Protecting the
GUT MICROBIOME
A Paradigm Shift in Managing GI Disorders

SUNDAY, MAY 7, 2017
FAIRMONT CHICAGO MILLENNIUM PARK

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Planning Committee Member

Barb Forney, CME Compliance – No Relevant Relationships
Julie Messick – No Relevant Relationships

Faculty

All faculty disclosures can be found in your meeting guide.
This program is supported by educational grants from Synthetic Biologics, Inc. and Commonwealth Diagnostics International, Inc.
Protecting the **GUT MICROBIOME**
A Paradigm Shift in Managing GI Disorders

**NICHOLAS J. TALLEY, MD (CHAIR)**
University of Newcastle, Australia
Callaghan, Australia

**ERIK R. DUBBERKE, MD**
Washington University
School of Medicine
Saint Louis, Missouri

**ROB KNIGHT, PhD**
University of California
at San Diego
La Jolla, California

**MARK PIMENTEL, MD**
Cedars-Sinai Medical Center
Los Angeles, California
Late Breaking News

Nicholas J. Talley, MD
University of Newcastle Australia
Callaghan, Australia
Ribaxamase Prevented *C. difficile* Infection and Protected Patients from Colonization: Phase 2B Trial

Patients hospitalized with LRT
N=412

- Ribaxamase 150 mg QID
- Ceftriaxone IV
- Placebo QID

LRT, lower respiratory tract.
Ribaxamase Prevented *C. difficile* Infection and Protected Patients from Colonization: Phase 2B Trial

- 43.9% RRR in new VRE colonization in ribaxamase group vs placebo ($P=0.0002$)
- Ribaxamase associated with significantly less change and loss of diversity in gut microbiome compared with placebo

**Confirmed CDI Cases**

<table>
<thead>
<tr>
<th></th>
<th>Confirmed CDI cases, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>7</td>
</tr>
<tr>
<td>Ribaxamase</td>
<td>2</td>
</tr>
</tbody>
</table>

$P=0.045$  

RRR, relative risk reduction; VRE, vancomycin-resistant enterococci.
Fecal Microbiota Transplantation in IBS: A Randomized Controlled Trial

Reduction in IBS-SSS From Baseline After FMT

4 week 8 week
-61.6 * -63.3 *

Reduction in GSRS-IBS total score

2 week 4 week
-0.46 * -0.71 * -0.79 *

Placebo (own feces material), n=8  Treatment (donor feces material), n=8

*P<0.01 vs baseline; †P<0.05 vs baseline.
GSRS-IBS, Gastrointestinal Symptom Rating Scale; IBS-SSS, IBS Symptom Severity Score.
Fecal Microbiota Transplantation in IBS: A Randomized Controlled Trial

Improvement in IBS-QoL From Baseline 8 Weeks After FMT

FMT has a beneficial effect on quality of life in IBS

*P<0.01 vs baseline.
Methanogenic Flora and Its Effects on Regional and Whole Gut Transit and Small Bowel pH

<table>
<thead>
<tr>
<th></th>
<th>Methanogenic Flora (n=70)</th>
<th>Non-methanogenic Flora (n=117)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GET (Hr)</td>
<td>6.1 ± 10.9</td>
<td>5.6 ± 10.5</td>
</tr>
<tr>
<td>SBTT (Hr)</td>
<td>5.2 ± 3.7</td>
<td>5.2 ± 2.5</td>
</tr>
<tr>
<td>CTT (Hr)*</td>
<td>59.43 ± 38.2</td>
<td>46.1 ± 34.2</td>
</tr>
<tr>
<td>Mean SB pH</td>
<td>6.7 ± 0.3</td>
<td>6.8 ± 0.3</td>
</tr>
<tr>
<td>Baseline CH$_4$*</td>
<td>26.66 ± 31.4</td>
<td>2.01 ± 1.1</td>
</tr>
<tr>
<td>Highest CH$_4$*</td>
<td>36.4 ± 39.7</td>
<td>3.62 ± 4.4</td>
</tr>
</tbody>
</table>

Data from 187 patients (mean age 46.6 ± 16.4 years, 151 females).

*P<0.05

The Gut Microbiome and Functional GI Disorders

Mark Pimentel, MD, FRCP(C)

Executive Director,
Medically Associated Science and Technology (MAST) Program
Cedars-Sinai Medical Center
Los Angeles, California
Defining IBS Over The Years

- 1960: Spastic colon
- 1970: Spastic colitis
- 1980: Irritable bowel syndrome
- 1990: Rome Criteria
- 2000: IBS-SIBO connection
- 2010: The word “microbiome”*
- 2015: Rome IV
- Now

*Lederberg and McCray 2001
IBS Pathophysiologic Sequence

1. **Food poisoning**
   - E. Coli
   - C. jejuni
   - Shigella
   - Salmonella

2. **Bacterial toxin**
   - Cytolethal Distending toxin (CDT B)

3. **Auto-immunity**
   - Anti-vinculin

4. **Gut nerve damage**
   - Reduced ICC
   - Reduced MMC

5. **Bacterial overgrowth**
   - Breath testing
   - Culture
   - qPCR
   - Deep sequencing

6. **IBS**

Odds Ratio for Abnormal Breath Test in IBS Patients (Age-Sex Matched Studies)

<table>
<thead>
<tr>
<th>Author</th>
<th>Type of Breath Test</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grover</td>
<td>Sucrose</td>
<td>2.29 (0.89, 5.87)</td>
</tr>
<tr>
<td>Lupascu</td>
<td>Glucose</td>
<td>10.89 (3.52, 33.71)</td>
</tr>
<tr>
<td>Pimentel</td>
<td>Lactulose</td>
<td>20.67 (5.29, 80.69)</td>
</tr>
<tr>
<td>Parodi</td>
<td>Glucose</td>
<td>4.30 (1.24, 14.98)</td>
</tr>
<tr>
<td>Scarpellini</td>
<td>Lactulase</td>
<td>24.27 (7.35, 80.15)</td>
</tr>
<tr>
<td>Collin</td>
<td>Lactulose</td>
<td>18.04 (6.55, 49.71)</td>
</tr>
</tbody>
</table>

Overall (I-squared = 67.9%, \( P=0.008 \))

9.64 (4.26, 21.82)

Cl, confidence interval; OR, odds ratio
Small Bowel Culture in IBS-D

- **Patients, %**
  - >10,000 coliforms
    - Control: 4 (P<0.05)
    - IBS: 24
  - >5,000 coliforms
    - Control: 12
    - IBS: 43 (P<0.001)

SIBO and IBS-D

Patients, %

Non-D-IBS

D-IBS

27.3

n=77

60

n=35

P=0.004

Single Organism PCR in IBS-D

Duodenal Aspirates

<table>
<thead>
<tr>
<th>Bacteria quantity $\log_{10}$</th>
<th>E. coli</th>
<th>Klebsiella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>$P&lt;0.05$</td>
<td>$P&lt;0.05$</td>
</tr>
<tr>
<td>Not-IBS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pyleris, et al. Scand J Gastroenterol. 2015
What is SIBO?

### TARGET 1 and 2: Rifaximin Primary Outcomes

<table>
<thead>
<tr>
<th>Efficacy Outcome</th>
<th>Study</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA-IBS Weekly</td>
<td>TARGET 1</td>
<td>1.53 (1.10, 2.12)</td>
<td>0.0125</td>
</tr>
<tr>
<td></td>
<td>TARGET 2</td>
<td>1.45 (1.05, 2.01)</td>
<td>0.0263</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.49 (1.18, 1.88)</td>
<td>0.0008</td>
</tr>
<tr>
<td><strong>Key Secondary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBS Bloating</td>
<td>TARGET 1</td>
<td>1.62 (1.46, 1.80)</td>
<td>0.0045</td>
</tr>
<tr>
<td>Weekly</td>
<td>TARGET 2</td>
<td>1.49 (1.08, 2.06)</td>
<td>0.0167</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.56 (1.23, 1.98)</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>Other Secondary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA-IBS Daily</td>
<td>TARGET 1</td>
<td>1.76 (1.62, 1.91)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>TARGET 2</td>
<td>1.59 (1.32, 2.47)</td>
<td>0.0072</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.61 (1.28, 2.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IBS Bloating</td>
<td>TARGET 1</td>
<td>1.41 (1.01, 1.97)</td>
<td>0.0486</td>
</tr>
<tr>
<td>Daily</td>
<td>TARGET 2</td>
<td>1.76 (1.26, 2.44)</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.62 (1.21, 1.92)</td>
<td>0.0004</td>
</tr>
<tr>
<td>IBS Ab Pain</td>
<td>TARGET 1</td>
<td>1.45 (1.05, 2.02)</td>
<td>0.0255</td>
</tr>
<tr>
<td>Daily</td>
<td>TARGET 2</td>
<td>1.46 (1.05, 2.03)</td>
<td>0.0232</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.42 (1.13, 1.78)</td>
<td>0.0028</td>
</tr>
<tr>
<td><strong>FDA Proposed</strong></td>
<td>Ab Pain &amp; Stool</td>
<td>1.40 (1.02, 1.92)</td>
<td>0.0401</td>
</tr>
<tr>
<td>Daily (FDA)</td>
<td>TARGET 1</td>
<td>1.55 (1.12, 2.13)</td>
<td>0.0077</td>
</tr>
<tr>
<td></td>
<td>TARGET 2</td>
<td>1.47 (1.17, 1.84)</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ab Pain Daily</td>
<td>TARGET 1</td>
<td>1.48 (1.05, 2.03)</td>
<td>0.0157</td>
</tr>
<tr>
<td>(FDA)</td>
<td>TARGET 2</td>
<td>1.46 (1.06, 2.00)</td>
<td>0.0194</td>
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<tr>
<td></td>
<td>Combined</td>
<td>1.46 (1.17, 1.93)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Stool Consist.</td>
<td>TARGET 1</td>
<td>1.80 (1.25, 2.59)</td>
<td>0.0015</td>
</tr>
<tr>
<td>Daily (FDA)</td>
<td>TARGET 2</td>
<td>1.57 (1.12, 2.21)</td>
<td>0.0096</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.67 (1.31, 2.14)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Outcomes:**
- Ab Pain
- IBS Bloating
- Stool Consist.

**Study:**
- TARGET 1
- TARGET 2
- Combined

**Odds Ratio and 95% CI:**
- Favors Placebo
- Favors Rifaximin
## TARGET 1 and 2: Durable Response (3 months)

<table>
<thead>
<tr>
<th>Efficacy Outcome</th>
<th>Study</th>
<th>Odds Ratio (95% CI) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA-IBS Weekly</td>
<td>TARGET 1</td>
<td>1.35 (1.00, 1.82) 0.0477</td>
</tr>
<tr>
<td></td>
<td>TARGET 2</td>
<td>1.52 (1.13, 2.03) 0.0053</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.44 (1.17, 1.77) 0.0007</td>
</tr>
<tr>
<td><strong>Key Secondary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IBS Bloating</td>
<td>TARGET 1</td>
<td>1.28 (0.95, 1.73) 0.1042</td>
</tr>
<tr>
<td>Weekly</td>
<td>TARGET 2</td>
<td>1.56 (1.16, 2.09) 0.0031</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.42 (1.15, 1.75) 0.0011</td>
</tr>
<tr>
<td><strong>Other Secondary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA-IBS Daily</td>
<td>TARGET 1</td>
<td>1.60 (1.18, 2.18) 0.0025</td>
</tr>
<tr>
<td></td>
<td>TARGET 2</td>
<td>1.47 (1.09, 1.99) 0.0127</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.48 (1.20, 1.83) 0.0003</td>
</tr>
<tr>
<td>IBS Bloating</td>
<td>TARGET 1</td>
<td>1.59 (1.10, 2.04) 0.0103</td>
</tr>
<tr>
<td>Daily</td>
<td>TARGET 2</td>
<td>1.67 (1.24, 2.25) 0.0068</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.53 (1.24, 1.89) &lt;0.0001</td>
</tr>
<tr>
<td>IBS Ab Pain</td>
<td>TARGET 1</td>
<td>1.35 (1.00, 1.83) 0.0485</td>
</tr>
<tr>
<td>Daily</td>
<td>TARGET 2</td>
<td>1.35 (1.01, 1.81) 0.0435</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.31 (1.06, 1.61) 0.0118</td>
</tr>
<tr>
<td><strong>FDA Proposed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ab Pain &amp; Stool</td>
<td>TARGET 1</td>
<td>1.36 (1.01, 1.83) 0.0396</td>
</tr>
<tr>
<td>Daily (FDA)</td>
<td>TARGET 2</td>
<td>1.44 (1.08, 1.92) 0.0141</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.40 (1.14, 1.72) 0.0014</td>
</tr>
<tr>
<td>Ab Pain Daily</td>
<td>TARGET 1</td>
<td>1.31 (0.98, 1.75) 0.0725</td>
</tr>
<tr>
<td>(FDA)</td>
<td>TARGET 2</td>
<td>1.37 (1.03, 1.83) 0.0288</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.33 (1.09, 1.64) 0.0059</td>
</tr>
<tr>
<td>Stool Consist.</td>
<td>TARGET 1</td>
<td>1.70 (1.24, 2.33) 0.0009</td>
</tr>
<tr>
<td>Daily (FDA)</td>
<td>TARGET 2</td>
<td>1.48 (1.09, 2.00) 0.0114</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.58 (1.27, 1.97) &lt;0.0001</td>
</tr>
</tbody>
</table>

Types of IBS

IBS-C 35%
IBS-M 23%
IBS-D 40%

Is IBS Really Two Diseases?

- IBS-C (35%)
- IBS-M (23%)
- IBS-D (40%)

Constipation IBS  Non-Constipation IBS
Methane Is Important in C-IBS

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>OR (98% CI)</th>
<th>% Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peled</td>
<td>1987</td>
<td>0.83 (0.20, 3.56)</td>
<td>8.43</td>
</tr>
<tr>
<td>Fiedorek</td>
<td>1990</td>
<td>4.32 (1.60, 11.68)</td>
<td>12.03</td>
</tr>
<tr>
<td>Pimentel</td>
<td>2003</td>
<td>5.58 (2.22, 14.03)</td>
<td>12.68</td>
</tr>
<tr>
<td>Pimentel</td>
<td>2003</td>
<td>44.23 (2.48, 788.51)</td>
<td>3.18</td>
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<tr>
<td>Majewski</td>
<td>2007</td>
<td>1.81 (0.70, 4.67)</td>
<td>12.46</td>
</tr>
<tr>
<td>Bratten</td>
<td>2008</td>
<td>2.22 (1.14, 4.33)</td>
<td>15.14</td>
</tr>
<tr>
<td>Parodi</td>
<td>2009</td>
<td>1.89 (0.79, 4.51)</td>
<td>13.17</td>
</tr>
<tr>
<td>Hwang</td>
<td>2009</td>
<td>47.67 (8.73, 260.41)</td>
<td>6.99</td>
</tr>
<tr>
<td>Attaluri</td>
<td>2009</td>
<td>3.70 (2.06, 6.66)</td>
<td>15.92</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>3.51 (2.006.16)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

NOTE: Weights are from random effects analysis

Methane Slows Intestinal Transit

Methane and Transit

Geometric center of colonic activity

- SIBO
- M-SIBO

Transit is slower when methane is present

P < 0.01
P = 0.01
P = 0.03

Transit based on wireless capsule.
Methane: Mechanism of Action

Conclusion: Methane likely affects enteric nervous system

**M Smithii and Methane on Breath**

*M. smithii* Counts in Methane and Non-Methane Producers in Stool

![Graph showing *M. smithii* counts in Methane and Non-Methane producers in stool with statistical significance.]

$p < 0.001$

Methane Positive C-IBS: Double-Blind, Placebo-Controlled Trial

![Graph showing constipation VAS score comparison between Neomycin + placebo (n=16) and Neomycin + rifaximin (n=15). The graph indicates a statistically significant difference (P=0.01) with a VAS score of 56.3 for Neomycin + placebo and 25.9 for Neomycin + rifaximin.]

After Treatment Bloating Level By Group

Constipation VAS Score

Neomycin + placebo

Neomycin + rifaximin

P<0.01

61.9

n=16

30.1

n=15

Final Visit Constipation Severity Based on Methane > 3 ppm

Analysis of Neomycin + Rifaximin Group

- Methane > 3 ppm: n=5, Constipation VAS Score: 67.2
- Methane ≤ 3 ppm: n=10, Constipation VAS Score: 25.6

P = 0.04

Statins and Methane

- Akiro Endo (1971) – discovered mevastatin, a chemical produced by certain fungi to defend against other organisms
- Mevastatin was toxic to humans
- Lovastatin later discovered from *Aspergillus* spp.
How Lovastatin Can Help

F420 is the key enzyme in path that makes methane in *M. smithii*

Methane (CH\(_4\))

Lovastatin

Hydrogen (H\(_2\))

F420

Syntroph

*M. smithii*
Lovastatin Lactone and F420 enzyme

Subjects who successfully completed Study 1 were transferred to active drug at Day 29.
Methane Correlates with Improved Symptoms on Lovastatin

Breath Methane AUC at Week 12 (ppm*h) vs. Weekly No. Complete Spontaneous Bowel Movements (CSBMs)

p = 0.0259

### Clinical Responders by Month

<table>
<thead>
<tr>
<th>Month</th>
<th>Placebo SYN-010 42 mg</th>
<th>SYN-010 21 mg</th>
<th>SYN-10 42 mg</th>
<th>SYN-010 42 mg</th>
<th>All SYN-010 42 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0%</td>
<td>5%</td>
<td>11%</td>
<td>46%</td>
<td>46%</td>
</tr>
<tr>
<td>2</td>
<td>40%</td>
<td>42%</td>
<td>33%</td>
<td>47%</td>
<td>47%</td>
</tr>
<tr>
<td>3</td>
<td>46%</td>
<td>47%</td>
<td>33%</td>
<td>43%</td>
<td>43%</td>
</tr>
</tbody>
</table>

Dose Months 1, 2, 3

Daily use of RLax® (bisacodyl; 5 mg) in Study 1

% Subjects using RLax® (bisacodyl; 5 mg) in Study 1

Hydrogen Sulfide

• Patients completed questionnaire during 120 minute breath test collection:
  – Gastrointestinal symptoms
  – Medical/surgical history
  – Demographics
Diarrhea and Hydrogen Sulfide

Lin, et al. Sp1102 Tuesday, DDW 2017
Post-Infectious IBS

- Meta-analysis of 45 studies
- Bacterial, Protozoal and viral
- Overall:
  - 1 in 9 people who acquire food borne illness develop IBS
  - Severity of diarrhea and pain predict this response
  - Psychological factors at time of event are also predictive

In 2017 the only known cause of IBS is post-infectious IBS

IBS Pathophysiologic Sequence

Food poisoning

E. Coli
C. jejuni
Shigella
Salmonella

Bacterial toxin

Cytolethal Distending toxin (CDT B)

Auto-immunity

Anti-vinculin

Gut nerve damage

Reduced ICC
Reduced MMC

Bacterial overgrowth

Breath testing
Culture
qPCR
Deep sequencing

IBS

Antibiotics

Post-Infectious Sequelae: Rat Model

Stool = Campy-
No Acute Gastroenteritis
n=33

Stool = Campy+
Acute Gastroenteritis
n=33

Stool consistency evaluation
3 months after recovery

Bacterial Quantitation by RT-PCR of
duodenum, jejunum, ileum

Development of SIBO In A Rat Model

Rate of SIBO 3 Months
After *C. jejuni* 81-176 Infection

- **Duodenum**: 6.7%
- **Jejunum**: 17%
- **Ileum**: 21%
- **Total**: 27%

ICC Density Predicts SIBO In A Rat Model

8 C+/SIBO+

8 C+/SIBO-

8 C-

CD117 immunostain of duodenal, jejunal, ileal cross-sections (Dako Cytomation, Inc; Carpinteria, CA)

Randomized and coded

2 independent, blinded readers reported as DMP ICC per villus

DMP, deep muscular plexus; ICC, interstitial cells of Cajal;
Reduction in ICC: Lowest in SIBO

* P<0.05 vs. C-; # P<0.001 vs. C-

DMP, deep muscular plexus; ICC, interstitial cells of Cajal;
Ileal ICC in Controls

Ileal ICC in C+/SIBO-

Ileal ICC in C+/SIBO+

The Role of CdtB

- Shigella
- Salmonella
- Campylobacter
- C. difficile
- E. coli

Cytolethal Distending Toxin B
Rat Study: Role of CdtB

- Wild type Campy
- CDtB (-) Campy
- Campy + Rifaximin

Daily stool for Campy (length of colonization)
3 months
Stool evaluation
Bacterial quantitative PCR of small bowel
# CDT and Rifaximin in IBS: Rat Model

<table>
<thead>
<tr>
<th></th>
<th>Campy</th>
<th>CdtB-</th>
<th>P-value</th>
<th>Rifaximin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool % wet weight</td>
<td>60.1±6.8</td>
<td>60.8±3.6</td>
<td>0.47</td>
<td>61.1±3.8</td>
<td>0.33</td>
</tr>
<tr>
<td>Consistency</td>
<td>1.51±0.37</td>
<td>1.23±0.27</td>
<td>&lt;0.00001</td>
<td>1.15±0.30</td>
<td>&lt;0.0000001</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>8.4±6.4</td>
<td>4.2±2.4</td>
<td>&lt;0.0001</td>
<td>4.1±2.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proportion with normal bowel form all 3 days</td>
<td>17.8%</td>
<td>50.0%</td>
<td>&lt;0.01 OR=4.63</td>
<td>59.3%</td>
<td>&lt;0.00001 OR=6.7</td>
</tr>
</tbody>
</table>

Molecular Mimicry

Pre-immune Serum  Anti-CDT Antibodies

Ganglia

DMP ICC
Vinculin: The Link to IBS
Vinculin

- Focal adhesion plaques
- Actin filaments
Molecular Mimicry/Autoimmunity

Cytolethal Distending Toxin B

Human Vinculin
Molecular Mimicry/Autoimmunity

Cytolethal Distending Toxin B

Human Vinculin
Immunization Trial

Campylobacter → Recombinant CdtB → Sprague-Dawley Rats

Serum Antibody Response to CdtB

Anti-CdtB

Before Immunization

After Immunization

*Paired t-Test

P<0.000001*

Optical Density

Anti-Vinculin

Before Immunization

After Immunization

P<0.01*

Optical Density

## Anti-CdtB Implications

<table>
<thead>
<tr>
<th>Factor</th>
<th>$R_s$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenal Microbial Counts</td>
<td>0.32</td>
<td>0.01</td>
</tr>
<tr>
<td>Ileal Microbial Counts</td>
<td>0.33</td>
<td>0.01</td>
</tr>
<tr>
<td>Vinculin expression</td>
<td>-0.28</td>
<td>0.03</td>
</tr>
<tr>
<td>Stool wet weight</td>
<td>0.26</td>
<td>0.04</td>
</tr>
<tr>
<td>TNF-$\alpha$ expression</td>
<td>-0.32</td>
<td>0.01</td>
</tr>
<tr>
<td>IL-1$\beta$ expression</td>
<td>-0.66</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-8 expression</td>
<td>0.06</td>
<td>0.64</td>
</tr>
<tr>
<td>$\beta$-defensin expression</td>
<td>-0.03</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Blood Test For IBS

• Validation study of serum biomarker in IBS-D patients (N=2,375)

• Comparison groups
  – Crohn’s disease (n=73)
  – Ulcerative colitis (n=69)
  – Celiac disease (n=121)
  – Healthy subjects (n=43)

• Anti-CdtB and anti-vinculin titers significantly higher in IBS-D compared with other groups (P<0.001)

<table>
<thead>
<tr>
<th>Optical Density</th>
<th>Specificity %</th>
<th>Sensitivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CdtB (cutoff ≥2.80)</td>
<td>91.6</td>
<td>43.7</td>
</tr>
<tr>
<td>Vinculin (cutoff ≥1.68)</td>
<td>83.8</td>
<td>32.6</td>
</tr>
</tbody>
</table>

CdtB, cytolethal distending toxin.
ROC for Serum Anti-vinculin and Anti-CdtB
Diagnosis of IBS in Subjects with *IBD*

AUC 0.81 (95% CI, 0.77-0.84)

AUC 0.62 (95% CI, 0.58-0.67)

IBS/Microbiome WORKSHEET 5.0

**Constipation IBS**

- Bloom of *M. smithii*
- Excessive CH$_4$
- SLOW TRANSIT
- C-IBS

**Diarrhea and Mixed IBS**

- Acute Gastroenteritis
- Immune response
- Anti-CDT Ab
- Molecular Mimicry
- Anti-vinculin antibody
- Reduced DMP-ICC
- DYSMOTILITY
- Dysbiosis
- D-IBS

**Fuel Source**

- HYDROGEN
- H$_2$S
Conclusions

• Acute gastroenteritis is the only known proven cause of IBS
• Animal models are proving that post-infectious IBS is a microbial disease
• Rifaximin is the first FDA approved microbiome drug
• New drugs are emerging to target methane as a cause of constipation
• Hydrogen sulfide may also be important in functional GI
• Autoimmunity from acute gastroenteritis may be the trigger for these changes in the microbiome
Acknowledgements

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Vera Cruz, Mexico: J-M Remes Troche, MD

Australia: Nicholas Talley, MD
London, UK: Anthony Hobson
India: Uday Ghoshal, MD
The Gut Microbiome and \textit{Clostridium difficile}

Erik R. Dubberke, MD, MSPH
Associate Professor of Medicine
Washington University School of Medicine
Saint Louis, Missouri
C. difficile is an “Urgent Threat”

- Over 450,000 cases per year
  - Over 29,000 associated deaths
- Most common cause of healthcare-associated infections in US

C. difficile is an “Urgent Threat”

Reported Causative Pathogens
(N=506 health care-associated infections)

- C. difficile
- S. aureus
- K. pneumoniae or K. oxytoca
- E. coli
- P. aeruginosa
- Candida spp.
- Streptococcus spp.
- Coagulase-negative staphylococcus spp.
- Enterobacter spp.

Current Pathogenesis Model for CDI

Acquisition of a toxigenic strain of *C. difficile* and failure to mount an anamnestic antibody response results in CDI.

Current Pathogenesis Model for CDI

Acquisition of a toxigenic strain of *C. difficile* and failure to mount an anamnestic antibody response results in CDI.

Microbiota Disruption, Antibiotics, and *C. difficile* Exposure Timing

- **Firmicutes** and **Bacteroidetes**
  - Likely combination of metabolic pathways more important than individual organisms
  - ? Bile salt metabolism

---

## Antibiotics and CDI Risk

<table>
<thead>
<tr>
<th>Very Commonly Related</th>
<th>Less Commonly Related</th>
<th>Uncommonly Related</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin</td>
<td>β-lactam inhibitors</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Macrolides</td>
<td>Metronidazole</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Carbapenems</td>
<td>Rifampin</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Tigecycline</td>
<td>Tetracyclines</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td></td>
<td>Daptomycin</td>
</tr>
</tbody>
</table>

The Host

- Immune response to toxins
  - Severity of underlying illness
    - >70% CDI cases with recent unexpected hospitalization
  - Physiological age vs. chronological age
  - Immunosuppression

Relation Between Serum Levels of IgG Antibody Against Toxin A and *C. diff* Diarrhea (N=47 patients with colonization)

CDI Treatment and the Microbiome

Metronidazole
Oral Vancomycin
Fidaxomicin

Vancomycin superior to metronidazole on multivariable analysis, including controlling for clinical severity ($P=0.013$)

**Fidaxomicin**

- Novel macrocyclic antimicrobial
- Narrow spectrum
  - No activity against Gram negatives
  - Sparing of *Bacteroides sp.*, Bifidobacterium, clostridial clusters IV and XIV

Patients with multiple recurrences were excluded.
Impact on Microbiome: Fidaxomycin vs Vancomycin

*Bacteroides* Group Counts in Feces
Before and After 10 days of Treatment

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Group Counts (N=45)</th>
<th>Group Counts (N=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fidaxomycin</td>
<td>200 mg BID</td>
<td>Day 0: 6.5 ± 1.2</td>
<td>Day 10: 5.8 ± 0.8</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>125 mg QID</td>
<td>Day 0: 8.0 ± 1.5</td>
<td>Day 10: 4.0 ± 0.5</td>
</tr>
</tbody>
</table>

Recurrent CDI

- Recurrence risk after first episode 10% to 30%
  - Risk increases with additional recurrences
- Associated with worse outcomes
  - Readmissions (RR = 2.5; 95% CI 2.2-2.9)
  - Costs ($11,631; 95% CI $8,937-$14,588)
  - Mortality (HR 1.3; 95% CI 1.1-1.6)

Management of Recurrent CDI

Restore the microbiome

- Vancomycin taper
- Fidaxomicin taper
- Rifaximin chaser
- Fecal microbial transplantation (FMT)
- Bezlotoxumab
Not Clear What Works “Best”

- **Vancomycin taper**
  - 50% relative reduction (recurrent CDI)
- **Fidaxomicin**
  - 45% relative reduction (first/second episode)
- **FMT**
  - Single dose, open label: 80% efficacy
  - Single dose, blinded: 60% efficacy
  - Recent notable failures
- **Bezlotoxumab**
  - 40% relative reduction (recurrent CDI)

CDI Prevention Today: Two Main Approaches

1. Decrease risk of transmission
   CDI Contact precautions
   - Gloves/gowns
   - Dedicated patient equipment
   Environment decontamination

2. Decrease risk of CDI if transmission occurs
   Antimicrobial stewardship

Prevention Is Effective, But Limitations Remain

*C. difficile* is ubiquitous, exposures will always occur. Most patients on antibiotics need them.

**Prevalence of CDI From Environmental Samples**

- **Shoes**: 40% CD-positive
- **Bathroom**: 33% CD-positive
- **Surface**: 19% CD-positive
- **Dust**: 33% CD-positive

Novel Approaches to Prevention

- Vaccination
- Protection of the microbiome
Vaccination

ELISA GMC Toxin B

Microbiome Protection: Ribaxamase

- **Achieved primary endpoint** of statistically significant (P=0.045) reduction of CDI
  - 71.4% relative risk reduction in CDI rates vs placebo
- Statistically significant (p=0.0002) reduction in new VRE colonization vs placebo
- Positive trend towards decreasing the incidence of AAD from all causes
- New VRE colonization predominantly occurred at the 72 hour time point

*New colonization was defined as a negative screening sample followed by a positive sample at 72 hours or 4 weeks*
Conclusions

• CDI is prototypical example of how microbiome disruption can lead to disease
• Risk of CDI and recurrent CDI related to:
  – Microbiome (antimicrobial exposures)
  – Host (immune response)
• Metronidazole is no longer first line treatment
• Current approach to prevent recurrence is with microbiome preservation / restoration
  – Selective antibiotics
  – FMT
  – Bezloxumab
• Novel prevention approaches being studied
  – Microbiome preservation
  – Vaccination
Thank you!