Bacterial Therapy: Fecal Transplants, Probiotics and Non-Toxigenic *Clostridium difficile* for *Clostridium difficile* Infection (CDI) Management

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Disclosures

DNG:
- holds patents for the treatment and prevention of CDI using non-toxigenic *C. difficile*, licensed to ViroPharma,
- is a consultant for Sanofi Pasteur, Merck, Rebiotix, ViroPharma, Roche, Novartis, Cubist, Cangene, and Actelion.
- holds research grants from GOJO, US Dept of Veterans Affairs and CDC.

Unapproved Use: Metronidazole, rifaximin, and nitazoxanide for treatment of CDI do not have US FDA approval, but are available for other indications.
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<th>Organism</th>
<th>Antibiotic</th>
<th>Dose</th>
<th>Alternatives</th>
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<tr>
<td><em>C. difficile</em> (mild to moderate)</td>
<td>Metronidazole</td>
<td>500 mg po tid X 10-14d</td>
<td>Vancomycin 125 mg po qid X 10-14d</td>
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<tr>
<td><em>C. difficile</em> (severe)</td>
<td>Vancomycin</td>
<td>125 mg po qid X 10-14d</td>
<td>Fidaxomicin 200 mg po bid X 10d</td>
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<tr>
<td><em>C. difficile</em> (severe complicated or fulminant)</td>
<td>Vancomycin + Metronidazole</td>
<td>500 mg po qid X 10-14d 500 mg iv tid X 10-14d</td>
<td>Tigecycline 50 mg iv bid X 10-21d in place of metronidazole or vancomycin Additional vancomycin via rectal retention enema, 500 mg in 100 ml NS q 6h if complete ileus present Colectomy or Ileostomy</td>
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<td><em>C. difficile</em> (first recurrence)</td>
<td>Same as primary CDI based on severity of disease</td>
<td>125 mg po qid X 10d, then 125 mg po bid X 7d, then 125 mg po qd X 7d, then 125 mg po qod or q3d X 14-28d, then</td>
<td>Fidaxomicin 200 mg po bid X 10d Fecal Transplant</td>
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<td><em>C. difficile</em> (&gt;1 recurrence)</td>
<td>Vancomycin taper</td>
<td>125 mg po qid X 10d followed by rifaximtin 400 mg po bid X 14d, Fidaxomicin 200 mg po bid X 10d</td>
<td>Vancomycin 125 mg po qid X 10d followed by rifaximtin 400 mg po bid X 14d, Fidaxomicin 200 mg po bid X 10d</td>
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Randomized Controlled Trial of Vancomycin vs. Metronidazole vs. Tolevamer:* Should we be using more Vancomycin?

Post hoc analysis of V vs. M, Pooled data from 2 Phase 3 studies

Multivariate logistic regression analysis of factors associated with clinical success

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95%CI); P value</th>
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<tr>
<td>Vancomycin vs Metronidazole</td>
<td>1.575 (1.035,2.396); P=.0338</td>
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<td>Primary Disease vs Recurrent Disease</td>
<td>1.552 (0.972,2.477); P=.0656</td>
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<td>Treatment naïve vs Treatment experienced</td>
<td>1.814 (1.196,2.753); P=.0051</td>
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<td>Mild-Moderate CDI vs Severe CDI</td>
<td>1.600 (1.032,2.479); P=.0356</td>
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Decreased chance of clinical success    
Improved chance of clinical success

* ID Week Presentation: Johnson S, et al. Poster #818, 10/19/12
The CDI Treatment Value Proposition

- **Metronidazole** tablets 10d @ 500 mg tid = ~$4 if on the list ($11-$27 range elsewhere)
- **Vancomycin** capsules 10d @ 125 mg qid =
  - Brand Name: $1,350.00
  - Generic (Many): $1,224.00
  - Liquid Injectable vancomycin orally: ~$30.00
- **Fidaxomicin**: $2,800*  
  
*Hospital pharmacy discount 25%, Additional CMS add-on payment of $868 to the hospital
New CDI Management Strategies: Inside and Outside the Box

- Use an antimicrobial treatment that spares the normal flora (Fidaxomicin, New agents)
- Avoid antimicrobial treatment entirely using luminal (oral) toxin binders, antibodies or germination inhibitors (toxin binding polymer = tolevamer, bovine milk-derived antibodies = MucoMilk, germination inhibitor = CamSA)
- Use a biotherapeutic approach to restore the protective effect of the microbiota (probiotics, fecal transplants, nontoxigenic C. difficile)
- Supplement or increase the antibody response to C. difficile toxins (active: vaccines, passive: monoclonal antibodies)

Gerding and Johnson, Clin Infect Dis 2010;51:1306-13
Novel CDI Therapies in Human Clinical Trials

• Monoclonal antibodies directed against toxin A and toxin B
  – Phase 2 clinical trial showed significant reduction of recurrence rates
  – Phase 3 trials underway

• Toxin A/B toxoid vaccine
  – Phase 1 immunogenicity and safety completed
  – Phase 2 trial to prevent primary CDI completed

• Biotherapeutics, FMT, Probiotics
  – Nontoxigenic C. difficile – Phase II trial completed
  – FMT- randomized trial published
  – Probiotics?
Sites of Attack for Prevention and Management of CDI

1. Keep patients out of the hospital.
2. Barrier precautions and environmental cleaning.
3. Stop unnecessary antimicrobial use.
4. Restore flora or colonize with nontoxigenic *C. difficile*
5. Bolster immunity with vaccines or passive antibody strategies.
6. Antibiotic Rx
   Nonantibiotic Rx

Now
Future

Asymptomatic *C. difficile* colonization

Gerding and Johnson Clin Infect Dis. 2010;51:1306-13
Biotherapeutics:
Fecal Transplants, Probiotics, and Nontoxigenic C. difficile May Prove to be Winners in the Biological Warfare Arena
Intestinal Microbiota Transplantation (IMT) for CDI Recurrences

• 27 case series, 317 patients reviewed in literature. **Overall reported disease resolution was 92%**. IMT administered by enema, nasojejunal tube, gastroscope or colonoscope.

• Effectiveness varied by route of instillation, relationship to stool donor, volume of IMT given, and treatment before infusion.

• Interest in doing IMT is high, but standardization and safety testing of stools have not been established.

Fecal Microbiota Transplant

- Donor feces were screened for parasites (including *Blastocystis hominis* and *Dientamoeba fragilis*), *C. difficile*, and enteropathogenic bacteria. Blood was screened for antibodies to HIV; human T-cell lymphotrophic virus types 1 and 2; hepatitis A, B, and C; cytomegalovirus; Epstein–Barr virus; *Treponema pallidum*; *Strongyloides stercoralis*; and *Entamoeba histolytica*.

- Feces were collected by the donor on the day of infusion and immediately transported to the hospital. Feces were diluted with 500 ml of sterile saline (0.9%). This solution was stirred, and the supernatant strained and poured in a sterile bottle. Within 6 hours after collection of feces by the donor, the solution was infused through a nasoduodenal tube (2 to 3 minutes per 50 ml).

- FMT subjects had 1-9 (median 3) prior CDI recurrences.

Rates of Cure without Relapse for Recurrent \textit{Clostridium difficile} Infection.

A Canadian doctor has treated 27 patients suffering from Clostridium difficile infections by giving them each between 24 and 30 handmade pills containing stool from one of their healthy relatives, curing each patient of their illness. What do you think?

“I don’t need the capsule. Just give me the feces.”
Manuel White – Technical Writer

“I could see eating 20, maybe 22 feces-filled pills. But 24?... Gross.”
Dana Masterson – Systems Analyst

“Did Jerry put you up to this? Because he’s been trying to trick me into eating his shit for months.”
Lyndell Thirlwell – Drying Oven Tender
Preventive Probiotics for *C. difficile* Diarrhoea: Cochrane Syst Review

• A complete case analysis of those trials investigating CDAD (23 trials, 4213 participants) suggests that probiotics significantly reduce CDAD risk by 64%.

• The incidence of CDAD was 2.0% in the probiotic group compared to 5.5% in the placebo or no treatment control group (RR 0.36; 95% CI 0.26 to 0.51).

• Our judgment is that the overall evidence warrants only “moderate” confidence of CDAD prevention due to imprecision.

• A secondary outcome, pooled complete case results from 13 trials (961 participants) and did not show a statistically significant reduction in *C. difficile* infection. The incidence of *C. difficile* infection was 12.6% in the probiotics group compared to 12.7% in the placebo or no treatment control group (RR 0.89; 95% CI 0.64 to 1.24).

Cochrane Database Syst Rev. May 31, 2013
Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial

- Pragmatic, efficacy trial of inpatients aged 65 years and older and exposed to one or more oral or parenteral antibiotics.
- Lactobacilli and bifidobacteria, with a total of $6 \times 10^{10}$ organisms, one per day for 21 days, or placebo.
- Outcomes were occurrence of AAD within 8 weeks and *C difficile* diarrhoea (CDD) within 12 weeks.
- 1470 and 1471 patients were included in the analyses; AAD (including CDD) occurred in 159 (10.8%) participants in the microbial preparation group and 153 (10.4%) participants in the placebo group (relative risk [RR] 1.04; 95% CI 0.84–1.28; p=0.71)
- CDD was an uncommon cause of AAD and occurred in 12 (0.8%) participants in the microbial preparation group and 17 (1.2%) participants in the placebo group (RR 0.71; 95% CI 0.34–1.47; p=0.35)

Allen SJ et al Lancet Published online August 8, 2013
A Novel Biotherapeutic Approach to Prevention and Treatment of *Clostridium difficile* Infection (CDI): Non-Toxigenic *C. difficile* (NTCD)
Original Hypothesis for *C. difficile* Hospital Infection Based on the MRSA and VRE Model

- Hospitalization
- *C. difficile* Acquisition
- Asymptomatic *C. difficile* Colonization
- Antibiotic(s)
- *C. difficile* Infection (CDI)
Reduced Risk of CDI in Patients Who are Colonized with *C. difficile*

- Data from four prospective studies in which rectal swab cultures were obtained weekly from hospitalized patients.
- Rate of CDI in colonized patients was **1.0%** compared to **3.6%** in patients not colonized with *C. difficile* in the past week. (P=0.021)
- The risk was also significantly decreased when only patients who received antibiotics were analyzed (1.1% vs. 4.5%). (P=0.024)

46% of 192 colonized patients had non-toxigenic strains and 44 (49%) belonged to **REA group M** and 17 (19%) belonged to **REA group T**.
Syrian Golden Hamster
General Outline of Hamster CDI Prevention Protocols

- Pretreat hamsters with oral clindamycin, 30 mg/kg

1. Establish disease mortality from toxigenic *C. difficile* by oral spore challenge at 5d post clindamycin

2. Establish ability and durability of non-toxigenic strains to colonize hamsters by following stool cultures on a daily and then weekly basis

3. Establish the ability of non-toxigenic strains to protect against challenge by toxigenic strains by giving non-toxigenic strains at 2d post clindamycin and challenging with toxigenic *C. difficile*
Non-Toxigenic REA* Type Frequency

*Typing by restriction endonuclease analysis (REA) JCM 1993;31:1870-1895.
Toxigenic Strains

B1 - outbreak strain in Minneapolis in 1982

J9 - principal strain in outbreaks in Albany, NY; Boston, MA; Somerset, NJ; Sarasota, FL; appearance in Chicago, IL 1990s

K14 - predominant strain at VA Lakeside in Chicago, IL in 1995

BI6 – epidemic strain from current US epidemic

BI1 – historic example of current US epidemic strain from 1984
Representative Hamster Study of CDI Prevention with NTCD M3

Establish protection from toxigenic B1 challenge by pretreating with non-toxigenic C. difficile M3

Day #2 Controls

Day #5

100 spores B1

2 48h

10

12 30 mg/kg clindamycin orally

10 10^5-10^6 spores of NTCD M3

100 spores B1

Alive to 99 Days
### Prevention of Fatal *C. difficile* Infection In a Hamster Animal Model

**Number of Animals that Survived Challenge**

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<tr>
<th>Toxigenic Strains used in Challenge</th>
<th>B1</th>
<th>J9</th>
<th>K14</th>
<th>All</th>
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<tr>
<td>M3 Non-toxigenic strains</td>
<td>10/10</td>
<td>9/10</td>
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<td>29/30</td>
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<td>M23</td>
<td>8/10</td>
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<td>9/10</td>
<td>26/30</td>
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<td>T7</td>
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<td>10/10</td>
<td>8/10</td>
<td>26/30</td>
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Sambol et al J Infect Dis 2002;186:1781-9
NTCD Protection Correlates with Colonization

Sambol et al J Infect Dis 2002;186:1781-9
NTCD strains M3 and T7 hamster protection against historic toxigenic BI1(027) *Clostridium difficile*.


Nagaro et al Antimicrob Agents Chemother online August 2013
**NTCD strains M3 and T7 hamster protection against epidemic toxigenic BI6(027) Clostridium difficile.**

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<tr>
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<th>Day 2 M3/T7 inoculation 10^6 cfu spores</th>
<th>Day 5 BI6 challenge 100 cfu spores</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Days 8-20</th>
<th>Day 36 End of Study</th>
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*White ovals; uncolonized hamster. Gray ovals; toxigenic colonized hamster. Striped ovals; non-toxigenic colonized hamsters. Gray striped ovals; co-colonized hamsters. Black ovals with X; dead hamsters.*

Nagaro et al Antimicrob Agents Chemother online August 2013
Phase 1: Non-Toxigenic *Clostridium difficile* Spores: VP20621

- Excellent Volunteer Safety Profile
  - Well tolerated at all doses; no serious adverse events (AEs) reported and no discontinuations due to AEs
  - No diarrhea or change of bowel habits
  - Headache (3), fatigue (2), burning tongue (2) most frequent AEs reported
  - No *C. difficile* found in stools following single low dose administration, but *C. difficile* was detected in stool following $10^8$ cfu twice a day × 5 days
  - All subjects were colonized following 5 days of vancomycin followed by 14 days VP20621

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</tbody>
</table>

(N) = Toxin A/B negative by EIA.  
(P) = Toxin A/B positive by EIA  
### NTCD VP 20621 C. difficile Stool Cultures following Vancomycin days -6 to -1

**Cohort 3: ≥60 y.o.; placebo or 10^8 spores QD days 1-14**

<table>
<thead>
<tr>
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</table>

(N) = Toxin A/B negative by EIA.

Phase II Clinical Trial: Prevention of CDI Recurrence (Relapse or Reinfection) Following Treatment

Acquisition of a new toxigenic *C. difficile* strain or regrowth of the original strain

Treatment with metronidazole or vancomycin

Asymptomatic non-toxigenic *C. difficile* colonization

Administer Non-Toxigenic *C. difficile* following treatment for 7-14 days to prevent recurrence of CDI

Recurrent *C. difficile* Infection

*C. difficile* Diarrhea
Background

- VP20621 is an oral liquid formulation of purified spores of a non-toxigenic strain of *C. difficile* [REA type M3], originally isolated from an asymptomatic patient
  - Lacks genes for expression of *C. difficile* toxins
  - Colonizes humans, based on observed prevalence among inpatients who are asymptomatic for CDI
  - Has undergone no genetic manipulation, i.e. it is “natural” and already circulating in hospitals, and not akin to genetically modified corn or soybeans
  - This Phase 2 study was the first use of VP 20621 in patients with CDI

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Study Objectives

• Evaluate safety and tolerability of VP 20621
• Characterize stool colonization with NTCD (VP 20621)
  – Primary colonization endpoint = NTCD in stool culture at any time after the end of study drug therapy to Week 6
• Evaluate clinical recurrence of CDI, defined as:
  – ≥3 unformed stools within 24 hours
  – Positive *C. difficile* stool assay
  – No other likely cause of diarrhea per investigator
  – Occurring after Day 1 through Week 6
• Select a dose regimen for future studies

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Key Entry Criteria

Inclusion

• Age ≥18 years
• Diagnosed with a qualifying episode of CDI (with symptoms that started within 28 days prior to randomization):
  • The CDI was either a primary episode or a first recurrence
  • The CDI was treated with a single course of metronidazole or oral vancomycin (or both) for a total of 10 - 21 days
• Subject had clinically recovered from the CDI (treatment cure)

Exclusion

• CDI Treatment other than metronidazole or oral vancomycin
• Known chronic GI disorders, or GI surgery in prior 6 wks
• Planned for oral or parenteral antibacterial therapy after randomization
• ANC <1000/mm³, immunodeficiency disorder, or use of myelosuppressive cancer chemotherapy
• Outpatient household contacts age <2 years (or with immunodeficiency)
• Anticipated need for mechanical ventilation or vasopressors for hemodynamic support during study
Study Design

Screening (Up to 28 days)

- CDI Onset: Vanco or Metro 10-21 days; CDI Resolves

Study Drug Administration (14 Days)

- Day 1 [1st dose], Wks 1, 2, 3, 6 [and for any events of diarrhea]
- Randomize to start study drug 1-2 days after end of Vanco/Metro
- Monthly until culture negative
- Stool C. difficile culture

Follow-Up

- Key efficacy assessments
- F/U to Week 26

Villano S et al IDWeek, San Francisco, CA Oct 4, 2013 Abstract #LB-7
<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Placebo</th>
<th>$10^4 \times 7$d</th>
<th>$10^7 \times 7$d</th>
<th>$10^7 \times 14$d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N Randomized and Treated = 168</td>
<td>n 43 41 43 41</td>
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<tr>
<td>Age (yrs)</td>
<td></td>
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<tr>
<td>Median (range)</td>
<td>58 (18-90)</td>
<td>58 (22-89)</td>
<td>58 (21-94)</td>
<td>64 (20-90)</td>
</tr>
<tr>
<td>≥65</td>
<td>33%</td>
<td>41%</td>
<td>37%</td>
<td>44%</td>
</tr>
<tr>
<td>Female</td>
<td>61%</td>
<td>63%</td>
<td>56%</td>
<td>68%</td>
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<tr>
<td>Race</td>
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</tr>
<tr>
<td>White</td>
<td>93%</td>
<td>98%</td>
<td>88%</td>
<td>90%</td>
</tr>
<tr>
<td>Black</td>
<td>7%</td>
<td>2%</td>
<td>9%</td>
<td>5%</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td>2%</td>
<td>5%</td>
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</tbody>
</table>

Villano S et al IDWeek, San Francisco, CA Oct 4, 2013 Abstract #LB-7
## Qualifying CDI Episode

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<tr>
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<th>$10^4 \times 7d$</th>
<th>$10^7 \times 7d$</th>
<th>$10^7 \times 14d$</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>43</td>
<td>41</td>
<td>43</td>
<td>41</td>
</tr>
<tr>
<td>Primary episode</td>
<td>81%</td>
<td>83%</td>
<td>88%</td>
<td>78%</td>
</tr>
<tr>
<td>First recurrence</td>
<td>19%</td>
<td>17%</td>
<td>12%</td>
<td>22%</td>
</tr>
<tr>
<td>Location at onset</td>
<td></td>
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<tr>
<td>Inpatient</td>
<td>28%</td>
<td>15%</td>
<td>30%</td>
<td>22%</td>
</tr>
<tr>
<td>Outpatient</td>
<td>72%</td>
<td>85%</td>
<td>70%</td>
<td>78%</td>
</tr>
<tr>
<td>CDI treatment</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Metronidazole only</td>
<td>60%</td>
<td>73%</td>
<td>53%</td>
<td>54%</td>
</tr>
<tr>
<td>Vancomycin only</td>
<td>14%</td>
<td>15%</td>
<td>21%</td>
<td>32%</td>
</tr>
<tr>
<td>Metro + Vanco</td>
<td>26%</td>
<td>12%</td>
<td>26%</td>
<td>15%</td>
</tr>
</tbody>
</table>

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**C. difficile Stool Culture (Central Lab)**

**Placebo**
- (n=39-43 subjects per visit)
- D1: 14% Cx+ Toxin+
- Wk1: 52%
- Wk2: 63%
- Wk3: 55%
- Wk6: 33%

**NTCD 10⁴ x 7d**
- (n=38-41 subjects per visit)
- D1: 2%
- Wk1: 29%
- Wk2: 54%
- Wk3: 47%
- Wk6: 36%

**NTCD 10⁷ x 7d**
- (n=39-43 subjects per visit)
- D1: 21%
- Wk1: 81%
- Wk2: 63%
- Wk3: 64%
- Wk6: 49%

**NTCD 10⁷ x 14d**
- (n=37-41 subjects per visit)
- D1: 22%
- Wk1: 67%
- Wk2: 70%
- Wk3: 61%
- Wk6: 27%
<table>
<thead>
<tr>
<th>Subjects with:</th>
<th>Placebo 10^4 x 7d</th>
<th>10^7 x 7d</th>
<th>10^7 x 14d</th>
<th>All NTCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTCD in stool at any time during the 14-day study drug administration</td>
<td>0 (0%)</td>
<td>22 (54%)</td>
<td>34 (79%)</td>
<td>30 (73%)</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NTCD in stool at any time after Day 14 (end of study drug) through Week 6</td>
<td>4 (9%)</td>
<td>26 (63%)</td>
<td>31 (72%)</td>
<td>29 (71%)</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Villano S et al IDWeek, San Francisco, CA Oct 4, 2013 Abstract #LB-7
## Clinical Endpoints (through Week 6)

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<tr>
<th></th>
<th>Placebo $10^4 \times 7$d</th>
<th>$10^7 \times 7$d</th>
<th>$10^7 \times 14$d</th>
<th>All NTCD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>43</td>
<td>41</td>
<td>43</td>
<td>41</td>
</tr>
<tr>
<td><strong>CDI Recurrence</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 (30%)</td>
<td>6 (15%)</td>
<td>2 (5%)</td>
<td>6 (15%)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.11</td>
<td>0.01</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Antibacterial Use for CDI</td>
<td>14 (33%)</td>
<td>6 (15%)</td>
<td>4 (9%)</td>
<td>7 (17%)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.07</td>
<td>0.02</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>Any Event of Diarrhea (of any</td>
<td>33 (77%)</td>
<td>23 (56%)</td>
<td>25 (58%)</td>
<td>23 (56%)</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.05</td>
<td>0.09</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*p values adjusted for pre-specified parameters: use of metronidazole, use of vancomycin, and primary episode vs. first recurrence*

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Time to CDI Recurrence

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# CDI Recurrence based on Colonization

|                       | Placebo | $10^4 \times 7$ | $10^7 \times 7$ | $10^7 \times 14$ | All
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</thead>
<tbody>
<tr>
<td><strong>CDI Recurrence:</strong></td>
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</tr>
<tr>
<td>Overall</td>
<td>13/43</td>
<td>6/41</td>
<td>2/43</td>
<td>6/41</td>
<td>14/125</td>
</tr>
<tr>
<td>Percent</td>
<td>30%</td>
<td>15%</td>
<td>5%</td>
<td>15%</td>
<td>11%</td>
</tr>
<tr>
<td><strong>Subjects Colonized with NTCD</strong></td>
<td>0/4</td>
<td>1/26</td>
<td>1/31</td>
<td>0/29</td>
<td>2/86</td>
</tr>
<tr>
<td>Percent</td>
<td>0%</td>
<td>4%</td>
<td>3%</td>
<td>0%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Subjects Not Colonized with NTCD</strong></td>
<td>13/39</td>
<td>5/15</td>
<td>1/12</td>
<td>6/12</td>
<td>12/39</td>
</tr>
<tr>
<td>Percent</td>
<td>33%</td>
<td>33%</td>
<td>8%</td>
<td>50%</td>
<td>31%</td>
</tr>
</tbody>
</table>

Colonization = NTCD in stool culture at any time after the end of study drug therapy to Week 6.
## Safety Summary through Week 6

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<tr>
<th></th>
<th>Placebo 10^4 x 7d</th>
<th>10^7 x 7d</th>
<th>10^7 x 14d</th>
<th>All NTCD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n 43</td>
<td>41</td>
<td>43</td>
<td>41</td>
</tr>
<tr>
<td><strong>Any AE</strong></td>
<td>88%</td>
<td>85%</td>
<td>91%</td>
<td>83%</td>
</tr>
<tr>
<td>Diarrhea b</td>
<td>65%</td>
<td>49%</td>
<td>54%</td>
<td>46%</td>
</tr>
<tr>
<td>Flatulence</td>
<td>16%</td>
<td>22%</td>
<td>23%</td>
<td>15%</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>37%</td>
<td>15%</td>
<td>21%</td>
<td>22%</td>
</tr>
<tr>
<td>Headache</td>
<td>5%</td>
<td>15%</td>
<td>9%</td>
<td>7%</td>
</tr>
<tr>
<td>Nausea</td>
<td>9%</td>
<td>12%</td>
<td>9%</td>
<td>5%</td>
</tr>
<tr>
<td>Abd. pain upper</td>
<td>2%</td>
<td>12%</td>
<td>5%</td>
<td>7%</td>
</tr>
<tr>
<td><strong>Any Serious AE</strong></td>
<td>7%</td>
<td>5%</td>
<td>7%</td>
<td>7%</td>
</tr>
<tr>
<td>Any AE causing Study Drug D/C</td>
<td>7%</td>
<td>2%</td>
<td>2%</td>
<td>5%</td>
</tr>
</tbody>
</table>

*a* Shown are events occurring in >6% of all NTCD; excludes reports of “CDI”, “CDI recurrence”, etc. since protocol-defined CDI is summarized separately

*b* Events that are coded to Diarrhea include “loose stool”, “watery stool”, “soft stools”, etc., in addition to any type of “diarrhea”.
Duration of Colonization

After Week 6, Stool Collected if \( C. \text{ difficile} \) Cx+ at Prior Visit

![Bar charts showing colonization rates over weeks for different treatment groups.]

- **Placebo**
  - (n=39 subjects at Wk 6)
  - Wk 6: 33%
  - Wk 10: 23%
  - Wk 14: 13%
  - Wk 18: 8%
  - Wk 22: 10%
  - Wk 26: 3%

- **NTCD 10^4 x 7d**
  - (n=39 subjects at Wk 6)
  - Wk 6: 36%
  - Wk 10: 10%
  - Wk 14: 8%
  - Wk 18: 3%
  - Wk 22: 3%
  - Wk 26: 3%

- **NTCD 10^7 x 7d**
  - (n=41 subjects at Wk 6)
  - Wk 6: 49%
  - Wk 10: 32%
  - Wk 14: 5%
  - Wk 18: 17%
  - Wk 22: 10%
  - Wk 26: 5%

- **NTCD 10^7 x 14d**
  - (n=37 subjects at Wk 6)
  - Wk 6: 19%
  - Wk 10: 14%
  - Wk 14: 5%
  - Wk 18: 5%
  - Wk 22: 5%
  - Wk 26: 5%
Conclusions

VP 20621 represents a novel non-antibiotic biotherapeutic agent that:

- can be administered orally once daily, starting immediately after completing antibiotic treatment; **one week of dosing appears to be sufficient**
- colonizes the GI tract during the vulnerable post-antibiotic treatment period; **higher dose of 10^7 spores is more effective than lower dose.**
- reduces CDI recurrence and associated need for further CDI antibiotic treatment by ≥50%
- has a very good safety profile – non-absorbed with no drug interactions and no need to alter dose for renal or hepatic impairment
Thank You to the Following

- The study subjects
- All 44 study sites that enrolled subjects in:
  - US
  - Canada
  - Spain
  - Germany
  - Belgium
  - Switzerland
- ViroPharma NTCD Project Team
- Barc USA Inc: Central laboratory for all *C. difficile* stool cultures and toxin assays

Villano S et al IDWeek, San Francisco, CA Oct 4, 2013 Abstract #LB-7
Collaborator Acknowledgement

Stuart Johnson

Susan Sambol

Adam Cheknis
Farida Siddiqui
Lorinda Wright

Eric Perdue
Lacey Petrella