Poster # 2567

Effect of broad vs. narrow spectrum *Clostridioides difficile* treatment on human stool bile acid composition over time

X. Qian[1,2], K. Yanagi[3], A. V. Kane[4], N. Alden[3], D. R. Snydman[4,5], K. Lee[3,*,#], C. M. Thorpe[4,5,*,#]

[1]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA, USA; [2]The Tufts Clinical and Translational Science Institute, Tufts University, Boston, MA, USA; [3]Department of Chemical & Biological Engineering, Tufts University, Medford, MA, USA; [4]Tufts Medical Center, Boston, MA, USA; and [5]Tufts University School of Medicine, Boston, MA, USA.

#These authors contributed equally to this work; presenting author email: cthorpe@tuftsmedicalcenter.org

BACKGROUND AND AIMS

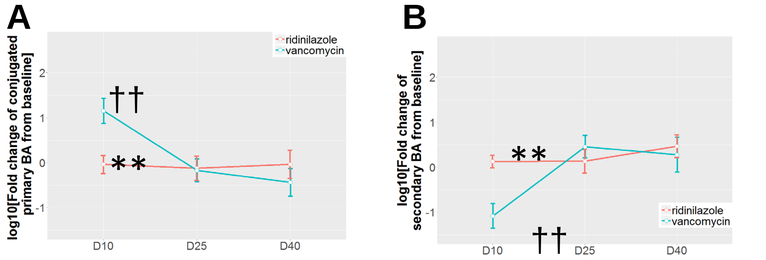
Secondary bile acid production by a diverse commensal flora may be a critical factor in preventing recurrence of *Clostridioides difficile* infection (CDI). Key enzymes involved are bacterial-encoded bile salt hydrolases (BSHs), felt to be "gatekeepers" to secondary bile acid synthesis. Ridinilazole, a novel narrow spectrum drug for CDI, demonstrated superior sustained clinical response compared to vancomycin in Phase 2. Longitudinal sampling during this trial allowed for assessment of metabolites differentially present in stools during/after therapy with either broad or narrow spectrum anti-CDI agent. Previous work characterizing subject's fecal microbiota in this trial showed that unlike vancomycin, ridinilazole has little effect on commensal flora during and after therapy. We hypothesized that ridinilazole's microbiota-preserving effect is associated with lack of accumulation of conjugated primary bile acids and/or reaccumulation/persistence of secondary bile acids over the course of CDI treatment, when compared with vancomycin-treated subjects. Furthermore, we hypothesized that we would observe correlations between treatment arms and predicted BSH gene abundances.

METHODS

Sequential stool samples were obtained from 44 subjects treated with either ridinilazole or vancomycin (22 in each arm), ranging from time of CDI diagnosis, at end-of-therapy, and up to 40 days after diagnosis. Bile acids were quantitated by liquid chromatography-mass spectrometry. Using the PICRUSt algorithm, metagenomic predictions of BSH gene abundances were performed using previously determined microbiota profiles. Control stool samples (n=31) were obtained from age-, gender- and location-similar volunteers enrolled in a separate study, and extracted similarly as the samples from CDI subjects in this study.

RESULTS

At end-of-treatment, vancomycin but not ridinilazole results in increased conjugated primary bile salts, and decreased secondary bile acids



*For both treatment groups, the log fold change in the indicated bile acid groups from baseline to subsequent time points was calculated for each individual subject , then averaged. Data shown are mean +/- SEM. Panel A: conjugated primary bile acids Panel B: secondary bile acids Asterisks indicate degree of significance of difference between treatment arms by Wilcoxon rank sum test. **=P<0.001. Daggers indicate significant difference from the baseline within treatment arms by Wilcoxon signed rank test. ††=P<0.001*

DISCLOSURES

This research was funded by Summit Therapeutics Plc. CMT, DRS, and AVK have previously received research funding from Summit Therapeutics. CMT has been on an Advisory Board for Summit Therapeutics. CMT and AVK have received travel grants from Summit Therapeutics.

Changes in stool bile acid composition over time following treatment with vancomycin or ridinilazole, and in healthy subjects.

RESULTS

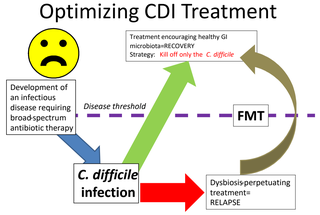
- Stool bile acid composition differed between ridinilazole-treated and vancomycin-treated subjects at end-of-treatment
- In vancomycin-treated subjects, stool composition became dominated by conjugated primary bile acids, with decreased levels of secondary bile acids compared with baseline.
- Bile acids and ratios predicted treatment arm in a random forest model.
- Metagenomic prediction of BSH's was higher in ridinilazole-treated subjects

CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions: In contrast to vancomycin, ridinilazole treatment preserves bile acid composition over the course of therapy. Because bile acid homeostasis affects *C difficile* germination and growth, this effect may have contributed to ridinilazole's higher rate of sustained clinical response in the Phase 2 trial.

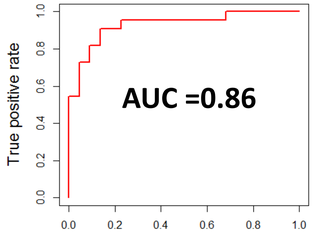
Future work:

- Explore correlations between specific taxa and bile acid composition.
- Assess bile acid features at end-of-treatment. predictive of recurrence (e.g., secondary, conj:sec)
- Assess other metabolites that may be differentially present between times/treatments.

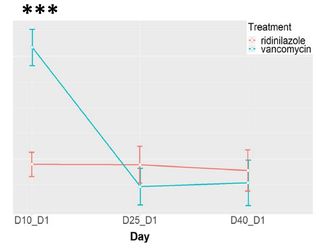


RESULTS

Random forest modeling based on bile acids and bile acid ratios at end-of-treatment accurately predicts treatment arm

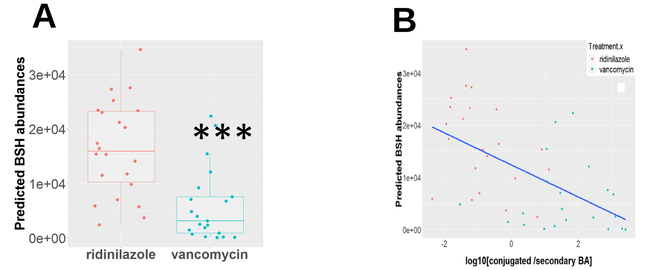


		Predicted		
		Rid	Van	Classification error rate
Actual	Rid	18	4	0.18
	Van	2	20	0.09



*Trajectory of the fold change of the ratio of conjugated to secondary bile acids, the most important predictor identified by the random forest model. ***P < 0.001 by Wilcoxon rank-sum test (van vs rid ΔD10-D1).*

At end-of-treatment, predicted bile salt hydrolase (BSH) gene abundances are higher in ridinilazole-treated subjects and correlate with the ratio of conjugated to secondary bile acids



*Panel A: BSH gene abundance predicted by PICRUSt at end-of-therapy. ***=P<0.0001 by Wilcoxon rank sum test. Median with interquartile range shown. Panel B: Relationship between log10(conjugated/secondary BA) and predicted BSH abundance; Pearson correlation coefficient (PCC= -0.57) is significant (p = <0.0001).*