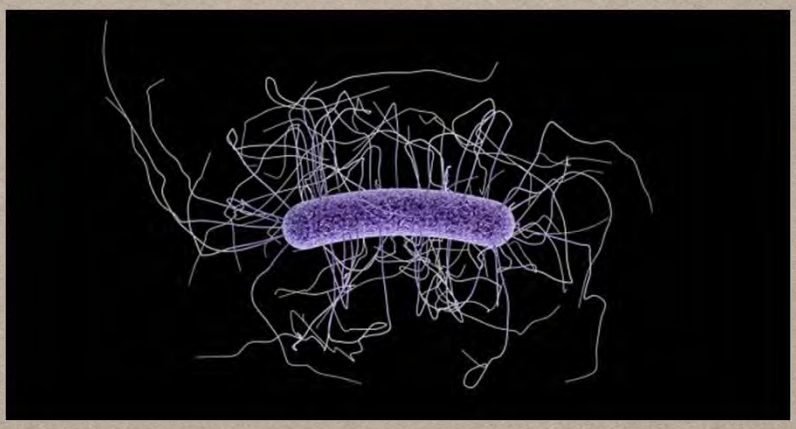

THE GUT MICROBIOME AND NON-TRADITIONAL THERAPIES FOR CLOSTRIDIOIDES DIFFICILE INFECTION

STATE OF THE FIELD AND A PATHWAY FORWARD

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1

DISCLOSURES

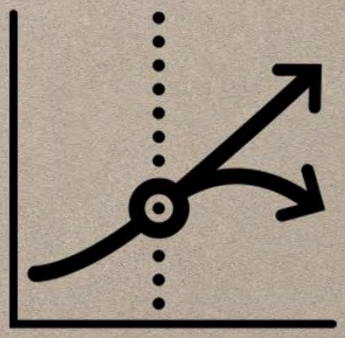
INVESTIGATOR-INITIATED GRANT:
- MERCK & CO, INC.

CONSULTING:
- SERES THERAPEUTICS, INC.
- REBIOTIX, INC.
- SUMMIT THERAPEUTICS, INC.

2

AN INFLECTION POINT IN CDI CARE?

EPIDEMIOLOGY
DIAGNOSIS
MONOCLONALS
LIVE BIOTHERAPEUTICS
FECAL TRANSPLANT



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AGENDA

- **Current state of CDI treatment including fecal microbiota transplantation**
- **Probiotics for CDI: back to the drawing board**
- **Conclusions and future directions**

4

CDI TREATMENT: CURRENT STATE OF THE FIELD

5

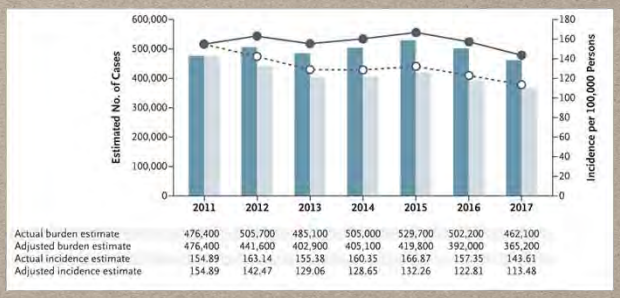
C. difficile INFECTION

- The organism: Gram-positive, anaerobic, spore-forming bacillus
- The syndrome: asymptomatic colonization -> acute self-limited colitis -> fulminant and sometimes fatal toxic megacolon

6

CDI = *Clostridioides difficile* / *Clostridium difficile* / *C. difficile* Infection

- A major healthcare-associated infection causing hospitalization, disability, and death
- Recurrence common even after initial cure: at least 1 in 6 will recur
- Costs over $1.5 billion annually to US healthcare system[1]
- Progress on prevention has stalled: Still have 462,000 cases/year in US alone[2]



	2011	2012	2013	2014	2015	2016	2017
Actual burden estimate	476,400	505,700	485,100	505,000	529,700	502,200	462,100
Adjusted burden estimate	476,400	441,600	402,900	405,100	419,800	392,000	365,200
Actual incidence estimate	154.89	163.14	155.38	160.35	166.87	157.35	143.61
Adjusted incidence estimate	154.89	142.47	129.06	128.65	132.26	122.81	113.48

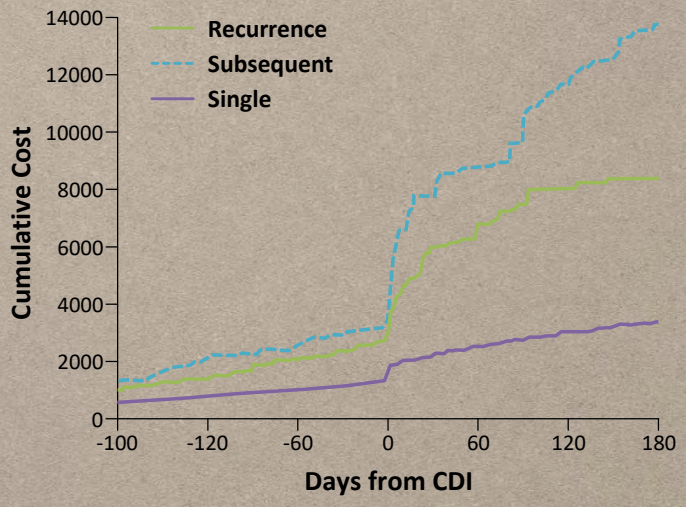
[1]Zimlichman E et al. Health care-associated infections: a meta-analysis of costs and financial impact on the US health care system. JAMA Intern Med. 2013;173(22):2039-46.

[2]Guh AY et al. Trends in U.S. Burden of Clostridioides difficile Infection and Outcomes. N Engl J Med. 2020;382(14):1320-30.

7

RECURRENT CDI: COSTS

- Each recurrent CDI patient:
  - Average 4.4 stool tests for CD
  - 2.5 prescriptions for vancomycin
- 84% required hospitalization
- 6% required urgent colectomy
- Average cost per patient
  - $34,104
- 83,000 cases of recurrent CDI in the US per year
  - $5 billion annual costs



Rodrigues R, et al. Infect Control Hosp Epi 2017;38:196
Singh H, et al. PLoS One 2019;14:e0224609
Zhang D, et al. Clin Infect Dis 2018;66:326

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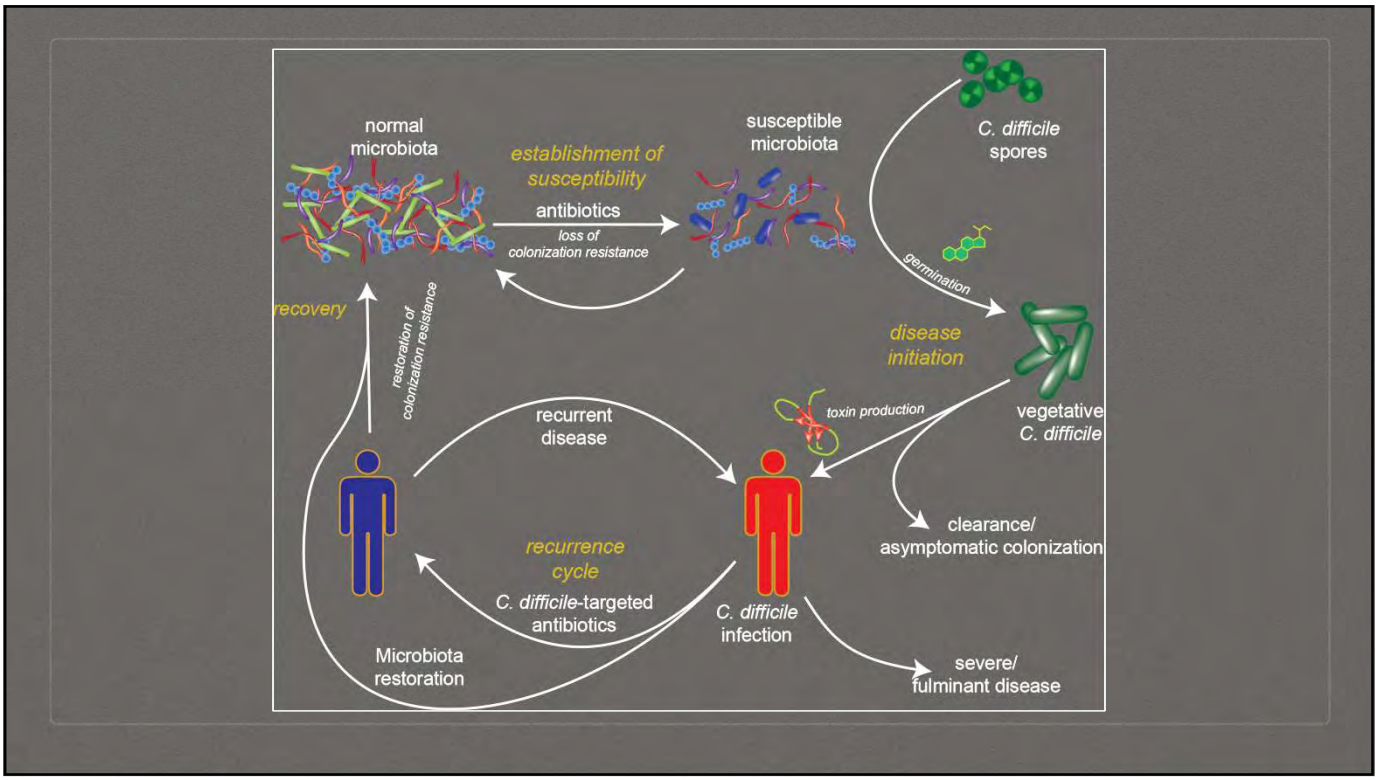

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TREATMENT OF INITIAL C. DIFFICILE INFECTION

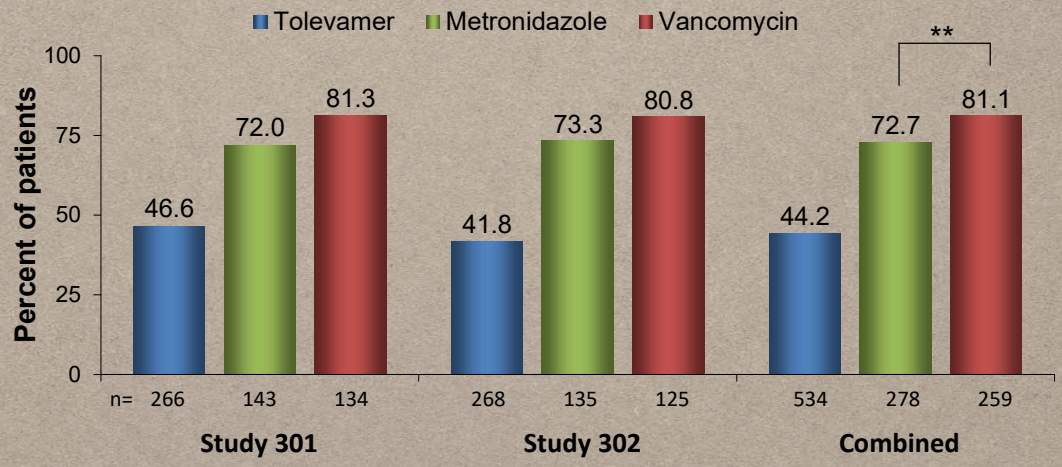
Infectious Disease Society of America – Society for Healthcare Epidemiology of America (IDSA-SHEA) 2017 guideline update

- **Vancomycin or fidaxomicin**
  - Vancomycin 125 mg orally 4 times a day x 10 days
  - **Fidaxomicin 200 mg orally 2 times a day x 10 days**
  - Continue to 14 days if still symptomatic at 10 days
- Metronidazole 500 mg orally 3 times a day x 10 days
  - Only with non-severe CDI when vancomycin or fidaxomicin are not available
  - Avoid repeat or prolonged exposure due to possible neurotoxicity

McDonald LC, et al. Clin Infect Dis. 2018;66:e1

10

TOLEVAMER VS. METRONIDAZOLE VS. VANCOMYCIN



*p<0.001, tolevamer (T) vs metronidazole (M) and T vs vancomycin (V)
**p=0.020, M vs V

Johnson S, et al. *Clin Infect Dis*. 2014;59:345-54.

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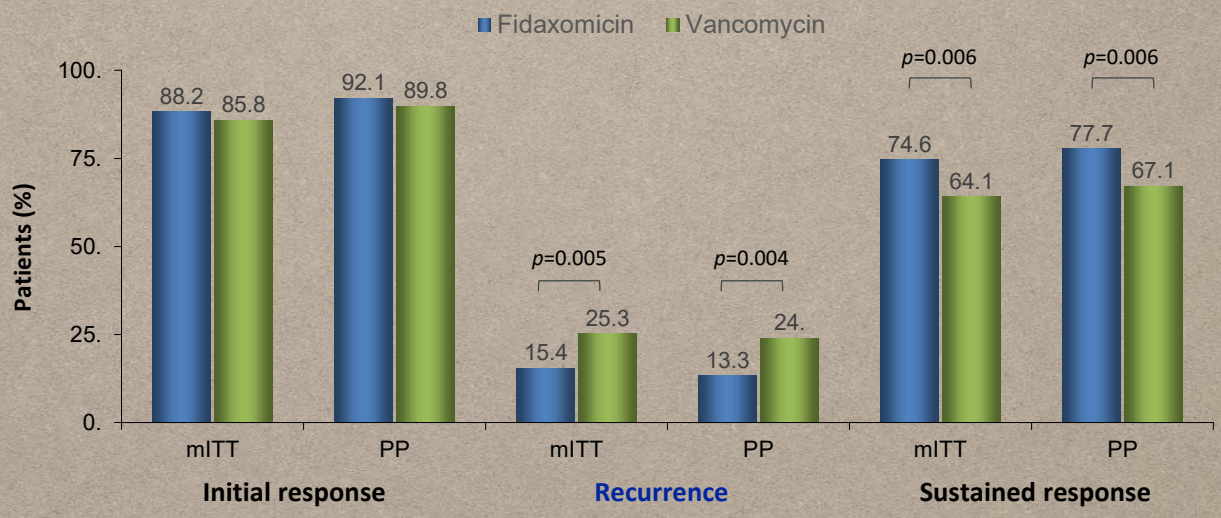
FIDAXOMICIN AND VANCOMYCIN FOR INITIAL *C. DIFFICILE* INFECTION



ITT: intention to treat; PP: per protocol

Louie et al. *N Engl J Med*. 2011;364(5):422-431.

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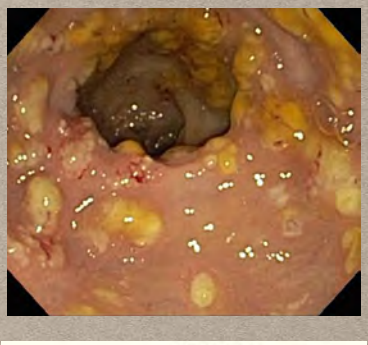
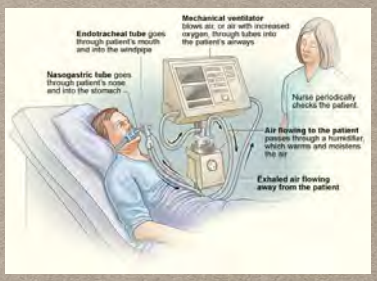
DRUGS FOR SEVERE CDI

- Severe
  - Vancomycin 125 mg PO QID x 14 days
  - **Fidaxomicin 200 mg PO BID x 10 days**
- Severe complicated
  - Vancomycin 500 mg PO QID PLUS
  - Vancomycin 500 mg PR QID PLUS
  - Metronidazole 500 mg IV q8h
    - Only situation where IV metronidazole is used/useful





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SURGERY CONSULT INDICATIONS?

- Complicated or suspected complicated CDI
- Clinical deterioration
  - Worsening abdominal distention/pain and/or peritonitis
  - Bowel obstruction
  - Intubation
  - Vasopressor requirement
  - Mental status changes
  - New or worsening Acute Kidney Injury
  - Worsening Lactate > 5mmol/L
  - Persistent or worsening leukocytosis (WBC ≥35,000 cells/mm3)
  - Hirschsprung's disease
- Failure to improve with standard therapy within 5 days as determined by resolving symptoms and physical exam, resolving WBC/band count

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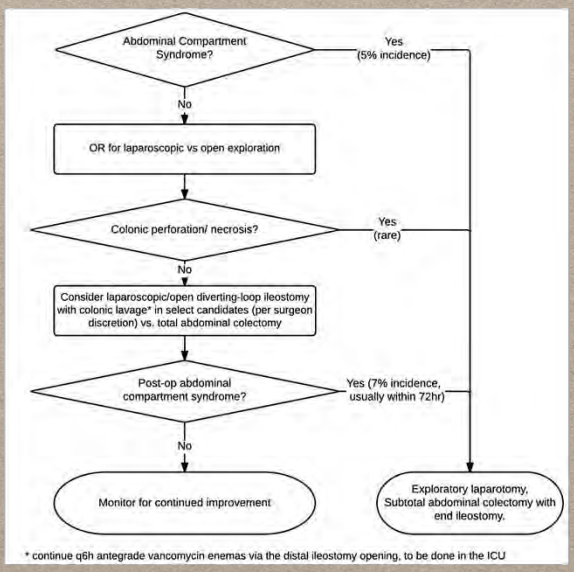
OPERATIVE MANAGEMENT STRATEGY



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SURGERY CONSULT OUTCOME

- Loop ileostomy + anterograde vancomycin enemas
  - New colectomy-sparing procedure
  - Non-inferior to colectomy
- Full/partial colectomy

Neal et al. 2011

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TREATMENT OF RECURRENT CDI: IDSA-SHEA GUIDELINES

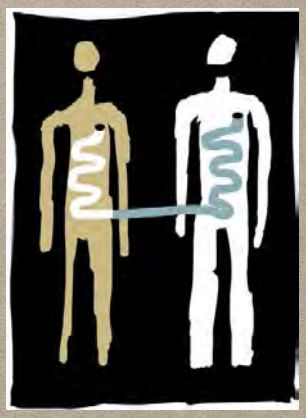
- First recurrence after 10-day course of vancomycin:
  - Vancomycin 125 mg PO qid x 10 days (if metronidazole used initially)
    - 36% chance of a 2nd recurrence
  - **Fidaxomicin 200 mg x 10 days**
    - 20% 2nd recurrence
  - Vancomycin tapered and pulsed regimen
    - 125 mg 4 times a day for 10-14 days
    - 125 mg 2 times a day for 1 week
    - 125 mg 1 time a day for 1 week
    - 125 mg 1 time a day every 2-3 days for 2-8 weeks

Cornely OA, et al. Clin Infect Dis 2012;55(2):S154
McDonald LC, et al. Clin Infect Dis. 2018;66:e1

17

TREATMENT OF RECURRENT CDI: IDSA-SHEA GUIDELINES

- 2nd or subsequent recurrent CDI:
  - Vancomycin tapered and pulsed regimen
  - **Fecal microbiota transplantation (FMT)**
  - **Fidaxomicin**
  - Standard vancomycin followed by rifaximin



McDonald LC, et al. IDSA 2018;66:E1
Garey KW, et al. J Antimicrob Chemo 2011;66:2850

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FIDAXOMICIN IN THE 2021 GUIDELINE UPDATE

- IN PATIENTS WITH AN INITIAL CLOSTRIDIOIDES DIFFICILE INFECTION EPISODE, SHOULD FIDAXOMICIN BE USED RATHER THAN VANCOMYCIN?
- For patients with an CDI episode, we suggest using **fidaxomicin** rather than a standard course of vancomycin
  - (conditional recommendation, moderate certainty of evidence). comment: This recommendation places a high value in the beneficial effects and safety of fidaxomicin, but its implementation depends upon available resources. Vancomycin remains an acceptable alternative.

Johnson S et al. Clinical Practice Guideline by the IDSA and SHEA: 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. Clin Infect Dis. 2021. doi: 10.1093/cid/ciab549.

19

FIDAXOMICIN IN THE 2021 GUIDELINE UPDATE

- In Patients With Recurrent CDI Episode(s), Should Fidaxomicin Be Used Rather Than Vancomycin?
- In patients with recurrent CDI episodes, we suggest **fidaxomicin** (standard or extended-pulsed regimen) rather than a standard course of vancomycin
  - (conditional recommendation, low certainty evidence). comment: Vancomycin in a tapered and pulsed regimen or vancomycin as a standard course are acceptable alternatives for a first CDI recurrence. For patients with multiple recurrences, vancomycin in a tapered and pulsed regimen, vancomycin followed by rifaximin, and fecal microbiota transplantation are options in addition to fidaxomicin.

Johnson S et al. Clinical Practice Guideline by the IDSA and SHEA: 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. Clin Infect Dis. 2021. doi: 10.1093/cid/ciab549.

20

PULSED FIDAXOMICIN?

Extended-pulsed fidaxomicin versus vancomycin for *Clostridium difficile* infection in patients 60 years and older (EXTEND): a randomised, controlled, open-label, phase 3b/4 trial

*Benoit Guery, Francesca Menichetti, Veli-Jukka Anttila, Nicholas Adomakoh, Jose Maria Aguado, Karen Bisnauthsing, Areti Georgopali, Simon D Goldenberg, Andreas Karas, Gbenga Kazeem, Chris Longshaw, Jose Alejandro Palacios-Fabrega, Oliver A Cornely, Maria J G T Vehreschild, for the EXTEND Clinical Study Group**

Lancet Infect Dis 2018; 18: 296–307
Published Online December 19, 2017
http://dx.doi.org/10.1016/

Summary

Background ***Clostridium difficile*** **infection causes severe complications and frequently recurs. An extended-pulsed fidaxomicin regimen might facilitate sustained clinical cure by prolonging *C difficile* suppression and supporting gut microbiota recovery. We aimed to compare clinical outcomes of extended-pulsed fidaxomicin with standard vancomycin.**

- Fidaxomicin 200 mg PO BID x 5 days
- Fidaxomicin 200 mg PO every other day x 20 days
- Same total of 20 tablets as usual prescription
- Clinical cure in 70% vs. 59%

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BEZLOTOXUMAB IN THE 2021 GUIDELINE UPDATE

- In Patients With a CDI Episode, Should Bezlotoxumab Be Used as a Co-intervention Along With Standard-of-Care Antibiotics Rather Than Standard-of-Care Antibiotics Alone?
- For patients with a recurrent CDI episode within the last 6 months, we suggest using **bezlotoxumab** as a co-intervention along with SOC antibiotics rather than SOC antibiotics alone
  - (conditional recommendation, very low certainty of evidence).
  - Other factors: age ≥65 years, immunocompromised host [per history or use of immunosuppressive therapy], and severe CDI on presentation
  - Avoid in CHF (volume overload)

Johnson S et al. Clinical Practice Guideline by the IDSA and SHEA: 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. Clin Infect Dis. 2021. doi: 10.1093/cid/ciab549.

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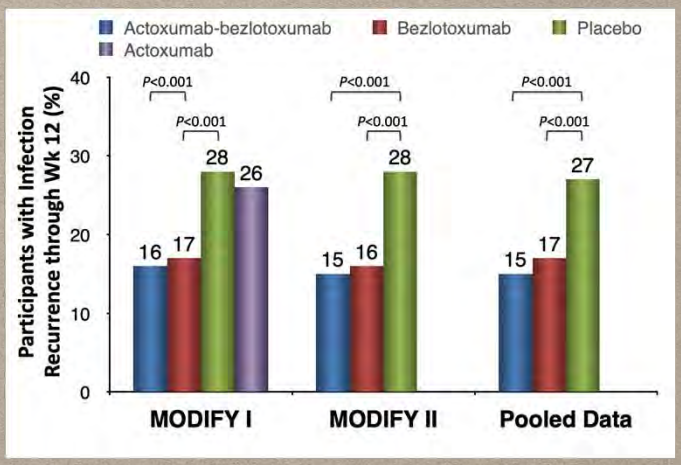
BEZLOTOXUMAB (ZINPLAVA)

- *C. difficile* produces two toxins: A & B
  - Damage epithelial cells in the GI tract leading to…
  - Increased gut permeability & acute inflammatory response
- FDA-approved human monoclonal antibody to toxin B, administered with standard CD antibiotic in patients at higher risk of recurrence



Wilcox MH, et al. N Engl J Med 2017;376:305

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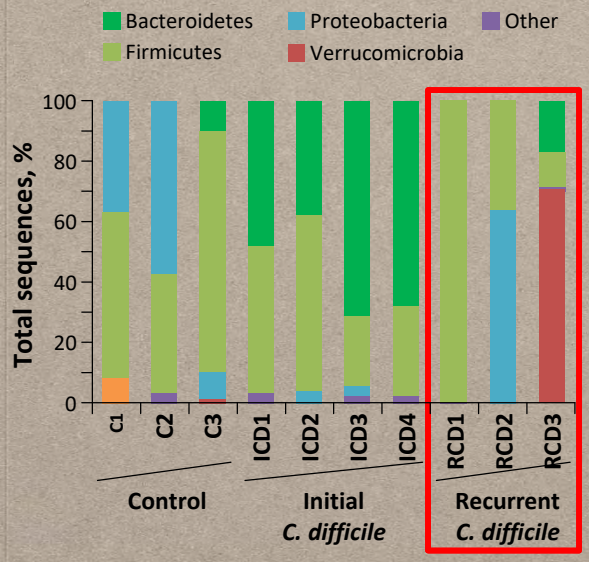
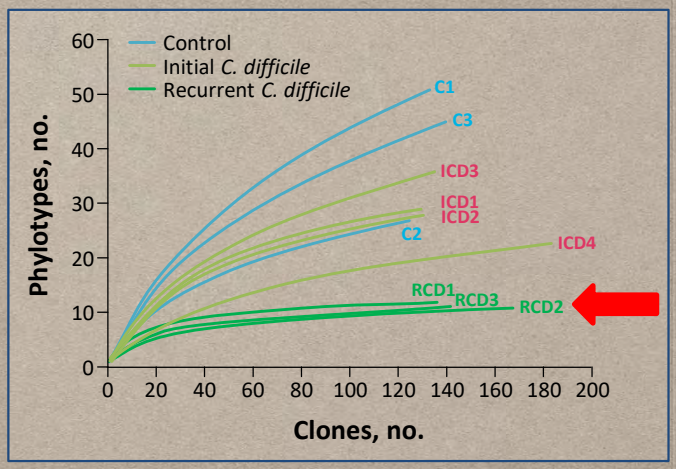
THE GUT MICROBIOME AS A THERAPEUTIC TARGET FOR CDI?





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STAW (STAGGERED AND TAPERED ANTIBIOTIC WITHDRAWAL) PROTOCOL

- Bakken et al. 2014, cured 25 patients w/o FMT
- Used staggered dosing (q72h) and tapering
- Lifeway kefir 5 ounces TID
  - Fermented dairy product
- On internal website

Antibiotic Time Course	Metronidazole Dose/Frequency		Vancomycin Dose/Frequency		Kefir
Weeks 1-2	250 mg Q 6h		125 mg Q 6h		150 mL TID
Weeks 3-4	750 mg Q 72h		375 mg Q 72h		150 mL TID
Weeks 5-6	500 mg Q 72h	OR	250 mg Q 72h	PLUS	150 mL TID
Weeks 7-8	250 mg Q 72h		125 mg Q 72h		150 mL TID
Weeks 9-15					150 mL TID

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WHAT ABOUT STOOL TRANSPLANT: …..WHAT IS STOOL?

Water and electrolytes

Fats, polysaccharides and protein

Undigested food

Microbiotic biomass

Any ingested pharmaceuticals

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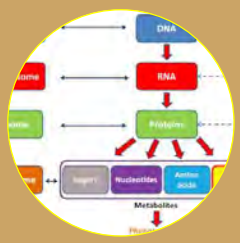
IMPORTANT DEFINITIONS

MICROBIOTA:
Microorganisms that live in an established environment

MICROBIOME:
Combination of microbiota + "theatre of activity", including local genetic material, chemistry, and environmental factors
(e.g. "gut microbiome")

DYSBIOSIS:
Derangement in the microbiota



METABOLOME:
Functional properties of gut microbiota

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WHAT CONSTITUTES THE INTESTINAL MICROBIOTA?

Bacteria

Archaea

Fungi

Viruses

Protozoa

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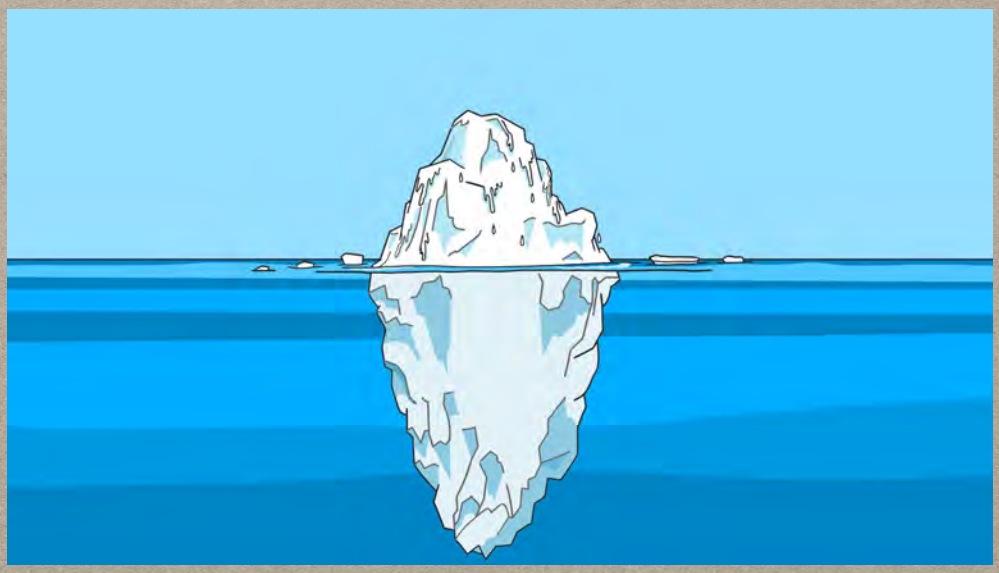
WE ARE LOOKING ONLY AT THE TIP OF THE ICEBERG



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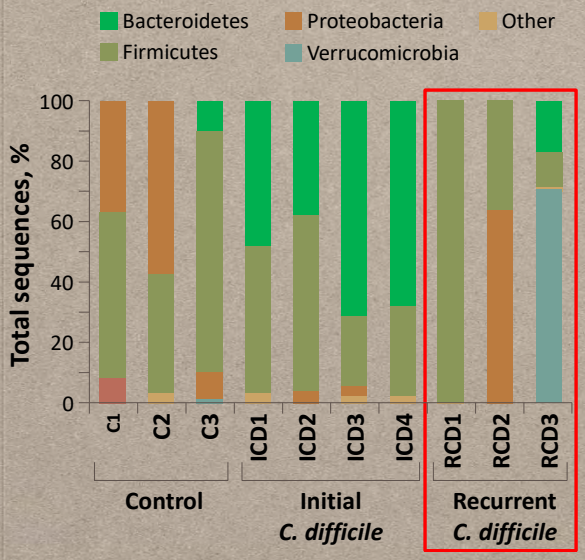
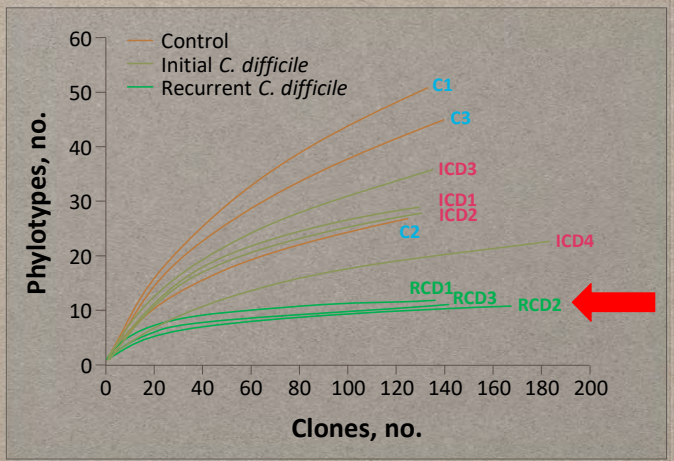
DIVERSITY OF MICROBIOME IN INITIAL AND RECURRENT CDI





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TREATMENT OF RECURRENT CDI: FECAL MICROBIOTA TRANSPLANTATION (FMT)

- IDSA: "Fecal microbiota transplantation is recommended for patients with multiple recurrences of CDI who have failed appropriate antibiotic treatments (strong recommendation, moderate quality of evidence)."
- Among recommended options for 2nd or subsequent recurrence of CDI
- Although there is little evidence, "the opinion of the panel is that appropriate antibiotic treatments for at least 2 recurrences (ie, 3 CDI episodes) should be tried" before FMT.

McDonald LC, et al. IDSA 2018;66:E1

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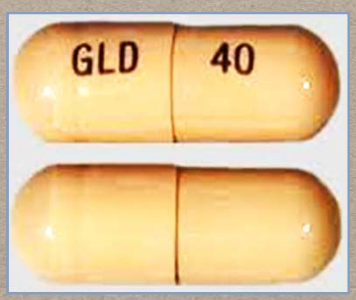
FMT FOR RECURRENT CDI



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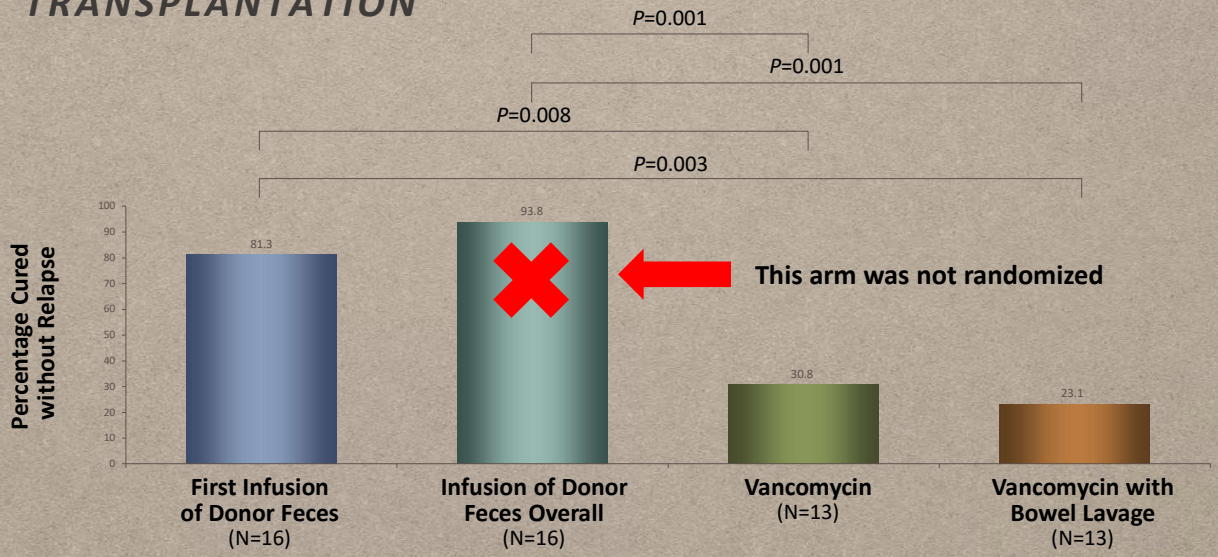
FMT LANDMARK STUDY:
FIRST RANDOMIZED TRIAL OF FECAL MICROBIOTA TRANSPLANTATION



Van Nood E, et al. *N Engl J Med*. 2013;368:407-15.

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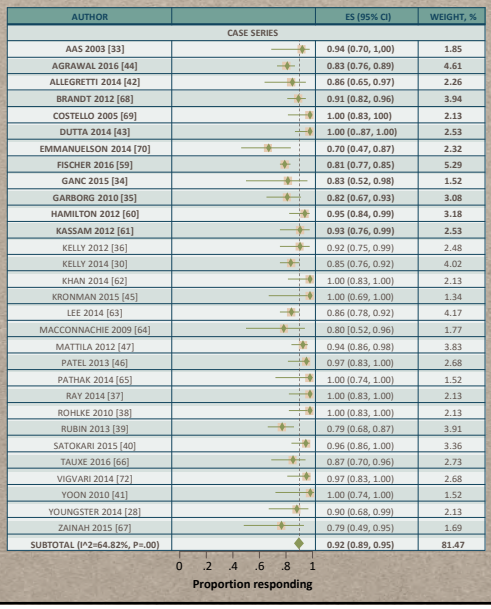
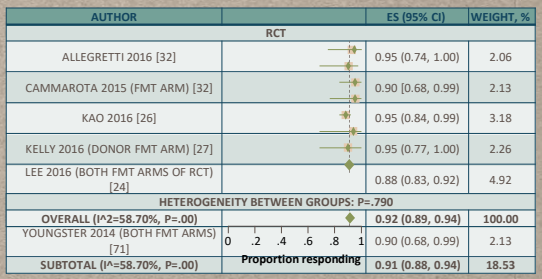
FMT EFFICACY META-ANALYSIS

- 37 studies
  - 7 RCT
  - 30 Case series
- Overall effectiveness 92%
- FMT more effective than vancomycin taper for recurrent/refractory CDI
- Lower administration more effective than upper administration
- No difference between fresh and frozen FMT





Quraishi et al. *Alim Pharm & Ther.* Sep;46(5):479-493. 2017

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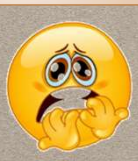
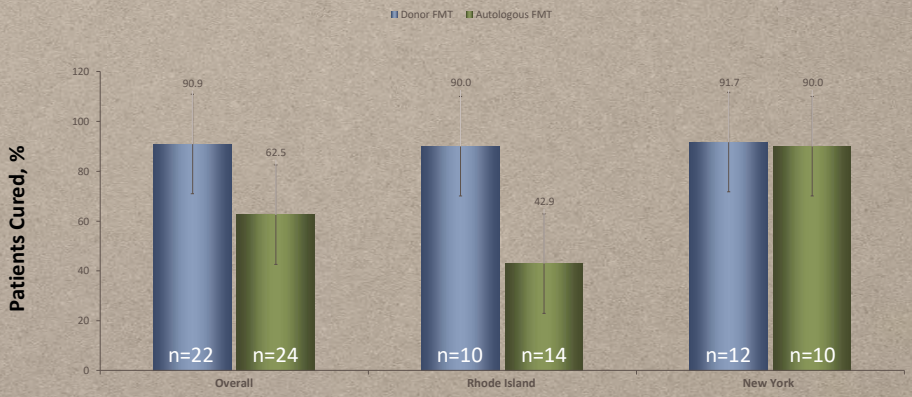
HOLD ON A SECOND: PLACEBO AND/OR NON-BACTERIAL FMT WORK?

Rates of clinical cure in the intention-to-treat population, overall and by site.

90% cured with patients' own stool in recent RCT





Kelly et al. 2016

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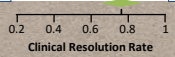
CURE RATES IN TRIALS LOWER THAN EXPECTED…

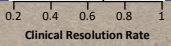
All RCTs

Study	Estimate (95% CI)	Cure/Treatment, No. of Patients	Weight, %
Youngster et al [17] (2014)	0.700 (.499 – .901)	14/20	6.58
Youngster et al [18] (2014)	0.700 (.499 – .901)	14/20	6.58
Kao et al [25] (2015)	0.977 (.932 – 1.000)	42/43	9.03
Khanna et al [27] (2016)	0.967 (.902 – 1.000)	29/30	8.85
Orenstein et al [26] (2016)	0.871 (.753 – .989)	27/31	8.09
Lee et al [19] (2016)	0.624 (.552 – .695)	111/178	8.77
Jiang et al [20] (2017)	0.875 (.799 – .951)	63/72	8.71
van Nood et al [21] (2013)	0.812 (.621 – 1.000)	13/16	6.76
Cammarota et al [22] (2015)	0.650 (.441 – .859)	13/20	6.43
Kelly et al [8] (2016)	0.909 (.789 – 1.000)	20/22	8.06
SER-109 [24] (2016)	0.559 (.433 – .686)	33/59	7.95
Dubberke et al [23] (2016)	0.639 (.535 – .742)	53/83	8.33
Hota et al [9] (2017)	0.438 (.194 – .681)	7/16	5.81
Overall (I^2=91.35%; *P*<.001)	**0.761 (.644 – .857)**	**439/610**	**100.00**



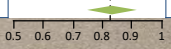
RCTs with non-FMT control group

Study	Estimate (95% CI)	Cure/Treatment, No. of Patients	Weight, %
van Nood et al [21] (2013)	0.812 (.621 – 1.000)	13/16	15.38
Cammarota et al [22] (2015)	0.650 (.441 – .859)	13/20	14.51
Kelly et al [8] (2016)	0.909 (.789 – 1.000)	20/22	18.90
SER-109 [24] (2016)	0.559 (.433 – .686)	33/59	18.59
Dubberke et al [23] (2016)	0.639 (.535 – .742)	53/83	19.67
Hota et al [9] (2017)	0.438 (.194 – .681)	7/16	12.93
Overall (I^2=78.88%; *P*<.001)	**0.677 (.542 – .813)**	**139/216**	**100.00**



Open-label RCTs

Study	Estimate (95% CI)	Cure/Treatment, No. of Patients	Weight, %
Youngster et al [17] (2014)	0.700 (.499 – .901)	14/20	11.15
Youngster et al [18] (2014)	0.700 (.499 – .901)	14/20	11.15
Kao et al [25] (2015)	0.977 (.932 – 1.000)	42/43	16.28
Khanna et al [27] (2016)	0.967 (.902 – 1.000)	29/30	15.83
Orenstein et al [26] (2016)	0.871 (.753 – .989)	27/31	14.24
Lee et al [19] (2016)	0.624 (.552 – .695)	111/178	15.71
Jiang et al [20] (2017)	0.875 (.799 – .951)	63/72	15.57
Overall (I^2=92.56%; *P*<.001)	**0.827 (.711 – .943)**	**300/394**	**100.00**



Tariq et al.,CID, 2019

36

CURRENT BIOPHARMACEUTICAL LANDSCAPE

Donor-Derived Consortium
- RBX2660 (Enema)
- CP-101 (Oral)

Narrow Consortium
- SER-109 (Oral)

Defined Consortium
- VE303 (Oral)

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LIVE BIOPHARMACEUTICAL TRIALS IN RECURRENT CDI

Product	Study Name	Phase	Primary Outcome
RBX2660 Enema	PUNCH CD 3	Approved 2023	Absence of CDI diarrhea without retreatment at 8 weeks
SER-109 Oral capsule	ECOSPOR III	Phase 3 / FDA review	CDI recurrence at 8 weeks
CP101	PRISM3	Phase 2	CDI recurrence at 8 weeks
VE303	CONSORTIUM	Phase 2	CDI recurrence at 8 weeks

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Similarities Among Clinical Trials of Live Biopharmaceuticals for Recurrent CDI

All patients:

- Had recurrent *C difficile* infection
- Received standard of care antimicrobial treatment
- Received a live biopharmaceutical product (LPB) intervention
- Had an 8-week initial follow-up (measured as rate of recurrence or absence of recurrence)

39

Differences Among Clinical Trials of Live Biopharmaceutical Products for Recurrent CDI

- Diagnostics used
- # of CDI recurrences
- Duration of antibiotic use before enrollment
- Antibiotic washout period
- Use of bowel purge prior to LBP
- Dosing of investigational LBP

40

RBX2660 PHASE 3 CLINICAL TRIAL
PUNCH CD3

Antibiotic washout period (24h-72h)

A
Treatment
1 Dose RBX2660

B
Control
1 Dose Placebo

8 weeks

CDI Resolved?

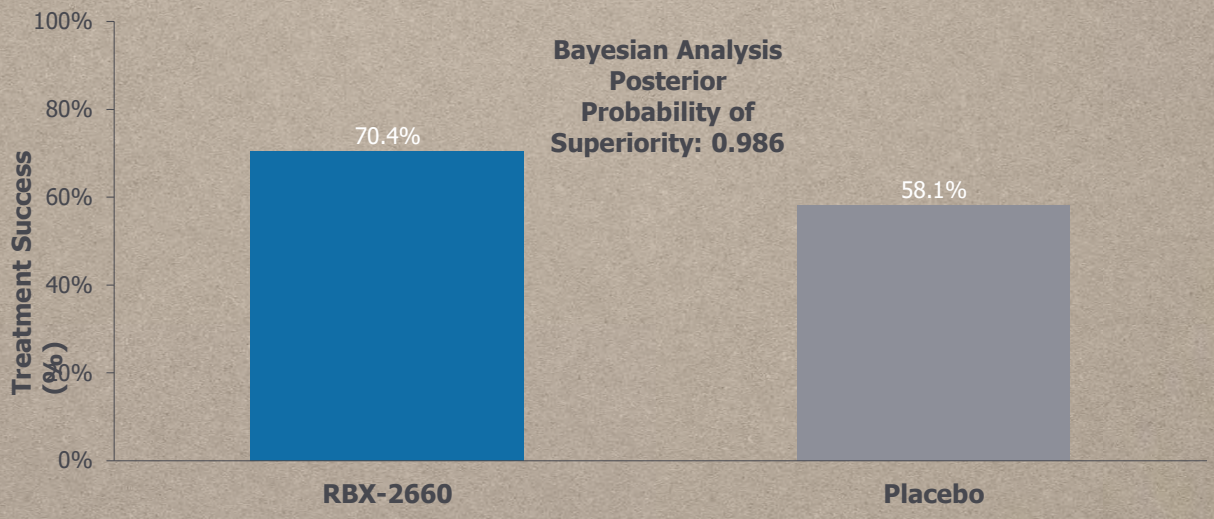
Yes → 6 months Follow-up

No → Option for second RBX2660 → 6 months Follow-up

Microbiota Restoration Therapy for Recurrent Clostridium Difficile Infection (PUNCHCD3). Accessed May 9, 2022. https://clinicaltrials.gov/ct2/show/NCT03244644

41

RBX2660 PHASE 3 CLINICAL TRIAL: PUNCH3 CD3
RATES OF TREATMENT SUCCESS AT WEEK 8

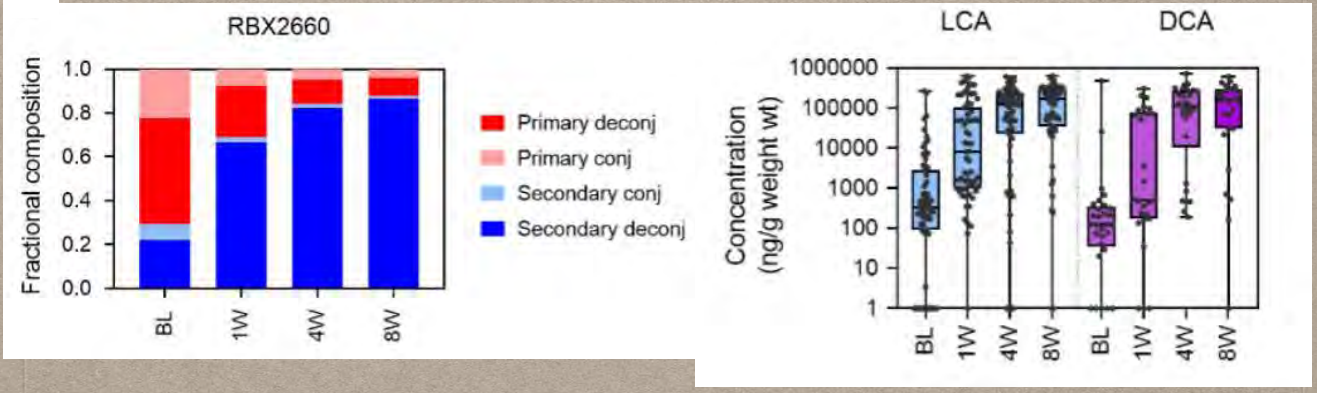


- Majority of TEAEs were similar between arms; most were mild to moderate in nature.

- Bancke LL, et al. ID Week Presentation 2021. Abstract 167.

42

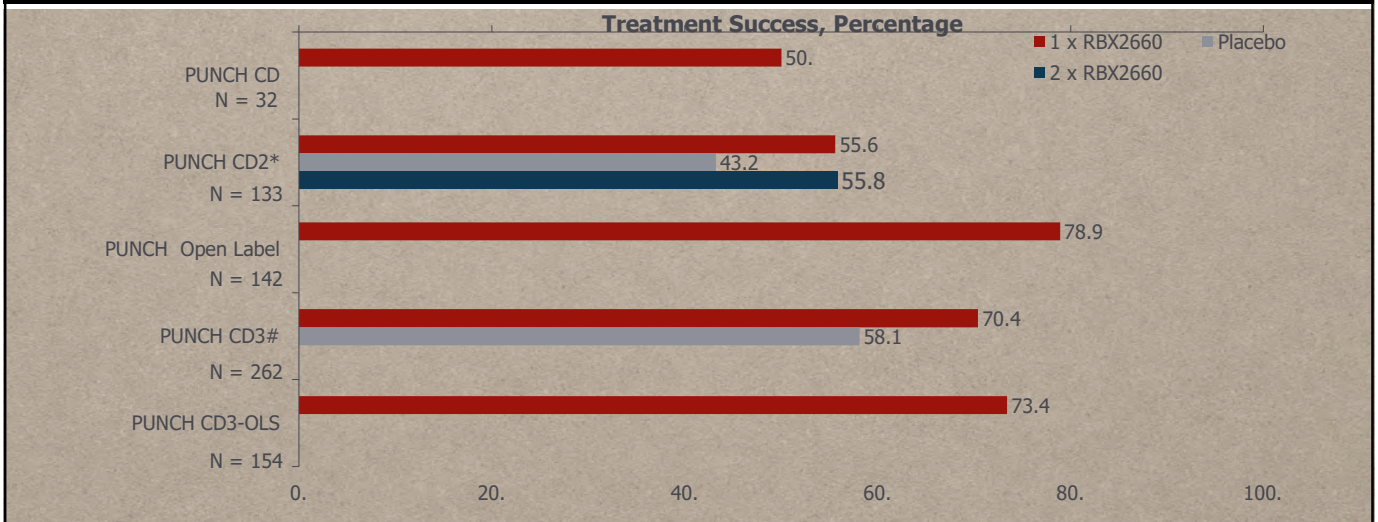
RBX2660 PHASE 3 CLINICAL TRIAL: PUNCH3 CD3
RESTORATION OF BILE ACID COMPOSITION



- Papazyan R, et al. ID Week Presentation 2021. Abstract 1039.

43

RBX2660
Reduction of CDI Recurrence Rates in a Series of Clinical Trials



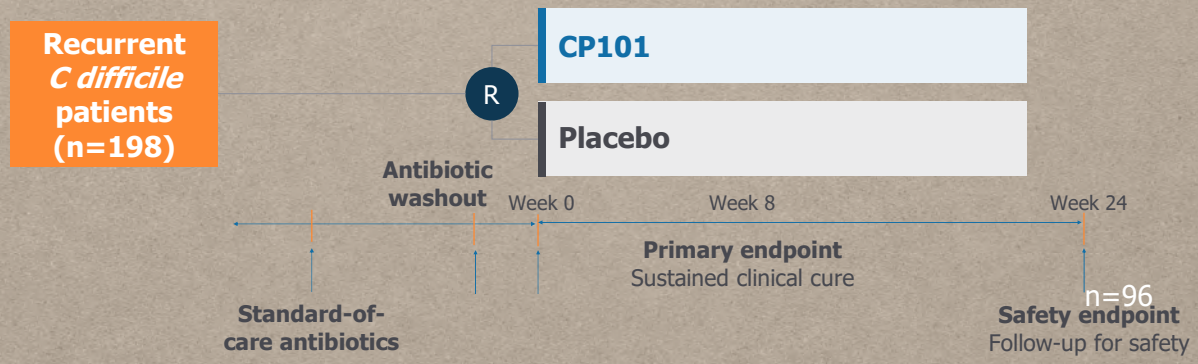
*Chi-square test; $P > .05$
#Bayesian hierarchical model; 98.6% (0.986) probability of superiority

Banke LL, et al. Open Forum Infect Dis. 2021;8:S100-S101.

44

CP101 Phase 2 Trial: PRISM3
Design

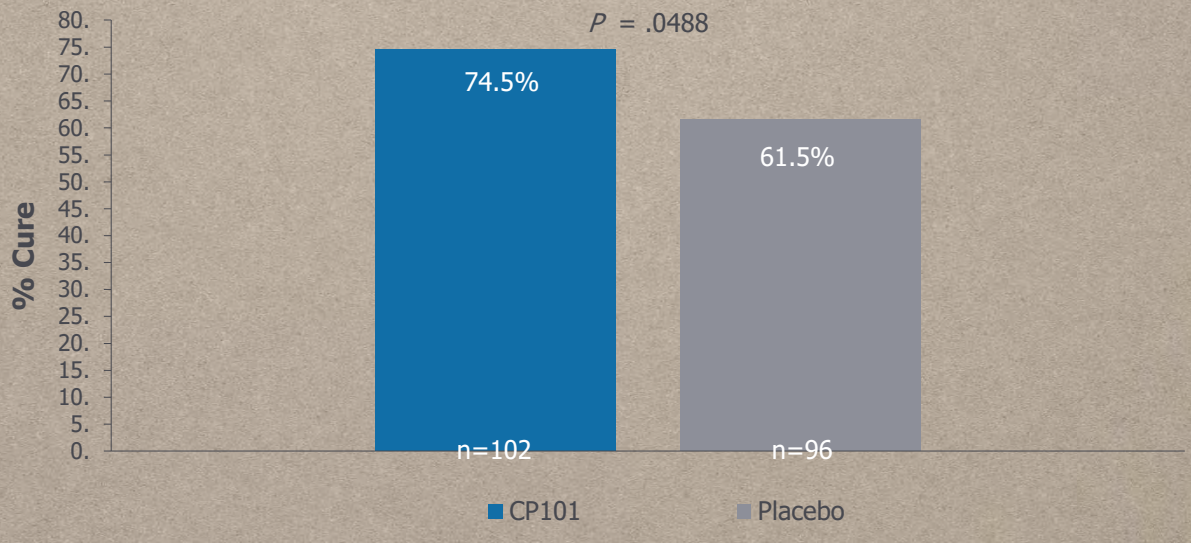
- Randomized, placebo- controlled phase 2 study
- Included patients with first recurrence and patients diagnosed by PCR alone



Allegretti J, et al. Presented at ACG 2020. Abstract LB2.

45

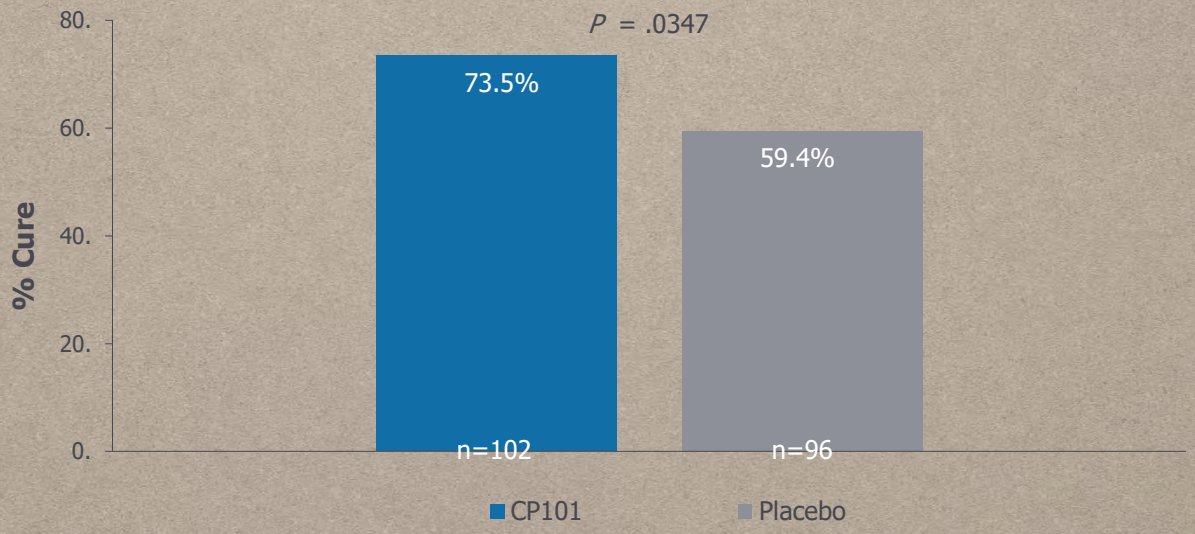
CP101 PHASE 2 TRIAL: PRISM3
SUSTAINED CLINICAL CURE THROUGH WEEK 8



- Allegretti J, et al. Presented at ACG 2020. Abstract LB2.

46

CP101 PHASE 2 TRIAL: PRISM3
ABSENCE OF CDI RECURRENCE THROUGH WEEK 24



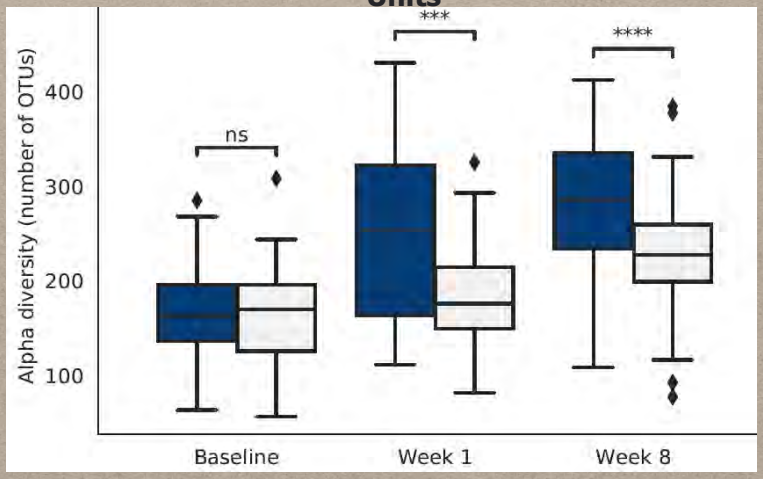
Adverse events were similar between both arms through week 24; no treatment-related severe AEs reported with CP101.

- Allegretti J, et al. Presented at ACG 2021. Abstract P0130.

47

CP101 Phase 2 Trial: PRISM3
Alpha Diversity

Number of Operational Taxonomic Units



Ns: P>.05; *** P<.001: ****P<.0001

Allegretti J, et al. Presented at ID Week 2021.

48

ECOSPOR-III: PHASE 3 DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL OF SER-109 FOR MULTIPLE RCDI

- 281 adult subjects with ≥2 CDI recurrences were screened
- 182 toxin+ subjects with symptom resolution on antibiotics at enrollment
- 10 oz magnesium citrate administered prior to randomization to minimize residual antibiotic
- Subjects stratified by age and antibiotic received

R

SER-109

4 Capsules once daily for 3 Days

Placebo

Recurrences evaluated through 8 weeks

Primary Endpoint

- Recurrence at 8 weeks

Safety

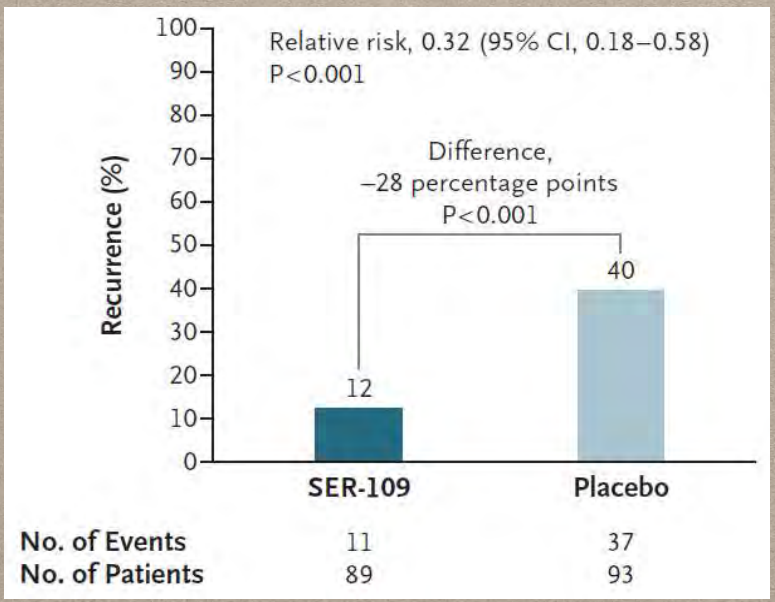
- Through 8 weeks

- Feuerstadt et al. N Engl J Med. 2022;386:220-229.

49

SER-109 PHASE 3 TRIAL: ECOSPOR-III

RECURRENCE IN OVERALL POPULATION THROUGH WEEK 8

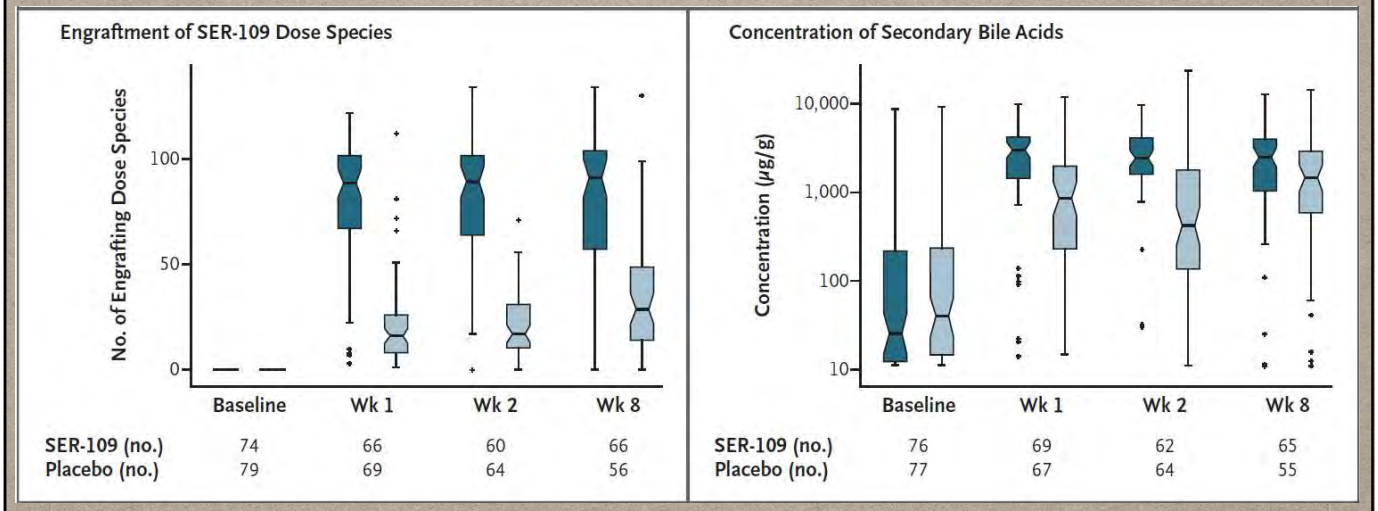


- Rates of adverse events possibly related to SER-109 or placebo were similar between arms
- Most common AEs were gastrointestinal in nature, mostly mild to moderate

- Feuerstadt P, et al. N Engl J Med 2022;386:220-229.

50

SER-109 PHASE 3 TRIAL: ECOSPOR-III
COMPOSITIONAL AND METABOLIC CHANGES



- Feuerstadt P, et al. N Engl J Med 2022;386:220-229.

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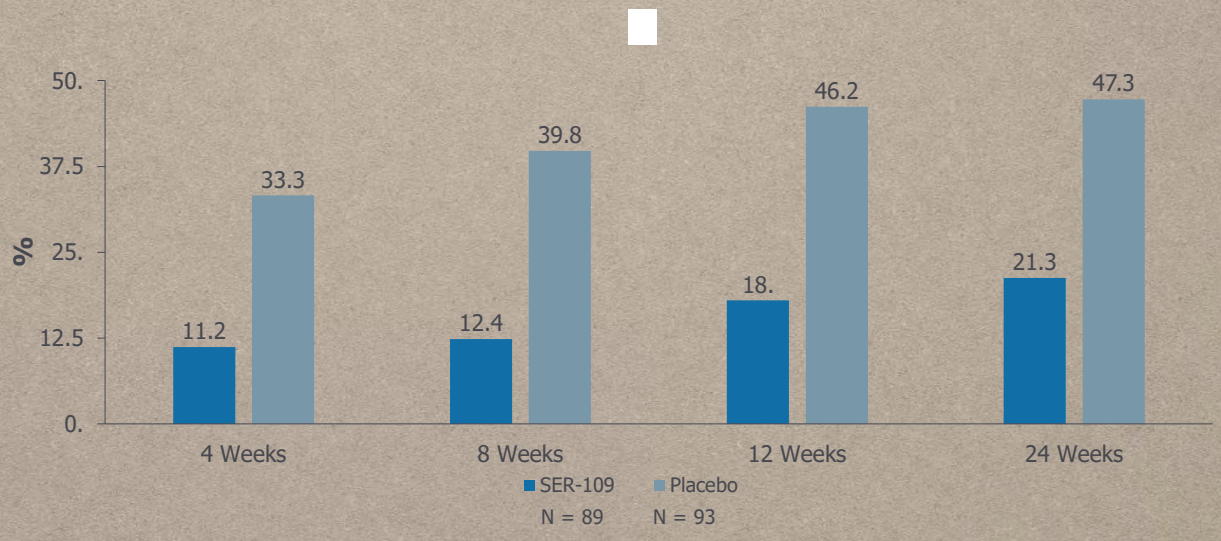
SER-109 PHASE 3 TRIAL: ECOSPOR-III
EFFICACY MAINTAINED THROUGH WEEK 24



- Week 8 was the primary efficacy endpoint. Weeks 4, 12 and 24 were secondary efficacy endpoints defined in the statistical analysis plan.
- Safety was comparable to placebo through week 24.
- Wilcox MH, et al. ECCMID 2022. Abstract 00689

52

VE303: Phase 2 Consortium Study

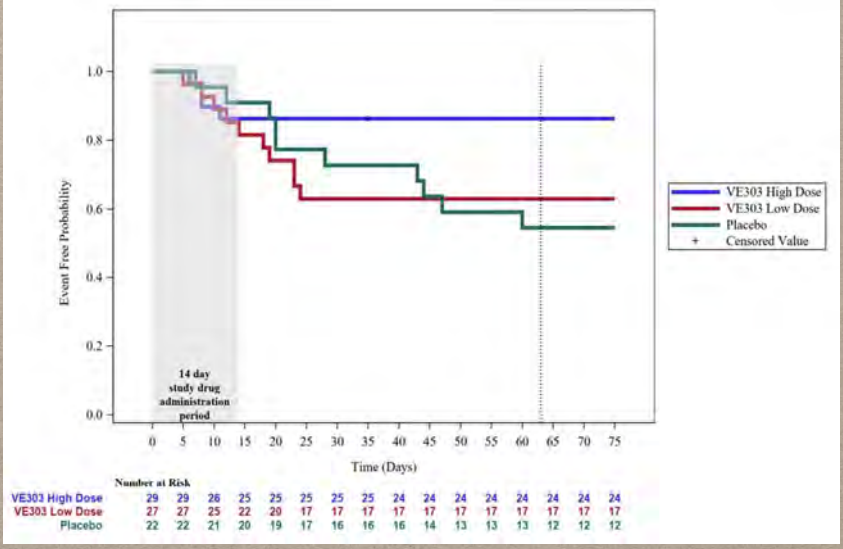
- Live biotherapeutic product containing 8 clonal human commensal bacterial strains
- Evaluated in a randomized, placebo-controlled phase 2 study for the prevention of subsequent CDI in high-risk patients or recurrent CDI (N=78):
  - VE303 high dose (10 capsules once daily; n = 29)
  - VE303 low dose (2 capsules once daily; n = 27)
  - Placebo (n = 22)

Louie TJ, et al. Presented at DDW 2022. Presentation 109.

53

VE303: Phase 2 Consortium Study
Rate of Recurrence Through Week 8

Rate of recurrence through week 8: 13.8% for high dose VE303 vs 45.5% for placebo (*P*=.0077)



Louie TJ, et al. Presented at DDW 2022. Presentation 109.

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CONCLUSIONS

- Treatment recommendations around CDI have shifted in the past decade
- Focus now is not just initial cure but preventing recurrence
- FMT is one of several modalities used now in treating initial or recurrent CDI, but questions around efficacy and regulation remain

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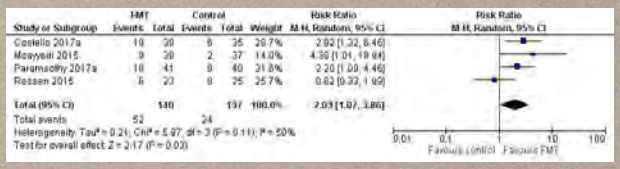
Yeah yeah FMT is great for CDI, but…

- Infection risk (MDROs, STEC, SARS-CoV-2)
- Variability in product
- Unknown long-term effects
- Tolerability / eligibility
- Ineffective for other conditions



Imdad et al., Cochrane Database Syst Rev, 2018; PMID: 30480772

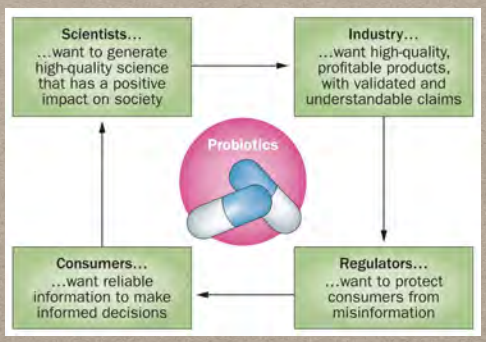
So what about probiotics?

56

What is a ~~probiotic~~ live biotherapeutic?

"live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"

- The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol, 2014



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Stretching the definition of a probiotic…

- Viruses like bacteriophages?

- Defined microbial communities?

- What about feces?
  - Microbes
  - Metabolites
  - Antibodies and inflammatory mediators

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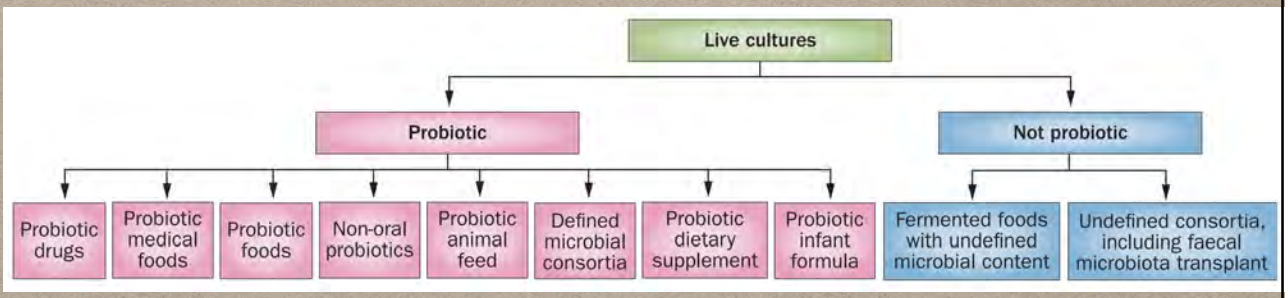
What is a probiotic?



59

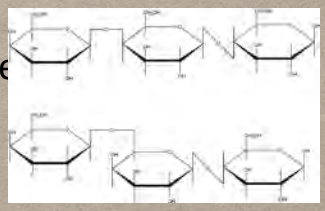
What is a probiotic?

- Include in the framework for definition of probiotics microbial species that have been shown ***in properly controlled studies*** to confer benefits to health
- Keep *live cultures*, traditionally associated with fermented foods and for which there is no evidence of a health benefit, *outside the probiotic framework*
- Keep undefined, *faecal microbiota transplants* outside the probiotic framework
- New commensals and consortia comprising ***defined strains*** from human samples, with adequate evidence of safety and efficacy, are 'probiotics'

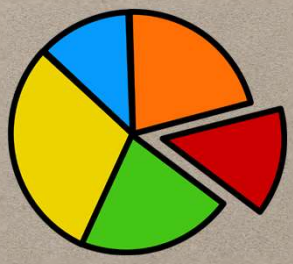
60

Prebiotics and Synbiotics

- Prebiotics
  - Non-digestible polysaccharides and oligosaccharide
  - Fermentation subtrates
  - Promote the growth of beneficial microbes



- Synbiotic = prebiotic + specific probiotic



61

Probiotic uses

- Prophylaxis
  - Primary and secondary
    - *Ex.* primary *C. difficile* infection vs. recurrent CDI
- Treatment
  - Adjunctive or primary
    - *Ex. C. difficile* infection vs. antibiotic associated diarrhea

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SO HOW TO PROBIOTICS EVEN WORK?

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How do probiotics even work? Feasibility?

- Many probiotics can easily make it into the lower gut
  - *L. acidophilus* = lover of acid. Tolerates low pH just fine.
- Detectable in feces
- Occasionally detectable at extra-intestinal sites (more on this later)
- Often selected to be resistant to certain antibiotics

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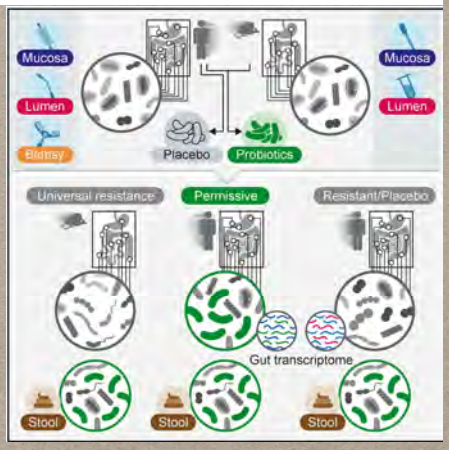
Cell

Article

Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features

- Sampled bacteria from several places along the GI tract & stool
- Subjects received cocktail of 11 probiotic species or placebo
- Half of subjects showed no difference
- All probiotics were detectable in stool
- Stool does not reflect state of "probiotic uptake"



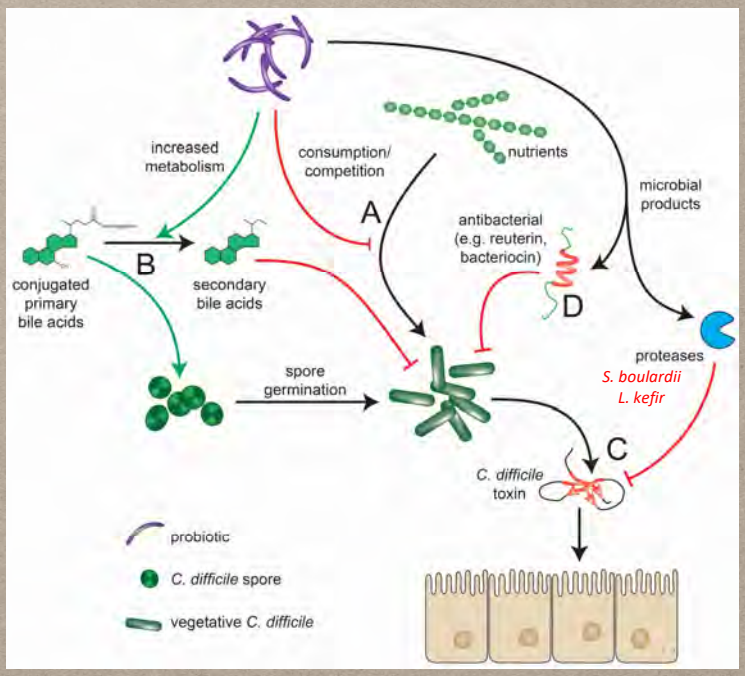
Zmora et al., Cell, 2018. PMID: 30193112

65

How could probiotics affect CDI?

Also:

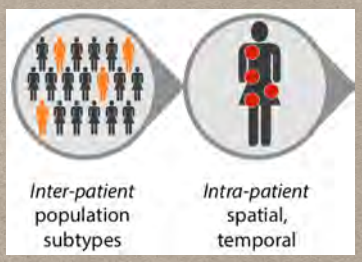
- ↑Mucin production
- Alteration of local:
  - pH
  - Inflammation
  - IgA (*L. casei*)



66

Are probiotics effective for CDI? Individual studies

- Many uncontrolled studies with poor quality evidence
- No one, large definitive RCT
- Heterogeneity
  - Strains
  - Doses
  - Regimens



67

Are probiotics effective for CDI? Individual studies

- PLACIDE trial: large, negative study*:
  - Insufficient power: only 1% of patients with CDI in the study
- Not focused on a high-risk population
- Ill-defined inclusion/exclusion criteria
  - Many did not exclude patients consuming fermented foods
- Other confounders (one study moved to a new hospital)
- Cooled interest in probiotics for CDI

**Allen et. al, Lancet, 382 (2013), pp*

68

Are probiotics effective for CDI? Meta-analyses

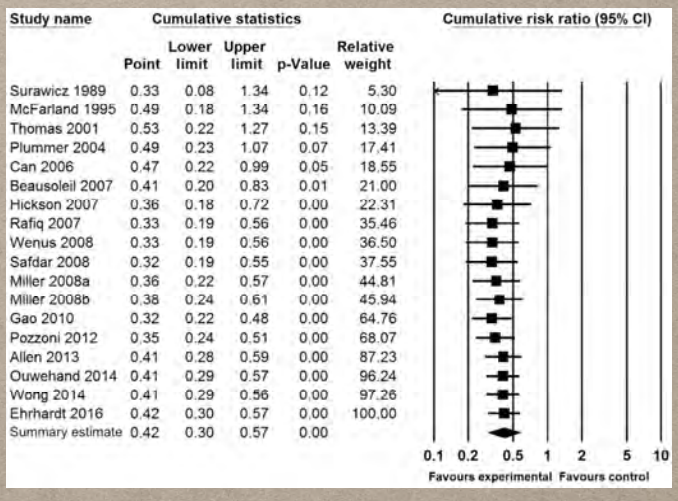
- Several conducted over the years*
- Different inclusion criteria for studies
- Did not always follow PRISMA best practice guidelines
- Broad criteria:
  - Weaker effect sizes and significance in general
  - More heterogeneity
- Narrow criteria
  - Focused on a high-risk population
  - Include only RCTs with placebo controls

**Lau et al., Int J Gen Med 2016;9:27-37*
McFarland et al., Antibiotics 2015;4:160-178
Johnston et al., Ann Intern Med 2012;157:878-888

69

Most salient meta-analysis in support of probiotics for CDI prevention?*

- Heterogeneous data in past
- Focused: RCTs, hospitalized patients on antibiotics
- Rigorous PRISMA adherence, missing data sensitivity analyses, etc.
- 19 trials with 6261 patients
  - RR of 0.42, NNT 43
  - Sens: ↑RR to 0.6, NNT 63



**Shen et al., Gastroenterol., 2017;152:1889-1900*

70

SO WHY AREN'T WE USING PROBIOTICS FOR CDI PREVENTION?

Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA)

L. Clifford McDonald,[1] Dale N. Gerding,[2] Stuart Johnson,[2,3] Johan S. Bakken,[4] Karen C. Carroll,[5] Susan E. Coffin,[6] Erik R. Dubberke,[7] Kevin W. Garey,[8] Carolyn V. Gould,[1] Ciaran Kelly,[9] Vivian Loo,[10] Julia Shaklee Sammons,[6] Thomas J. Sandora,[11] and Mark H. Wilcox[12]

XXVII. What is the role of probiotics in primary prevention of CDI?

Recommendation

1. There are insufficient data at this time to recommend administration of probiotics for primary prevention of CDI outside of clinical trials *(no recommendation)*.

71

Maybe they aren't safe?

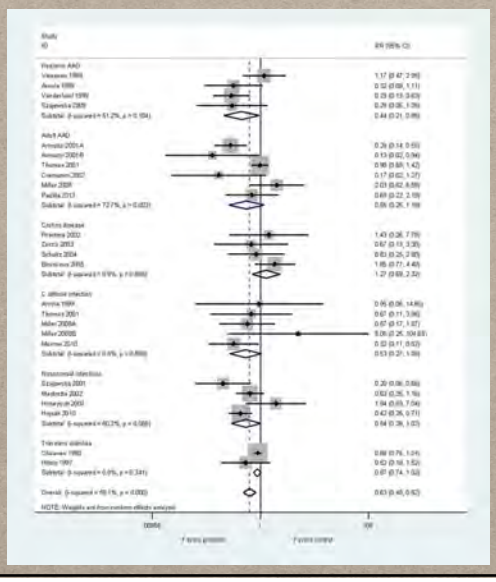
- Officially GRAS
- IBS-like symptoms can occur
- Bacteremia and endocarditis reported with *Saccharomyces*[1] and *Lactobacillus*[2]
- Increased mortality in acute pancreatitis[2]
- Most trials excluded immunocompromised, IBD, and ICU patients
  - Among those at highest risk for CDI

[1]*Herbrecht et al., CID 2005;40:1635-1637*
[2]*Kato et al., Int J Cardiol 2016;224:157-161*
[3]*Besselink et al., CID 2014;59:858-861*

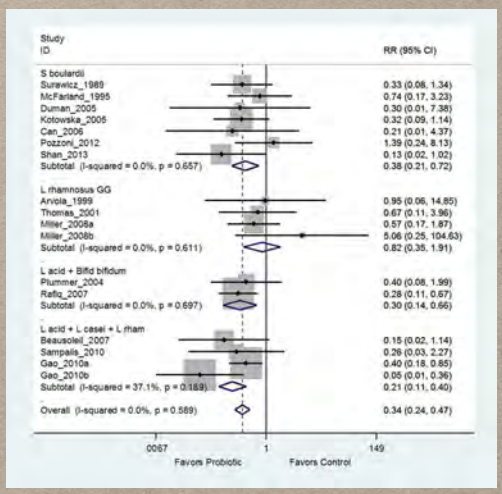
72

Strain and disease specificity in clinical literature

Lactobacillus rhamnosus GG vs. disease state



CDI vs. different strains



McFarland et al., Front. Med. 2018; doi: 10.3389/fmed.2018.00124

73

Too much heterogeneity: Evidence in favor ≠ a specific recommendation

- We need clinical trials based on basic, mechanistic research
  - Moving from bench to bedside
  - Major challenge: translation of models with different microbiomes to humans
  - Strains used in basic studies to-date, though, are similar to ones already showing efficacy in meta-analyses

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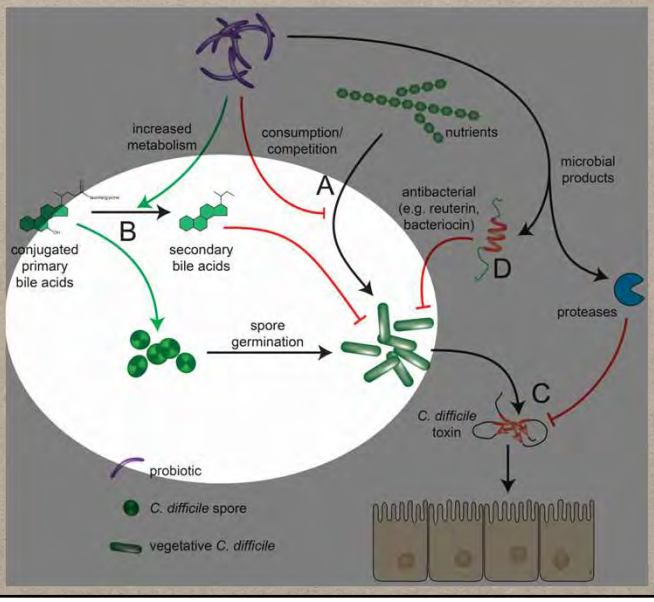
FINE, SO WHAT BASIC, MECHANISTIC RESEARCH IS BEING CONDUCTED?

75

Mechanism spotlight: Bile acid homeostasis



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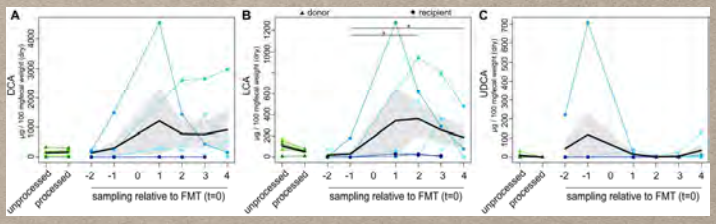
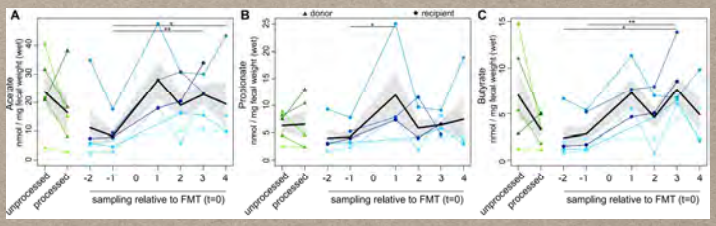
Effect of FMT on bile acids/short chain fatty acids

- 6 patients with FMT
- 2° bile acids increased
- SCFAs increased
- Direction of causality?



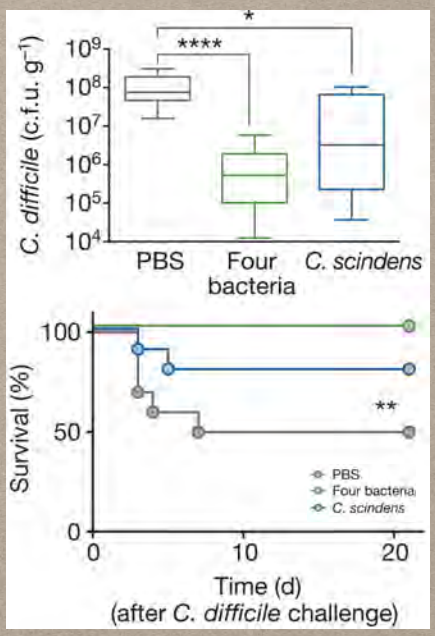


Seekatz et al, Anaerobe, 2018

77

Bile acid homeostasis

- Mice treated with antibiotics and microbiota assessed
- Identified specific taxa conferring resistance to CDI
  - *Clostridium scindens*: 7α-dehydroxylase
- *C. scindens* + 3 others attenuate CDI

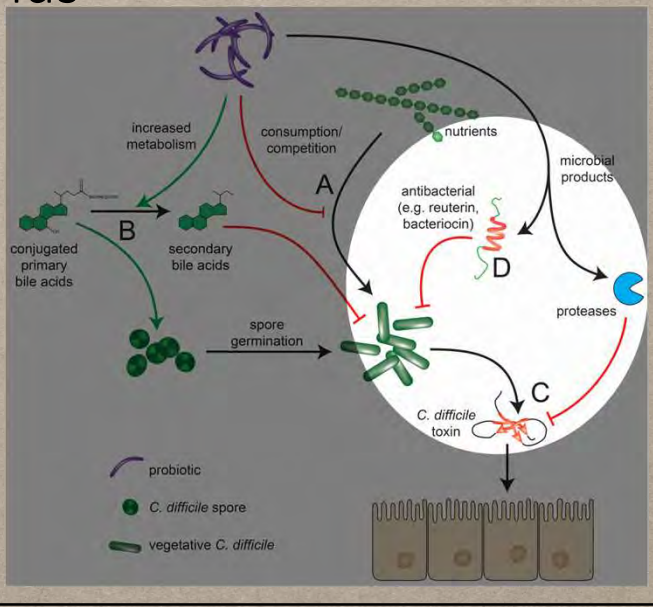


Buffie et al., Nature, 2015

78

Mechanism spotlight: Production of antimicrobial compounds

- Bacteriocins
  - Mostly small polypeptides with narrow spectrum
  - Bacterio-cidal or -static
  - Mostly target related species



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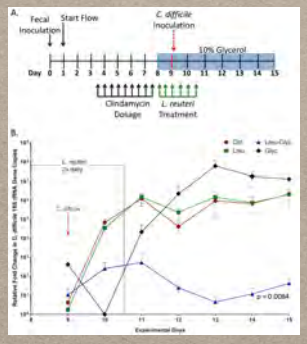
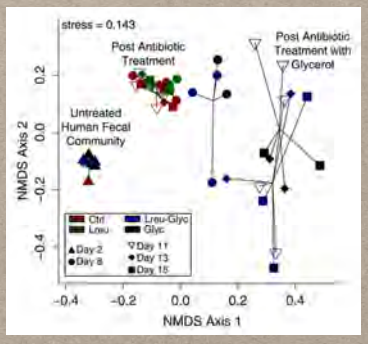
Production of antimicrobial compounds

Lactobacillus reuteri: makes reuterin from glycerol

- Resistant to many antibiotics
- Reduces *C. difficile* growth in a bioreactor model
- Alters the microbiota



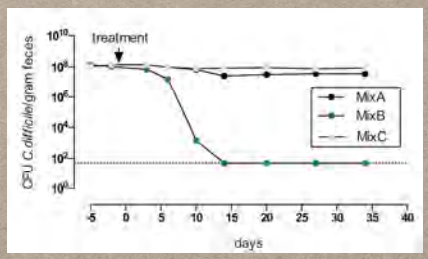


Spinler et al., Infect. Immun., 2017

80

Defined communities

- FMT treated CDI in mice effectively
- Studied community of healthy feces
- Tested many combinations of the bacterial phyla in lieu of FMT
- Most of these mixtures did not work….
- Mixture B:
  - *Bacteroidetes* novel species
  - *Lactobacillus reuteri*
  - *Enterococcus hirae*
  - *Anaerostipes* novel species
  - *Staphylococcus warneri*
  - *Enterorhabdus* novel species



Lawley et al., PLOS Pathogens, 2

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ANY SUCCESS STORIES YET?

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ARTICLE

doi:10.1038/nature23480

A randomized synbiotic trial to prevent sepsis among infants in rural India

Pinaki Panigrahi[1,2], Sailajanandan Parida[3], Nimai C. Nanda[4], Radhanath Satpathy[3], Lingaraj Pradhan[6], Dinesh S. Chandel[7], Lorena Baccaglini[1], Arjit Mohapatra[3], Subhranshu S. Mohapatra[3], Pravas R. Misra[3], Rama Chaudhry[8], Hegang H. Chen[9], Judith A. Johnson[10], J. Glenn Morris Jr[10], Nigel Paneth[11] & Ira H. Gewolb[12]

- *Lactobacillus plantarum* is immunomodulatory in cell cultures
- Blocks adherence and translocation of Gram-negative bacteria from the intestinal lumen into the bloodstream
- *L. plantarum* + FOS colonizes the neonatal gut
- *L. plantarum* + FOS prevents neonatal sepsis

Table 2 | Effect of synbiotic treatment on sepsis and other morbidities in the first 60 days of life

Outcome variables	Control $n = 2,278$ (%)	Synbiotic $n = 2,278$ (%)	RR (95% CI)	NNT (95% CI)	P value
Death and sepsis (primary outcome)	206 (9.0)	123 (5.4)	0.60 (0.48, 0.74)	27 (19, 47)	<0.001
Deaths	4 (0.2)	6 (0.3)	1.50 (0.42, 5.31)	NA†	0.526†
Sepsis (A + B + C)	202 (8.9)	117 (5.1)	0.58 (0.46, 0.72)	27 (19, 44)	<0.001

$1/day

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Future of probiotic design: mechanistic, reductionist, and complementary

- Inclusion of taxa that produce *secondary bile acids*
- Screening bacteria for production of *antimicrobial compounds*
- Screening bacteria for useful specific or broad *proteases*
- Select bacteria that *compete for nutrients*
- Experiment with coformulations: *synbiotics*
- Defined communities with *complementary actions*

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Conclusions

- Probiotics are generally safe and well tolerated
- Regulation as supplements and safety concerns impede deployment
- Much data are preliminary / preclinical
- Clinical data: Heterogeneity, low-quality of data, and safety concerns have precluded widespread deployment and recommendation by guidelines
- Clinical trials are expensive when outcomes are rare
- Newer directions focused on mechanisms of action show promise

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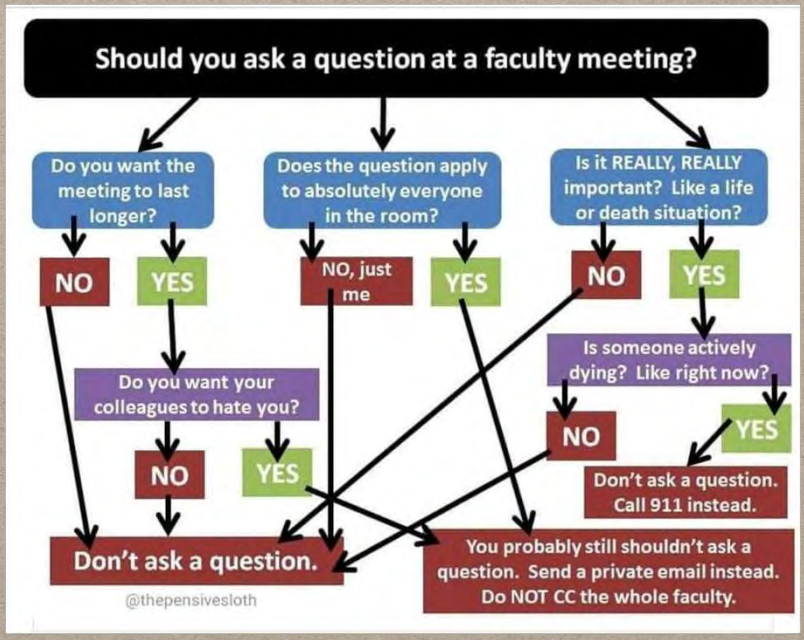
QUESTIONS? (THIS IS NOT A FACULTY MEETING)



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