Ridinilazole for *Clostridium difficile* Infection: Human Gut Microbiome Profiles from a Phase 2 Clinical Trial

ID Week; Abstract 1774
October 2018
Ridinilazole for *Clostridium difficile* Infection (CDI)

Human Gut Microbiome Profiles from a Phase 2 Clinical Trial

Recurrence of CDI (rCDI) remains a central unmet medical need in the management of CDI. Each episode associated with:
- Increased morbidity and mortality
- Increased healthcare cost
- Limited treatment options

Recurrent CDI associated with increased perturbation of the gut microbiota
- Mainstay CDI therapy (metronidazole and vancomycin) associated with increased dysbiosis, potentially increasing the risk of rCDI

Ridinilazole is a novel, GI restricted, *C. difficile* targeting antibacterial. Designed to preserve the microbiome.

Being developed to treat CDI and reduce the recurrence of CDI

Risk of Disease Recurrence (%)

1st Infection Risk: ~25%
2nd Infection Risk: ~45%
3rd Infection Risk: ~65%

*(1) Kelly, Clin Micro Infect, 2012*
In vitro Susceptibility Profile

Potent growth inhibition of *C. difficile*; targeted spectrum of activity

*C. difficile* MIC range (≈600 clinical isolates) = 0.015 – 0.5µg/mL; MIC<sub>90</sub> = 0.125µg/mL

- No differences in MICs between ribotypes
- No increase in MIC against isolates with reduced vancomycin or metronidazole susceptibility
- No cross resistance to other classes of antibiotics

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Ridinilazole (1)</th>
<th>Fidaxomicin (1)</th>
<th>Vancomycin (1)</th>
<th>Surotomycin (2)</th>
<th>Metronidazole (1)</th>
<th>Cadazolid (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bifidobacterium</em> spp.</td>
<td>&gt;512</td>
<td>0.125</td>
<td>1</td>
<td>2</td>
<td>&gt;512</td>
<td>128</td>
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<tr>
<td><em>Lactobacillus</em> spp.</td>
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<td>&gt;512</td>
<td>&gt;512</td>
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<td>&gt;512</td>
<td>128</td>
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<tr>
<td><em>Eggerthella lenta</em></td>
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<td>8</td>
<td>0.5</td>
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<td>0.5</td>
<td>1</td>
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<td>0.5</td>
<td>&gt;512</td>
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<td>128</td>
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<td><em>Streptococcus</em> spp.</td>
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<td>128</td>
<td>1</td>
<td>-</td>
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<td>512</td>
<td>&gt;512</td>
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(1) Goldstein et al: AAC 2013
(2) Snydman et al. AAC 2012; Citron et al. AAC 2016
(3) Tyrrell et al. Anaerobe, 2016, # PIII-16
Phase 2 Proof of Concept Study

Study design and efficacy outcomes

Double blind, randomised, active controlled clinical trial
- 100 CDI patients recruited across 34 North American sites
- Subjects randomised (1:1) to 10 days treatment
- Ridinilazole 200mg BID; vancomycin 125mg QID
- 30 day follow-up to monitor for rCDI

Primary endpoint: Sustained Clinical Response (SCR)
- Defined as clinical cure at the end of therapy (EOT) and no recurrence to 30 days after treatment

Key secondary endpoint: Clinical response at TOC*

Exploratory analysis: Detailed microbiome assessment
- During course of therapy and in the post dosing follow up period

*TOC = test of cure visit; 2 days post EOT
Microbiome Analysis

Study design and controlling for confounding factors

Faecal samples collected from all subjects at baseline, day 5, EOT, days 25 and 40, and at recurrence
  • Relative and quantitative impact on the gut microbiota assessed through qPCR and sequencing of the V4 region of 16S rDNA
  • Analyses performed by assessing each individual over the course of the study
  • Data from 50 healthy, age and gender similar subjects included as control (separate study)
A primary analysis population established to account for potentially confounding factors

Randomised Population
N = 100 (50 RDZ: 50 VAN)

qPCR and Microbiota Analysis
N = 82 (41 RDZ: 41 VAN)

Primary Analysis
N = 44 (22 RDZ: 22 VAN)

• Subjects excluded due to < 3 samples provided
• Subjects excluded due to prior SoC CDI therapy or concomitant antibiotic therapy at randomisation
• Any sample post concomitant antibiotic therapy or at rCDI excluded
Microbiome Analysis

Even limited exposure to CDI standard of care potentially results in perturbation of the microbiome

Subjects could be enrolled into the study with up to 24 hours of CDI therapy prior to randomisation

- 22 of 82 subjects received either vancomycin and/or metronidazole
- Quantitative and relative abundance comparisons of those who did/did not receive prior SoC demonstrated significant differences
  - Other factors, such as in-patient status or underlying co-morbidities, may also have contributed

Quantitative (qPCR) assessment of baseline samples in subjects who did, or did not, receive prior CDI treatment

Cladogram showing taxa at baseline with significantly higher relative abundance when comparing participants who did (green) or did not (red) receive prior CDI treatment.
Quantitative Changes in Key Bacterial Groups

qPCR analysis demonstrated significant reductions following completion of vancomycin treatment.

- **Bacteroides**
- **C. leptum**
- **C. coccoides**
- **Prevotella**
- **Enterobacteriaceae**
- **Eubacteria**

**Data**
- Baseline (BL)
- End of therapy (EOT)
- Vancomycin
- Ridinilazole

**Notation**
- NS: Not significant
- **: P < 0.05
- ***: P < 0.01
- ****: P < 0.0001

**Changes**
- Δ = -2.94
- Δ = -3.42
- Δ = -2.83
- Δ = -1.97
- Δ = 0.51
- Δ = -0.53

**Notes**
- Data excluded prior CDI SOC or concomitant antibiotic use.
Alpha and Beta Diversity Changes Baseline to End of Therapy

Minimal impact with ridinilazole; significant loss of diversity with vancomycin
Relative Changes in Taxonomic Composition

Significantly greater shifts in relative abundance following vancomycin treatment

Red – higher abundance at baseline
Green – higher abundance at EOT
Changes in Taxonomic Composition

Impact on the Bacteroidetes and Firmicutes

Vancomycin resulted in significant decreases in members of the Firmicutes and Bacteroidetes

- Firmicute families including Ruminococcaceae, and Lachnospiraceae often reduced to below the limit of detection (2-3 log reduction)
- Bacteroidetes decreased by over 3 logs, to <0.05% relative abundance

Ridinilazole’s impact restricted to modest decreases in selected Firmicutes families

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Fold change</th>
<th>Taxa</th>
<th>Fold Change BL to EOT</th>
<th>Vancomycin</th>
<th>Ridinilazole</th>
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<tbody>
<tr>
<td>Actinobacteria</td>
<td></td>
<td>Bacteroidetes</td>
<td>1773.5</td>
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<td></td>
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<td>Bacteroides</td>
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<td>Porphyromonadaceae</td>
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<td>Lactobacillaceae</td>
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<td>Clostridaceae</td>
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<td>Lachnospiraceae</td>
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<td>Erysipelotrichi</td>
<td>46.6</td>
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</table>
Vancomycin treatment resulted in significant expansion of the Proteobacteria

- Primarily observed in the Enterobacteriaceae with ≈220 fold increase by EOT
- Taxa including *Citrobacter*, *Enterobacter* and *Serratia* increased 10 fold from below detection at baseline
- *Klebsiella spp.* abundance expanding by over 3 logs.

<table>
<thead>
<tr>
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<th>VAN</th>
<th>RDZ</th>
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<tr>
<td>Actinobacteria</td>
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<tr>
<td>Bacteroidetes</td>
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<td>Firmicutes</td>
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<td>Proteobacteria</td>
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<table>
<thead>
<tr>
<th>Taxa</th>
<th>Fold Change BL to EOT</th>
<th>Vancomycin</th>
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<tr>
<td>Proteobacteria</td>
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<tr>
<td>Gammaproteobacteria</td>
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<td>Enterobacteriaceae</td>
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<td>Citrobacter</td>
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<td>Serratia</td>
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<td>Haemophilus sp.</td>
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<td></td>
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<td>4.5</td>
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</table>

- Increase
- Decrease

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Impact on the Proteobacteria
Microbiota Composition Associated with rCDI
Profile of rCDI subjects associated with enhanced dysbiosis of the microbiota

The limited numbers of rCDI cases entering the primary analysis prevented a between treatments comparison. However, considering all cases of rCDI in the study, recurrence associated with a state of increased dysbiosis at end of therapy:
- Reduced diversity and changes in taxa (↓Firmicutes; ↑Proteobacteria)
Conclusions

Ridinilazole preserved the microbiota of CDI patients

*In vitro*, ridinilazole showed potent growth inhibition of *C. difficile* and a targeted spectrum of activity
- Hypothesis was minimal collateral damage to the gut microbiota of CDI patients would translate into reduced rates of rCDI
- Efficacy data from Phase 2 showed superior Sustained Clinical Response rates with ridinilazole compared to vancomycin
- Improved Sustained Clinical Response driven by marked reduction in rates of rCDI

Microbiota analysis of the trial subjects revealed significant differences between ridinilazole and vancomycin treatment
- Vancomycin induced microbiota wide changes with significant reductions in key phyla and concomitant expansion of Proteobacteria
- Ridinilazole’s impact was modest and restricted to selected families from the Firmicutes
- Reductions in diversity were significantly greater after 10 days therapy with vancomycin

Profile of rCDI subjects associated with reduced diversity, reduced abundance of Firmicutes and increased abundance of Proteobacteria

Ridinilazole treatment did not induce significant gut microbiota perturbation which likely contributed to improved Sustained Clinical Response rates when compared with vancomycin
- Further microbiome and metabolome studies will be undertaken in Phase 3
Acknowledgments

Microbiome Analysis

Tufts Medical Center

Professor David Snydman
Dr Cheleste Thorpe
Dr Anne Kane
Dr Justin Chang
Dr Albert Tai

In vitro Susceptibility

R. M. Alden Research Laboratory

Professor Ellie Goldstein
Dr Diane Citron

Clinical Trial

CoDIFy

Investigators and participants
clinicaltrials.gov: NCT02092935

Funding

Translation Award: 099444

Enhanced preservation of the human intestinal microbiota by ridinilazole, a novel Clostridium difficile-targeting antibacterial, compared to vancomycin

Comparative In Vitro Activities of SMIT9609, a New Antimicrobial Agent, against Clostridium difficile and 350 Gram-Positive and Gram-Negative Anaerobic and Aerobic Intestinal Bacteria

Comparative In Vitro Activities of SMIT9609, a New Antimicrobial Agent, against 162 Strains From 35 Less Frequently Recovered Intestinal Clostridium Species: Implications for Clostridium difficile Baculoneness

Efficacy and safety of ridinilazole compared with vancomycin for the treatment of Clostridium difficile infection: a phase 2, randomised, double-blind, active-controlled, non-inferiority study