp/Resistant Bac | Amp+P1A

Pip/Tazo+P1A | Pip/Tazo+P1A | Amp+P1A | Amp+P1A


However, P1A does not degrade cephalosporins, a major risk factor for *Clostridium difficile* infection.
Ceftriaxone Is an Important Antibiotic Target

### IV β-Lactam Use

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>EU</th>
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<tbody>
<tr>
<td>Patients</td>
<td>23 million</td>
<td>17 million</td>
</tr>
<tr>
<td>Days on Therapy</td>
<td>170 million</td>
<td>115 million</td>
</tr>
<tr>
<td>Total IV Abx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV β-lactams</td>
<td>17 million</td>
<td>10 million</td>
</tr>
<tr>
<td>% of Total</td>
<td>72%</td>
<td>70%</td>
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</table>

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

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

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

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 One amino acid substitution: D276N

*E. coli* growth microtiter plate assay

SYN-004 efficiently degrades cephalosporins
SYN-004 is Stable in Human Chyme

SYN-004 Enteric-Coated Pellets

**pH Dissolution Profile**

- pH 1.0
- pH 6.8
- pH 5.8
- pH 5.5

**Stability in Human Chyme**

- Relative SYN-004 Activity (ΔM405 nm)

SYN-004 Degrades CRO in the Dog GI Tract

Six fistulated dogs received IV ceftriaxone +/- oral SYN-004. Chyme was collected and assayed for ceftriaxone (CRO) and SYN-004.
SYN-004 Dog Toxicity Studies

**Study Design**

Ceftriaxone intravenously 1X per day for 14 days  
SYN-004 orally 3X per day  
Three cohorts (n=6)  
  - CRO alone  
  - CRO plus SYN-004 (6.6 mg/kg/day)  
  - CRO plus SYN-004 (57 mg/kg/day)

**Results**

Safe and well tolerated  
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 was safe and well tolerated with a single dose of up to 750 mg and multiple doses of 300 mg 4X per day for 7 days.

Phase 2a clinical studies were initiated in 1H 2015
- Ileostomy studies
  - To confirm that SYN-004 removes CRO from the chyme without altering CRO plasma levels

A Phase 2b clinical study is on track to be initiated in 3Q 2015
- Endpoints include CDI
P4A Further Expands the Antibiotic Degradation Profile

P4A was engineered from SYN-004 using random mutagenesis and rational design


E. coli growth microtiter plate assay

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>SYN-004</th>
<th>P4A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>CRO</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>CTX</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>CFZ</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>CXM</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>CFP</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>FEP</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>CAZ</td>
<td>100</td>
<td>10</td>
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</tbody>
</table>

Amp: ampicillin
CRO: ceftriaxone
CTX: cefotaxime
CFZ: cefozolin
CXM: cefuroxime
CFP: cefoperazone
FEP: cefepime
CAZ: ceftazidime

P4A further improves the degradation of cephalosporins

However, P4A does not degrade carbapenems
P2A, NDM, and KPC are Broad-Spectrum Carbapenemases

**P2A**
Isolated 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
P2A, NDM, and KPC Were Produced in *E. coli*

Over 100 *E. coli* strains were generated

P2A and NDM were caught in inclusion bodies

But Zn\(^{2+}\) shifted their expression to the soluble cytoplasmic fraction

**Purification**

<table>
<thead>
<tr>
<th></th>
<th>P2A</th>
<th>NDM</th>
<th>KPC</th>
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<tbody>
<tr>
<td>Size</td>
<td>21.5 kDa</td>
<td>31.0 kDa</td>
<td>21.5 kDa</td>
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**Activity**

CENTA Chromogenic Assay

**SYN-004**

**NDM**

**KPC**

**P2A**
Antibiotic Degradation Profiles of the Purified Carbapenemases

P2A, NDM, and KPC were compared to SYN-004

NDM displayed the broadest antibiotic degradation profile. P2A was a close second.
P2A was incubated in human chyme and activity was assessed with the CENTA assay. P2A displayed sustained biological activity in human chyme but was sensitive to low pH.
NDM and KPC were incubated in human chyme and activity was assessed with the CENTA assay.

Why was NDM less stable in chyme?
Purified NDM was incubated in 2% human chyme and the initial cleavage sites were mapped.

NDM contains a limited number of sites at which protease digestion starts.
P2A Degrades Meropenem in the Dog GI Tract

6 fistulated dogs received IV meropenem
3 received oral P2A in liquid formulation
Chyme was assayed for meropenem and P2A and serum was assayed for meropenem

Chyme Meropenem and P2A

<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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<tbody>
<tr>
<td>Meropenem Alone</td>
<td>1</td>
<td>NA</td>
<td>3.0</td>
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<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>
</tr>
<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 meropenem in the dog GI tract without altering the systemic levels
SYN-004 is intended as an orally-delivered β-lactamase to protect the gut microbiome from IV penicillins and cephalosporins to prevent *C. difficile* infection.

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

SYN-004 is progressing though Phase 2 clinical trials.

SYN-004 and P4A are broadly acting cephalosporinases that do 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.

Currently formulating P2A for evaluation in a pig model.
## Acknowledgements

### Synthetic Biologics, Inc.

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<thead>
<tr>
<th>Research</th>
<th>Manufacturing</th>
<th>Clinical</th>
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<tbody>
<tr>
<td>John Monahan</td>
<td>J. Andrew Bristol</td>
<td>Joe Sliman</td>
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<td>Sheila Connelly</td>
<td>Steven Hubert</td>
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<td>Heather McFall</td>
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### Ipsat Therapies, Ltd

- Pertti Koski

### SynPhaGen, Inc.

- Todd Parsley