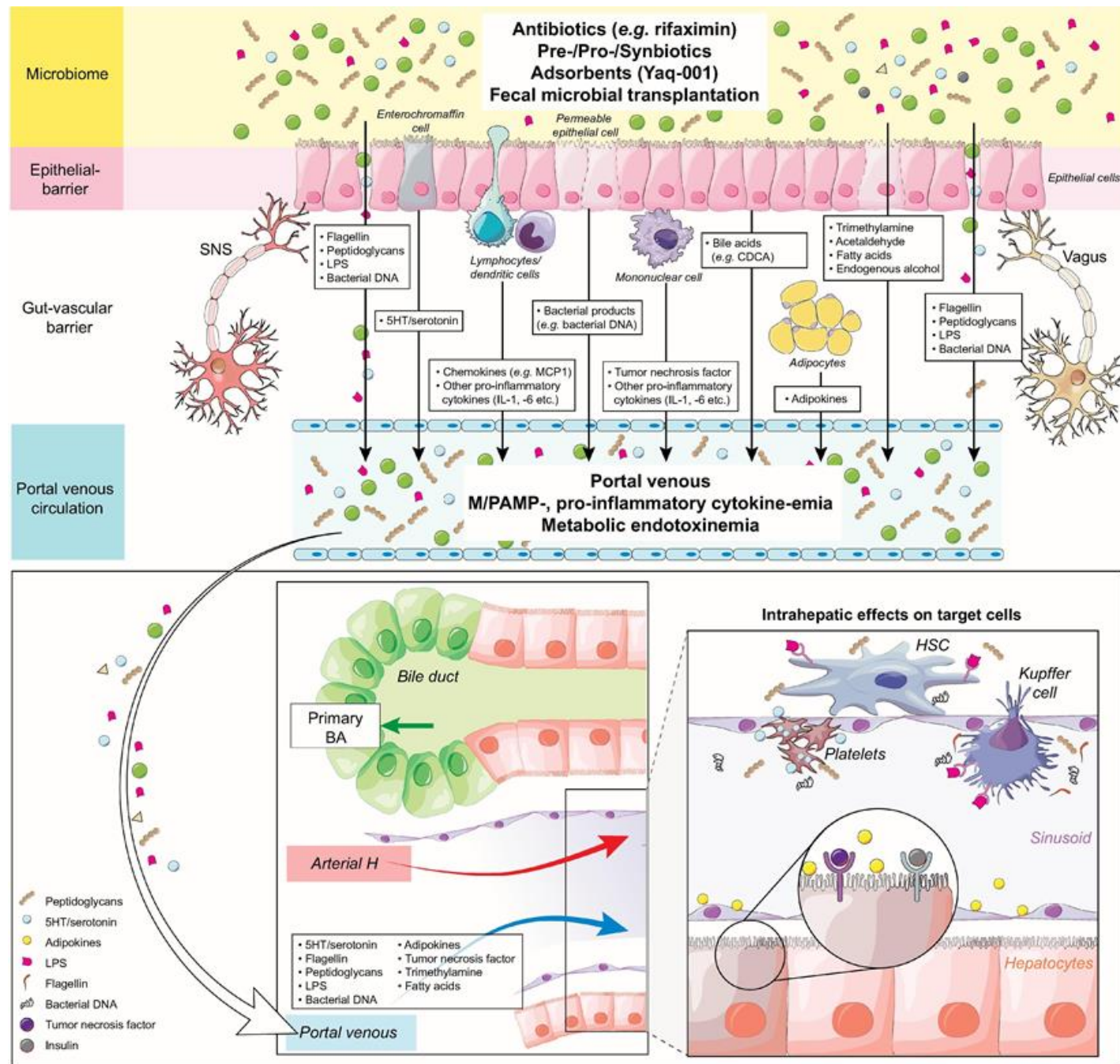


Pre and Probiotics in NASH

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Center,
Richmond, Virginia, USA

Outline

- Gut-Liver Axis changes in NAFLD
- Changes before and after cirrhosis
- Longitudinal changes
- Modulation using therapies
 - Probiotics
 - Prebiotics
 - Diet
 - Gastric bypass
 - Fecal microbiota transplant

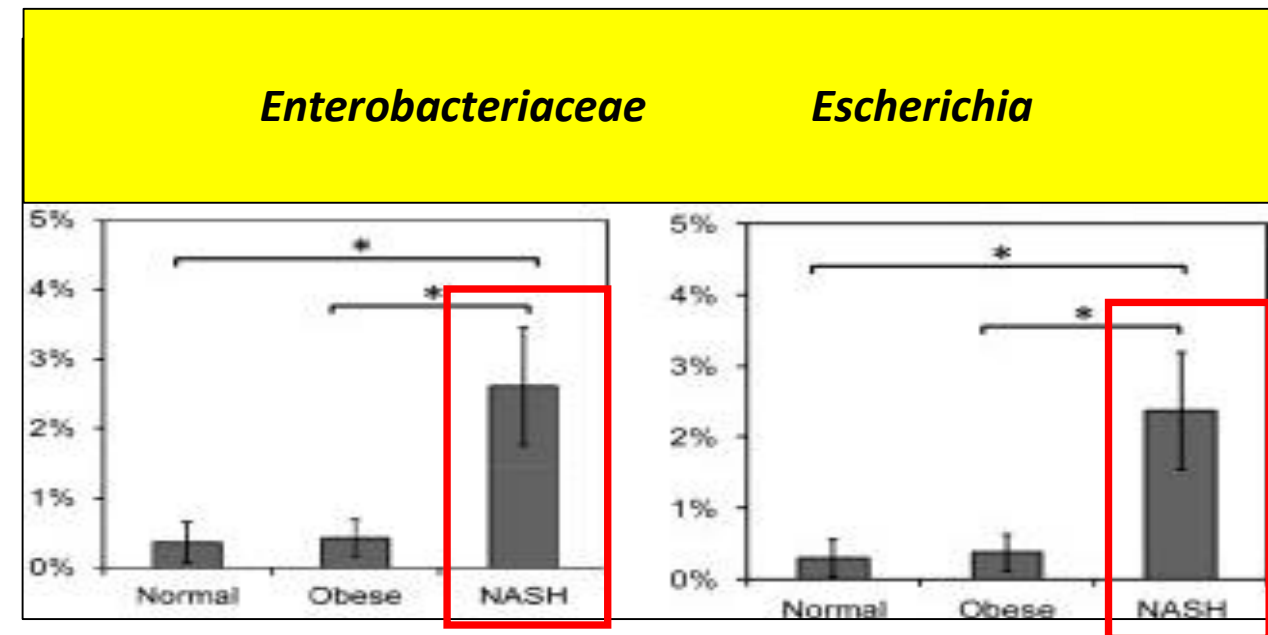
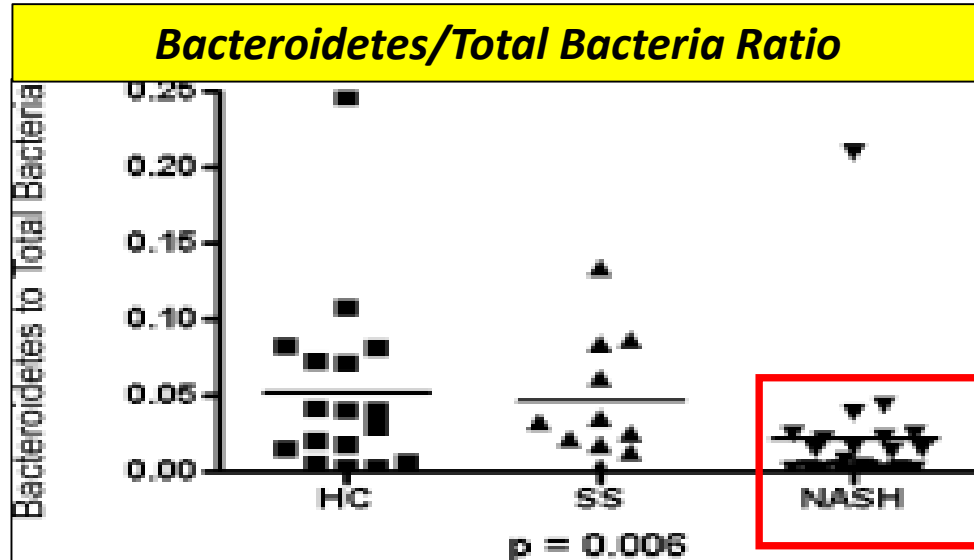


Altered gut-liver axis

Wiest, Albillos, Trauner, Bajaj and Jalan
J Hepatology 2018

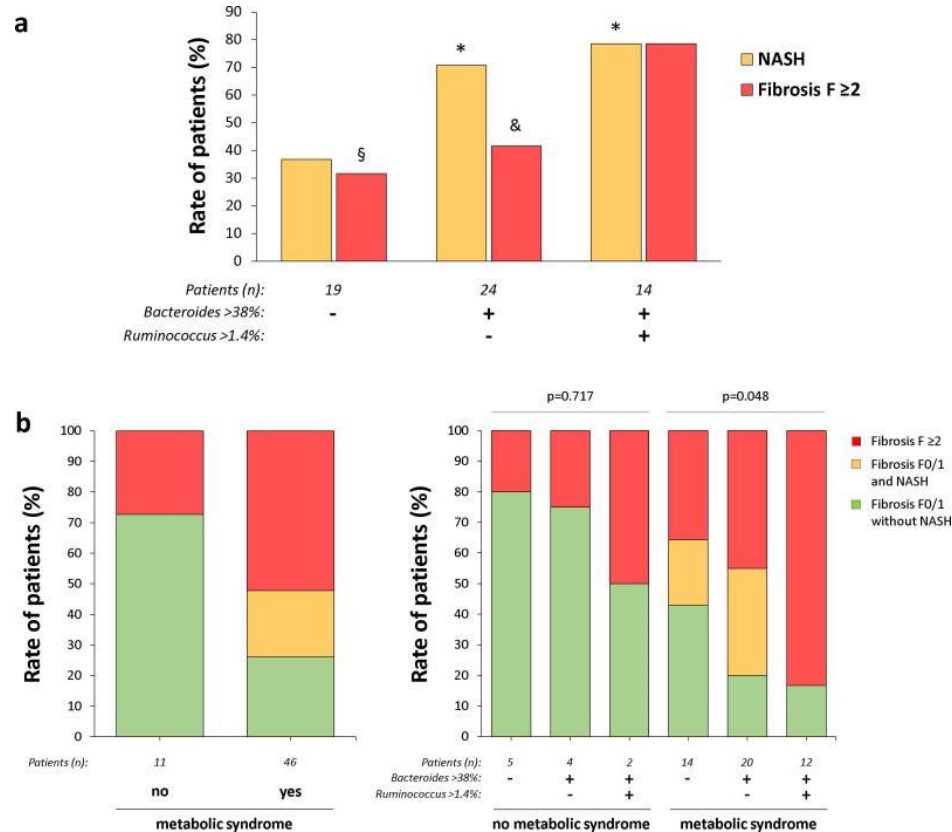
Stool microbiota composition is altered in pre-cirrhotic stages of NAFLD

Stool Microbial Families	Mean (NAFLD)	Mean (control)
Phylum_class_order_family		
Proteobacteria_Alphaproteobacteria_Kiloniellales_Kiloniellaceae	0.00000	0.00106
Proteobacteria_Gammaproteobacteria_Pasteurellales_Pasteurellaceae	0.00000	0.00572
Firmicutes_Bacilli_Lactobacillales_Lactobacillaceae	0.00000	0.01376
Firmicutes_Clostridia_Clostridiales_Lachnospiraceae	0.16210	0.22631
Firmicutes_Clostridia_Clostridiales_Ruminococcaceae	0.22077	0.15240
Bacteroidetes_Bacteroidia_Bacteroidales_Porphyromonadaceae	0.04519	0.02062
Firmicutes_Clostridia_Clostridiales_Veillonellaceae	0.01934	0.04953

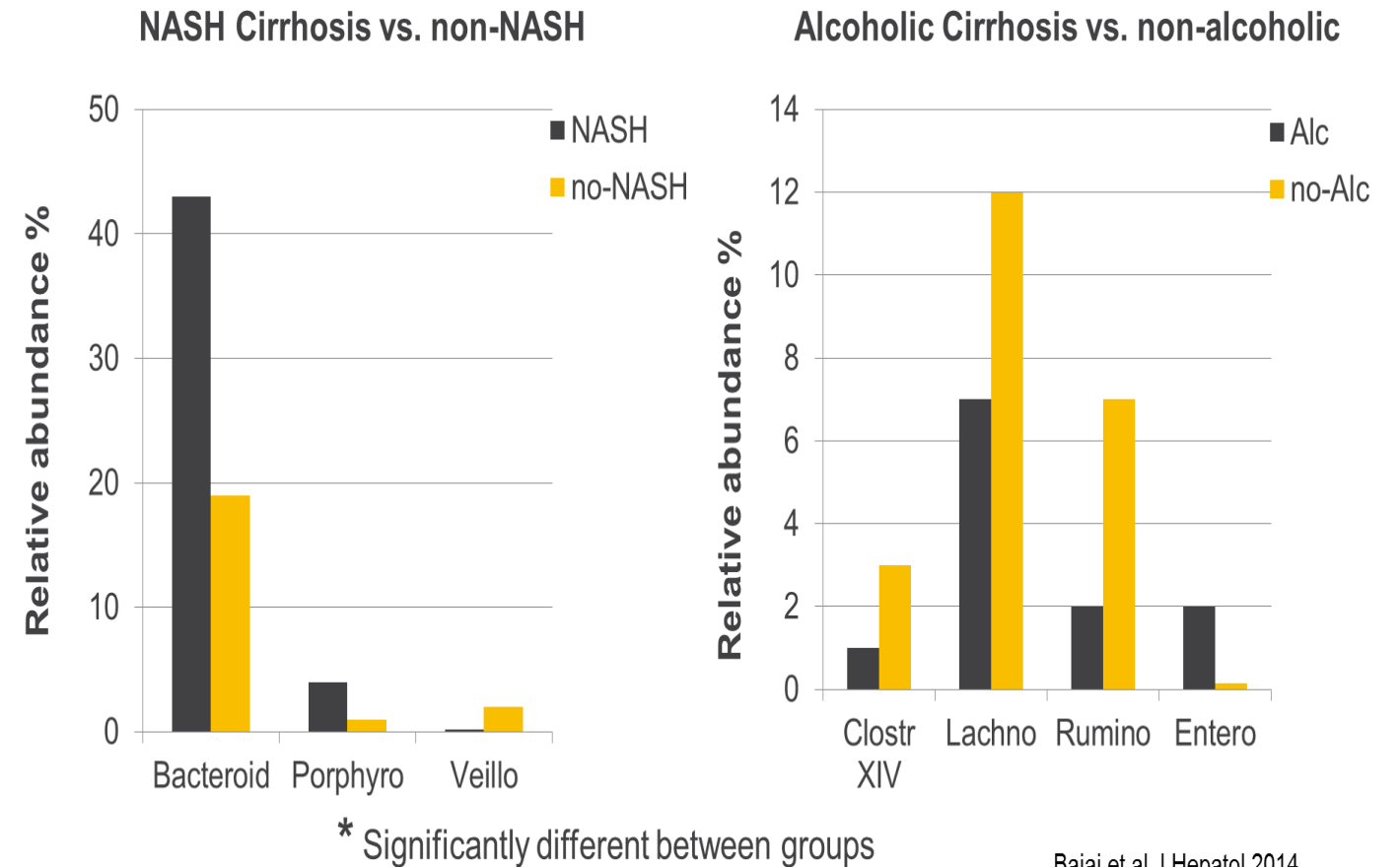


NASH-associated microbiota changes worsen with advancing fibrosis and are found even after cirrhosis development

NASH with/without Fibrosis F2



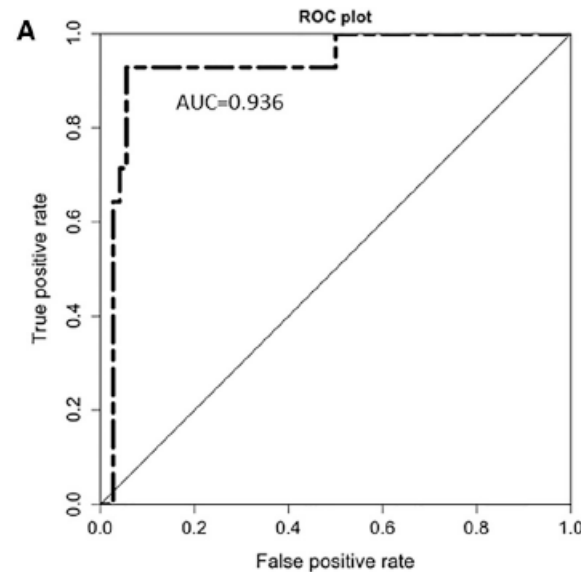
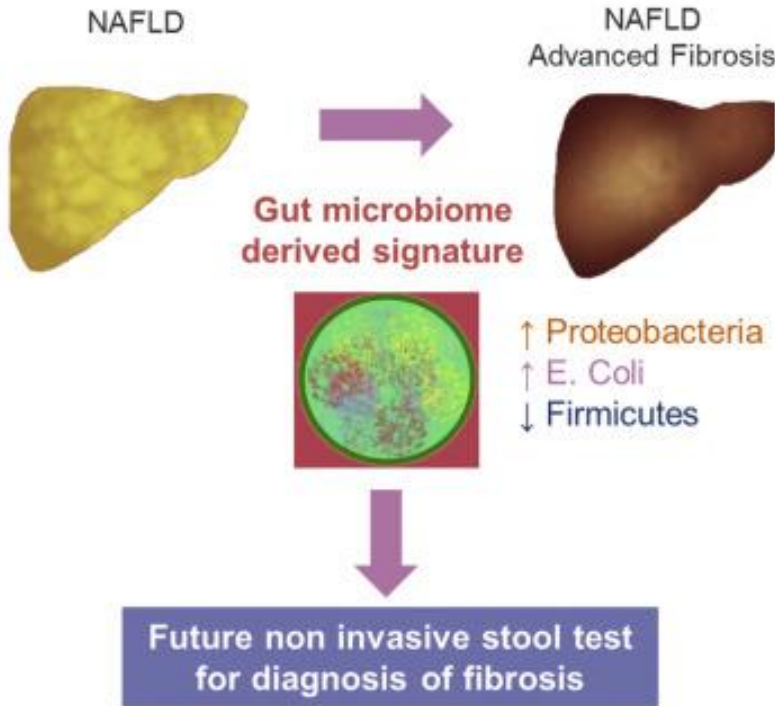
After cirrhosis development



Bajaj et al J Hepatol 2014

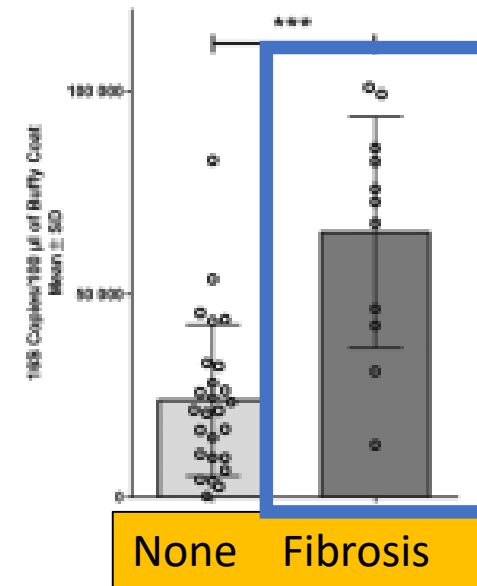
Stool microbiota and Blood microbiota can predict the presence of NAFLD in pilot studies

Gut Microbiota can detect advanced fibrosis in a small sample

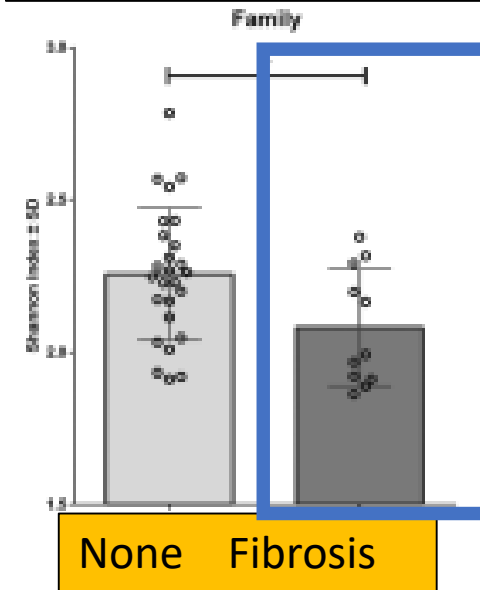


Blood Microbiota: Higher in quantity but lower in diversity in patients with NAFLD fibrosis

Copies in serum



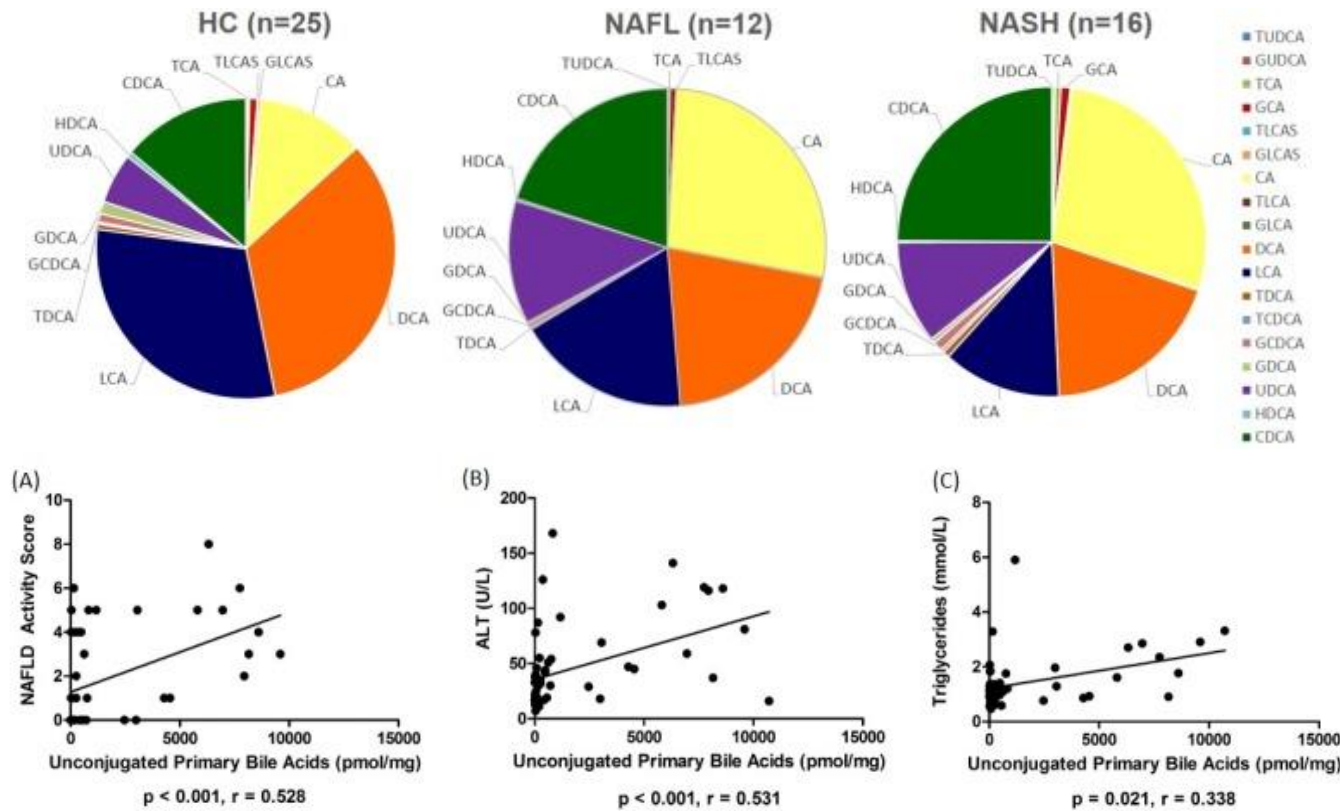
Serum Diversity



Need larger numbers and multi-center studies to increase the uptake of this methodology

Lelouvier et al 2015
Loomba et al 2017

Fecal Bile Acids change with worsening fibrosis but this may not be specific for NASH but rather for fibrosis

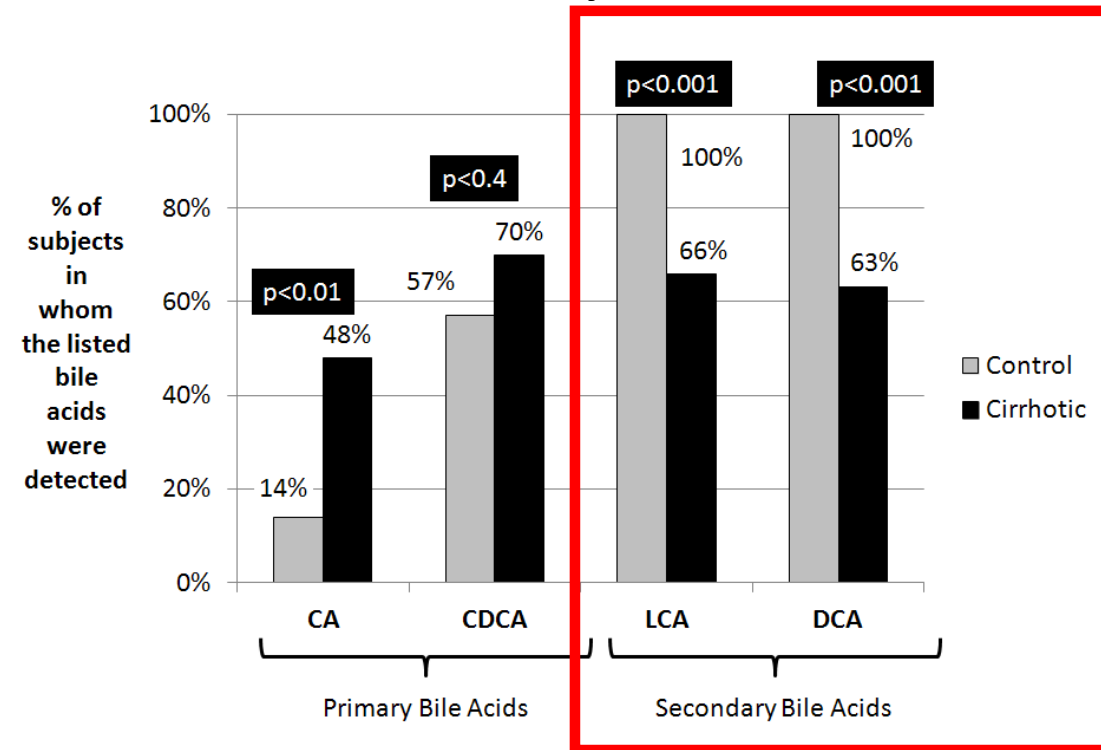


Fecal BAs in NAFLD compared to controls

- Higher total BAs
- Higher primary BAs
- Lower secondary BAs

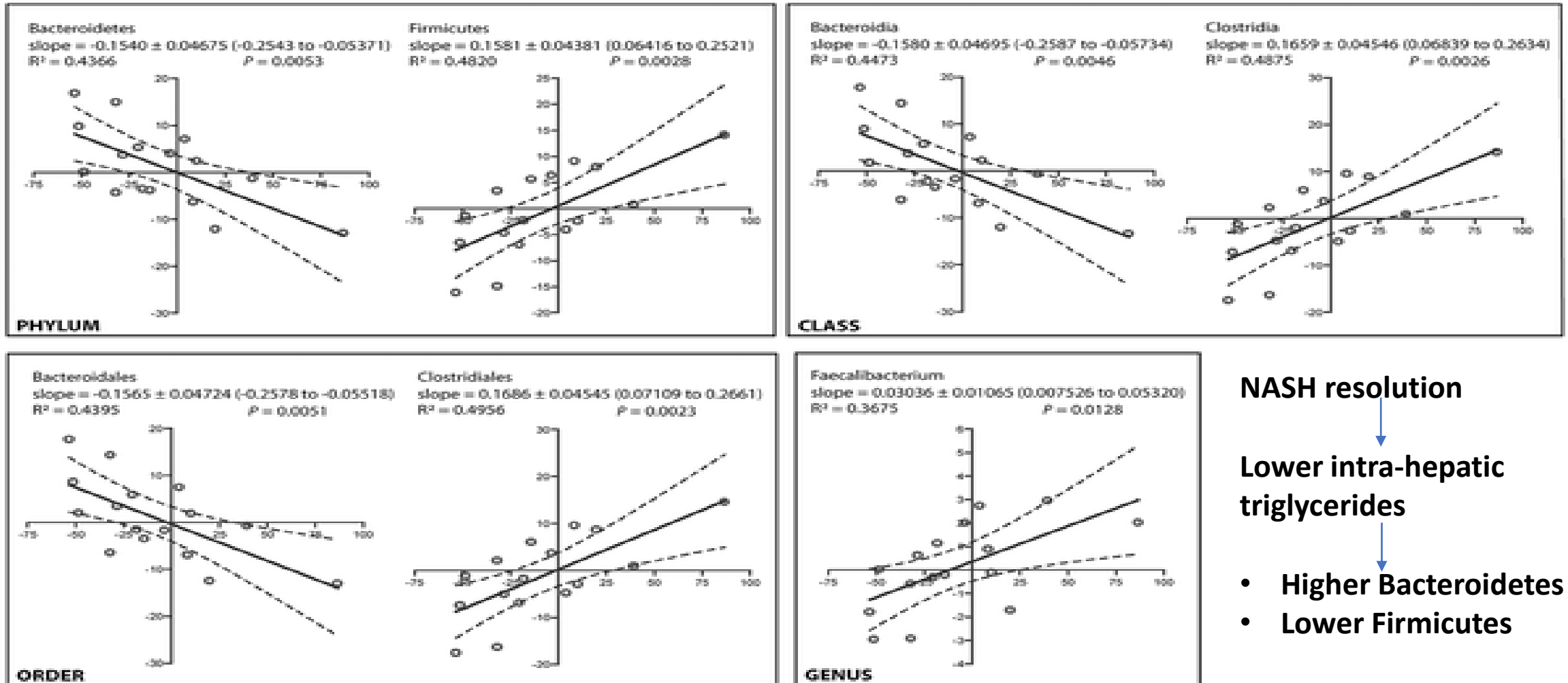
Cirrhosis due to any etiology in serum and stool

- Higher primary BAs
- Lower secondary BAs



Longitudinal change in microbiota in NASH

Correlation between the changes in intrahepatic triglyceride content and fecal bacterial abundance over 6 months.



NASH resolution

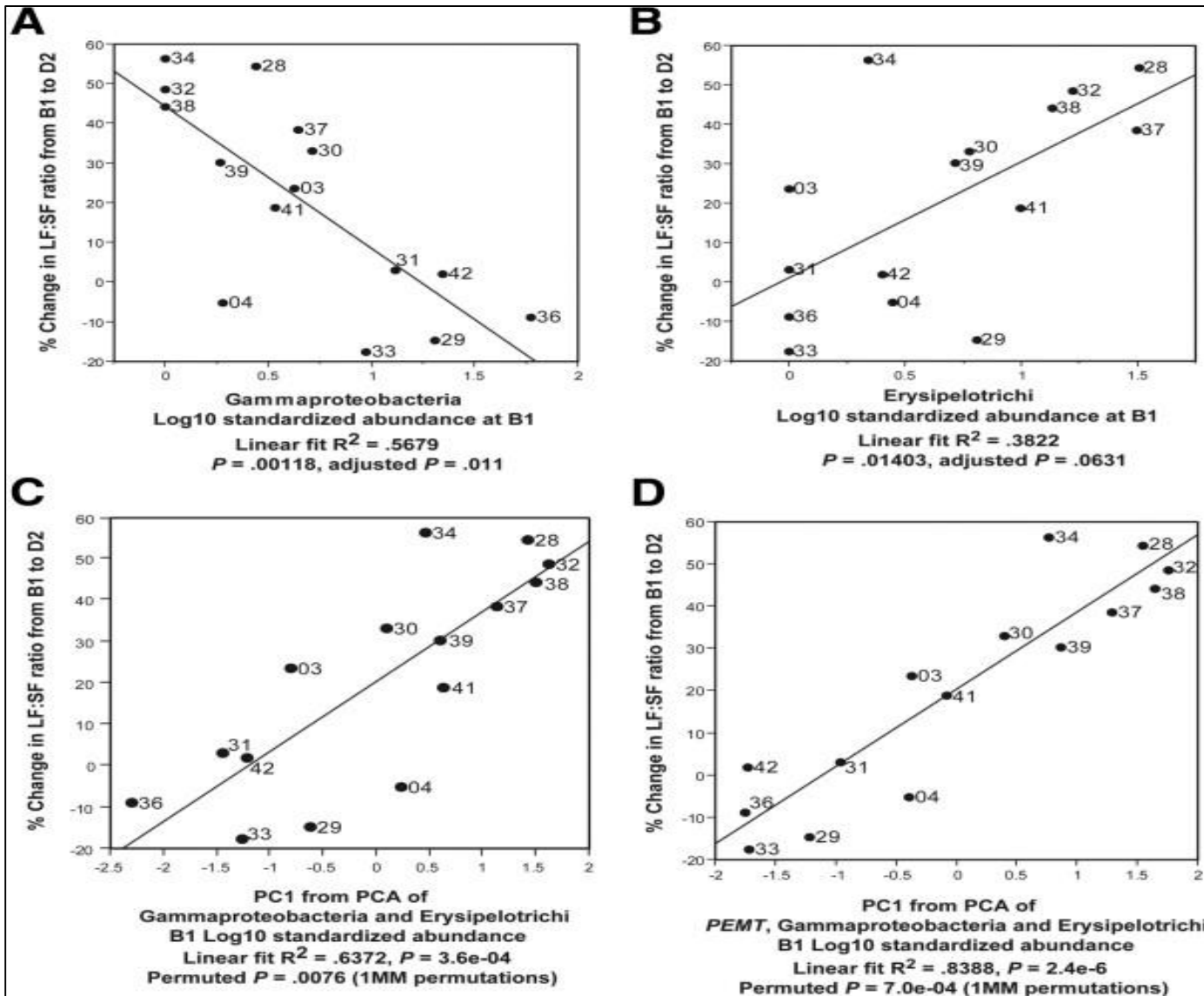
Lower intra-hepatic triglycerides

- **Higher Bacteroidetes**
- **Lower Firmicutes**

X Axis: Intra-hepatic TG content, Y-axis: relative abundance of microbiota

Wong VWS et al 2013

Choline deficiency-associated human NASH is associated with over-abundance of *Gammaproteobacteria*



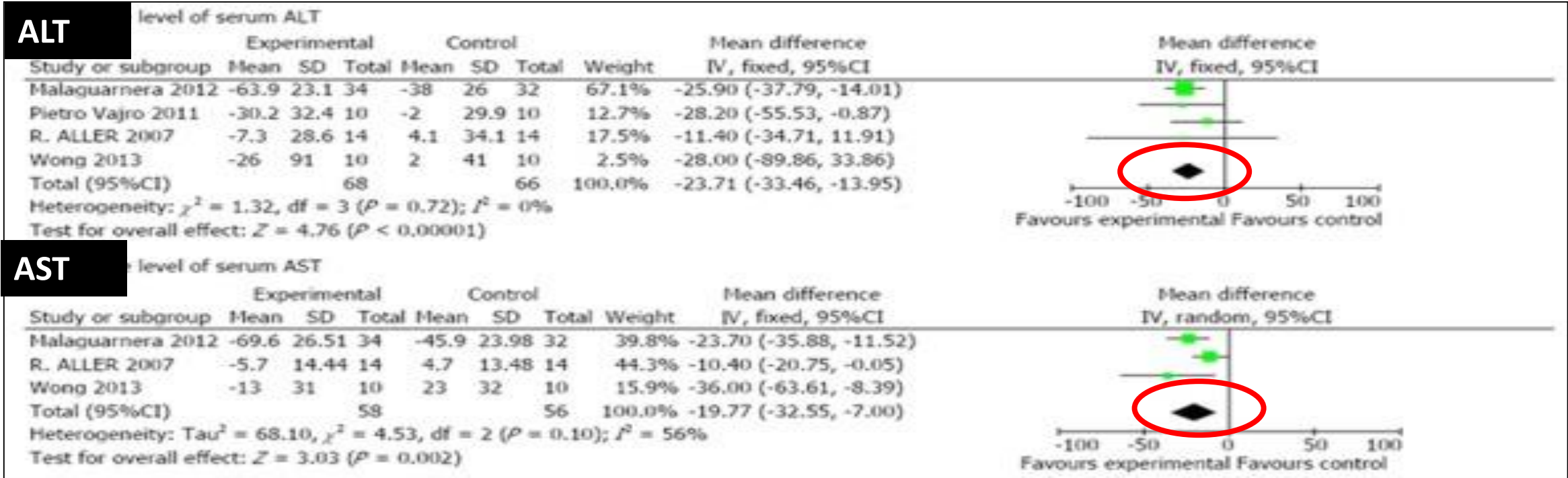
Gammaproteobacteria and *Erysipelotrichi* abundance, particularly when combined with subject genotype, predicts choline deficiency–induced fatty liver.

Pre/pro/syn/post Biotics and
NASH

Prebiotic, Probiotic, Synbiotic and Postbiotic

- **Prebiotic**: a substrate that is selectively utilized by host microorganisms conferring a health benefit
- **Probiotic**: live microorganisms that, when administered in adequate amounts, confer a health benefit on the host
- **Synbiotic**: Prebiotic+Probiotic
- **Postbiotic**: are non-viable bacterial products or metabolic byproducts from probiotic microorganisms that have biologic activity in the host.
 - Soluble factors (products or metabolic byproducts), secreted by live bacteria, or released after bacterial lysis, such as enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins, and organic acids.

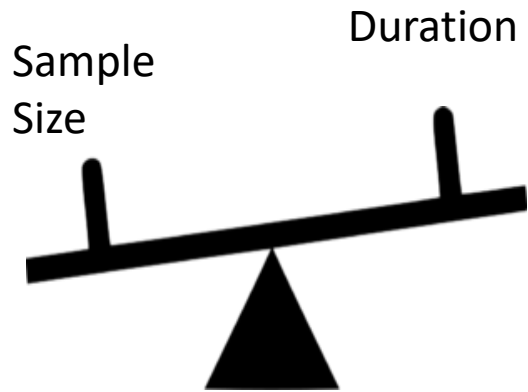
Probiotics and NASH: improvement in AST/ALT but different bugs used



4 Studies: 4 different pro/synbiotic preparations

1. Bifidobacterium longum with fructo-oligosaccharides
2. Lactobacillus rhamnosus strain GG,
3. Lepicol probiotic formula contained Lactobacillus plantarum, Lactobacillus deslbrueckii, Lactobacillus acidophilus, Lactobacillus rhamnosus and Bifidobacterium bifidum,
4. Lactobacillus bulgaricus and Streptococcus thermophilus

Probiotics and NASH: Similar trends continue



Few studies with histological endpoints

Koopman et al
APT 2019

Reference	Treatment	Subjects	Main findings	Limitations
Probiotics				
Vajro et al, (2011)	8 wk with <i>L. Rhamnosus</i> GG	Obese children with NAFLD (n = 20)	Significant decrease in ALT and anti-peptidoglycan-polysaccharide antibodies No effect on visceral adiposity	No liver biopsies taken Effect on intestinal permeability and LPS levels not assessed
Monem, (2017)	1 mo with <i>L. Acidophilus</i>	NASH patients (n = 30)	Significant decrease in ALT and AST	
Wong et al, (2013)	6 mo with a mixture of <i>L. Plantarum</i> , <i>L. Delbrueckii</i> spp. <i>Bulgaricus</i> , <i>L. Acidophilus</i> , <i>L. Rhamnosus</i> and <i>B. Bifidum</i>	NASH patients (n = 20)	Significant reduction in liver fat content and AST	
Aller et al, (2011)	3 mo with <i>L. Bulgaricus</i> and <i>S. Thermophilus</i>	NAFLD patients (n = 30)	Significant reduction of ALT, AST and γ -glutamine transferase	
Famouri et al, (2017)	12 wk with <i>L. Acidophilus</i> , <i>B. Lactis</i> , <i>B. Bifidum</i> and <i>L. Rhamnosus</i>	Obese children with NAFLD (n = 64)	Significant decrease of ALT and AST	No liver biopsies taken
Nabavi et al, (2017)	8 wk with a probiotic yoghurt containing <i>L. Acidophilus</i> and <i>B. Lactis</i>	NAFLD patients (n = 72)	Significant decrease in ALT and AST	No liver biopsies taken Absence of control group without yoghurt intake
Alisi et al, (2014)	4 mo with VSL#3	Obese children with NAFLD (n = 48)	Improved liver function and increased levels of GLP 1/ active GLP-1. No improvement in triglycerides, HOMA and ALT	
Loguercio et al, (2005)	3 mo with VSL#3	NAFLD patients (n = 22)	Improved plasma levels of lipid peroxidation markers malondialdehyde and 4-hydroxynonenal Improvement in oxidative stress	
Solga et al, (2008)	4 mo with VSL#3	NAFLD patients (n = 4)	Increase in hepatic fat accumulation in 3 out of 4 subjects, after washout, a decrease in liver fat was observed	Small study size

Prebiotics and NASH: Small number of studies

Prebiotics

Daubioul et al, (2005)	8 wk with oligofructose	NASH patients (n = 7)	Decreased serum ALT and AST Decreased insulin levels	No post-treatment liver histology Small study size
Parnell et al, (2011) Review of different studies	Inulin and/or oligofructose	Healthy subjects (5 studies) T2DM patients (8 studies)	Two studies reported a decrease in serum triglycerides or cholesterol after supplementation in healthy subjects. In patients with T2DM, improvements were more robust and reported in six of eight studies. Oligofructose supplementation resulted in weight loss in humans and improvements in glycaemia and modifications in plasma GLP-1, PYY and ghrelin	Not all studies included histological measurements

Koopman et al
APT 2019

Synbiotics and NASH:

Similar trends but some studies are for a longer duration

Synbiotics				
Eslamparast et al, (2014)	28 wk with a combination of <i>L Casei</i> , <i>L Rhamnosus</i> , <i>S Thermophilus</i> , <i>B Breve</i> , <i>L Acidophilus</i> , <i>B Longum</i> ,	NAFLD patients (n = 52)	Significantly decreased ALT levels, inhibition of NF- κ B activation and reduced TNF- α	No liver biopsies taken Effects on gut microbiota composition not evaluated
Malaguarnera et al, (2012)	24 wk with <i>B Longum</i> and oligofructose	NASH patients (n = 66)	Reduced TNF- α , CRP, endotoxin and AST levels. Improvement of insulin resistance and NASH activity index	Effects on gut microbiota composition not evaluated
Mofidi et al, (2017)	28 wk with a combination of <i>L Casei</i> , <i>L Rhamnosus</i> , <i>S Thermophilus</i> , <i>B Breve</i> , <i>L Acidophilus</i> , <i>B Longum</i> , <i>L Bulgaricus</i> and oligofructose	Lean individuals with NAFLD (n = 50)	Improvements in levels of fasting blood glucose, TAG, and inflammatory cytokines	No liver biopsies taken
Asgharian et al, (2016)	8 wk with a combination of <i>L Casei</i> , <i>L Rhamnosus</i> , <i>S Thermophilus</i> , <i>B Breve</i> , <i>L Acidophilus</i> , <i>B Longum</i> , <i>L Bulgaricus</i> and oligofructose	NAFLD patients (n = 80)	Reduction in hepatic steatosis measured by ultrasound No changes in ALT and AST	Limited duration of treatment No liver biopsies taken
Manzhalii et al, (2017)	12 wk with <i>L Casei</i> , <i>L Rhamnosus</i> , <i>L Bulgaris</i> , <i>B Longum</i> and <i>S Thermophilus</i> and oligofructose	NASH patients (n = 75)	Significant reduction of ALT and liver stiffness	No liver biopsies taken Non-blinded trial

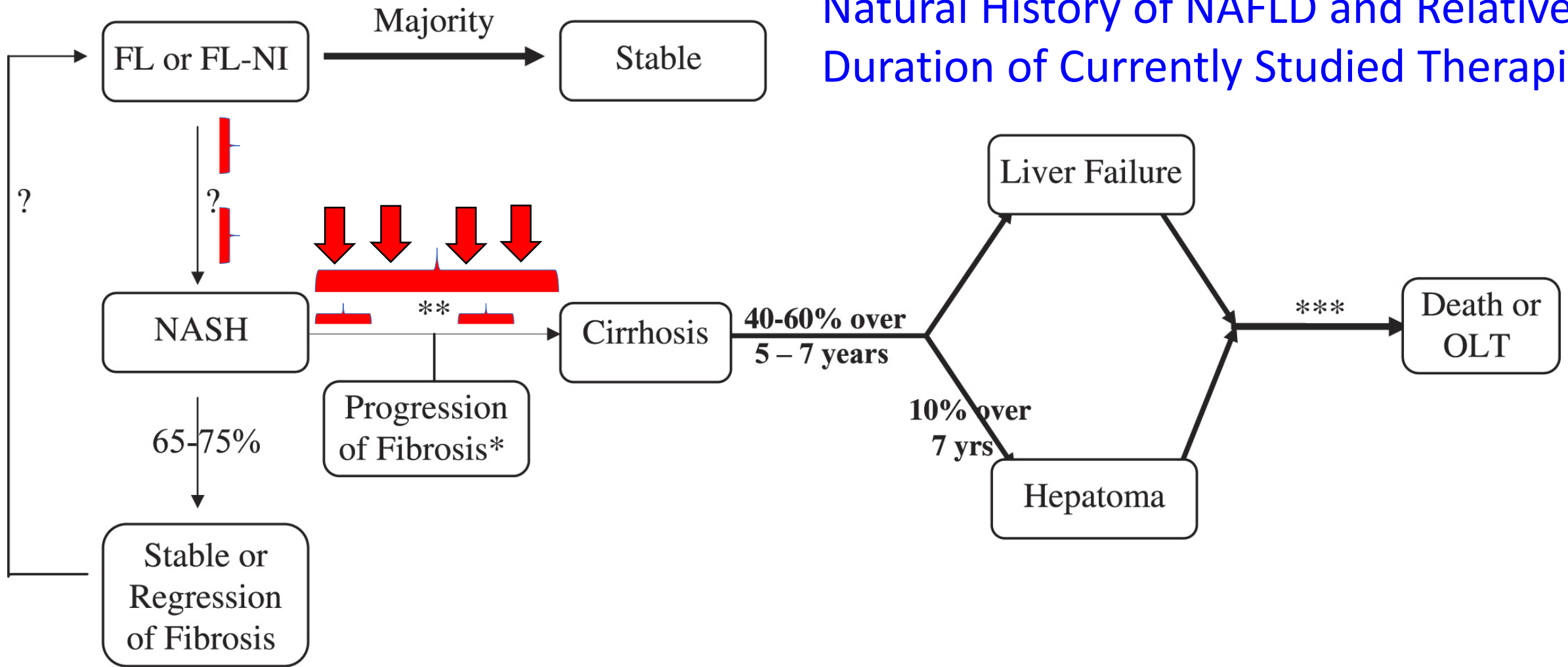
Non-cirrhosis NASH therapy outlines per FDA

- Early Phase 2: +/- histology with proof of concept
- Late Phase 2: 12-18 months + histological change and biomarkers

Ideal Probiotic Study

- Defined strains with minimal variations in CFU per dose/batches
- Engraftment, localized and systemic actions known
- Changes in microbiota composition and function assessed throughout
- Probiotic(s) organisms recovered from stool
- Clinically relevant endpoint

Natural History of NAFLD and Relative Duration of Currently Studied Therapies



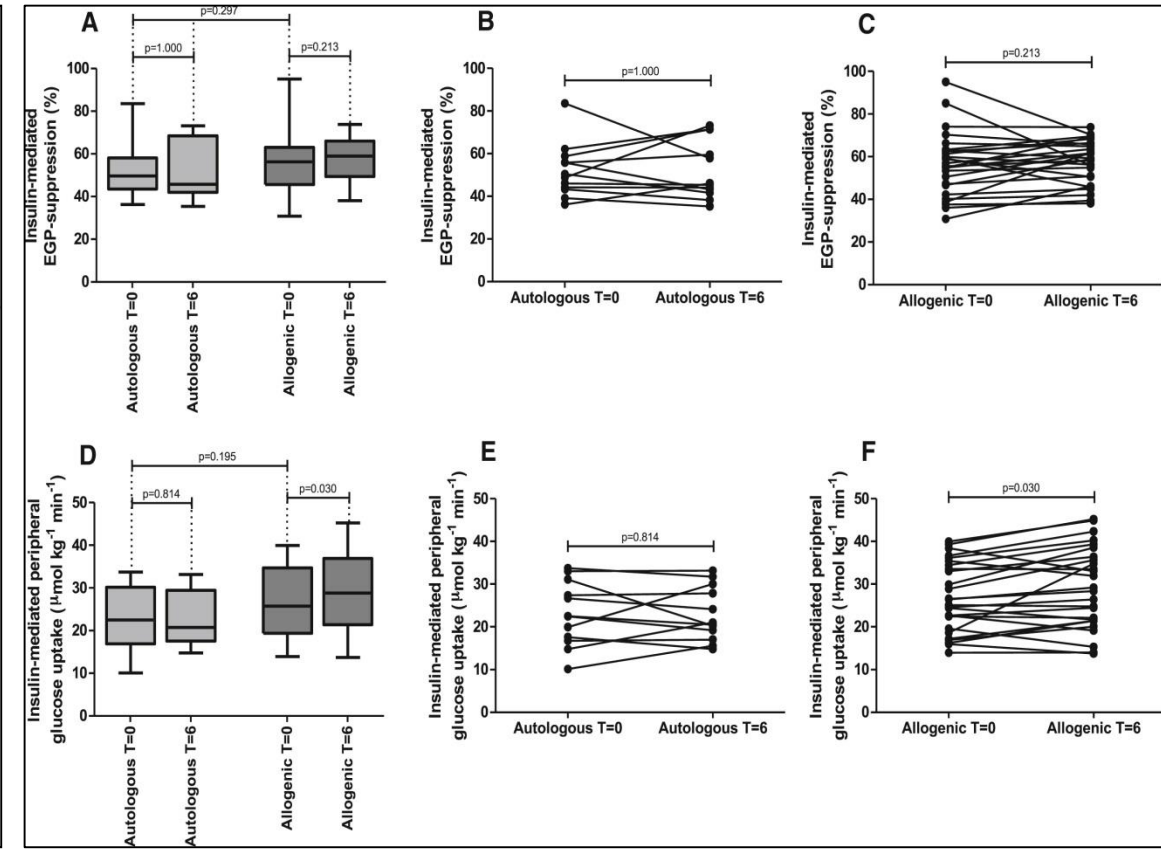
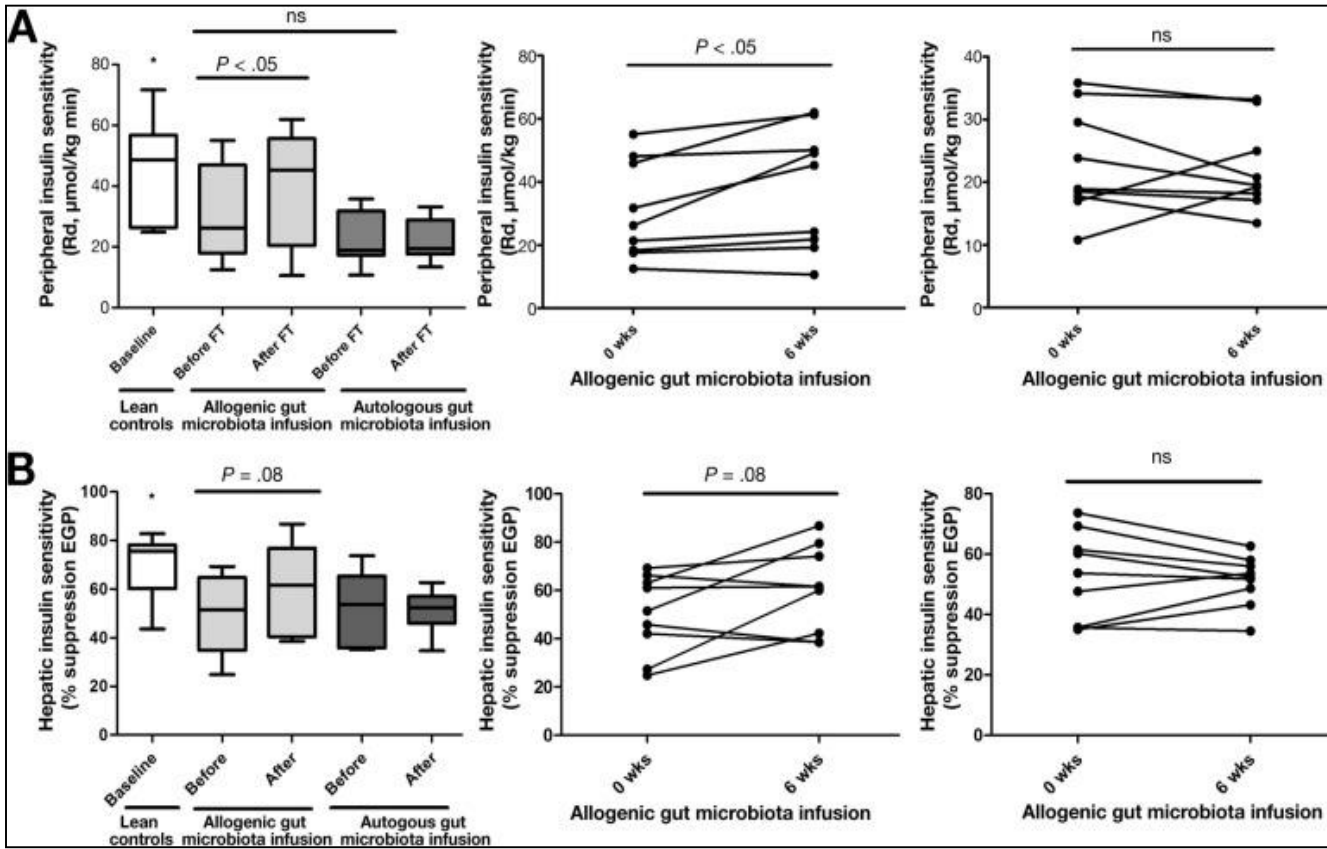
Ong and Younossi
CLD 2018

* 25% to 35% of NASH patients have progression of fibrosis

** 9% to 20% of NASH patients progress to cirrhosis

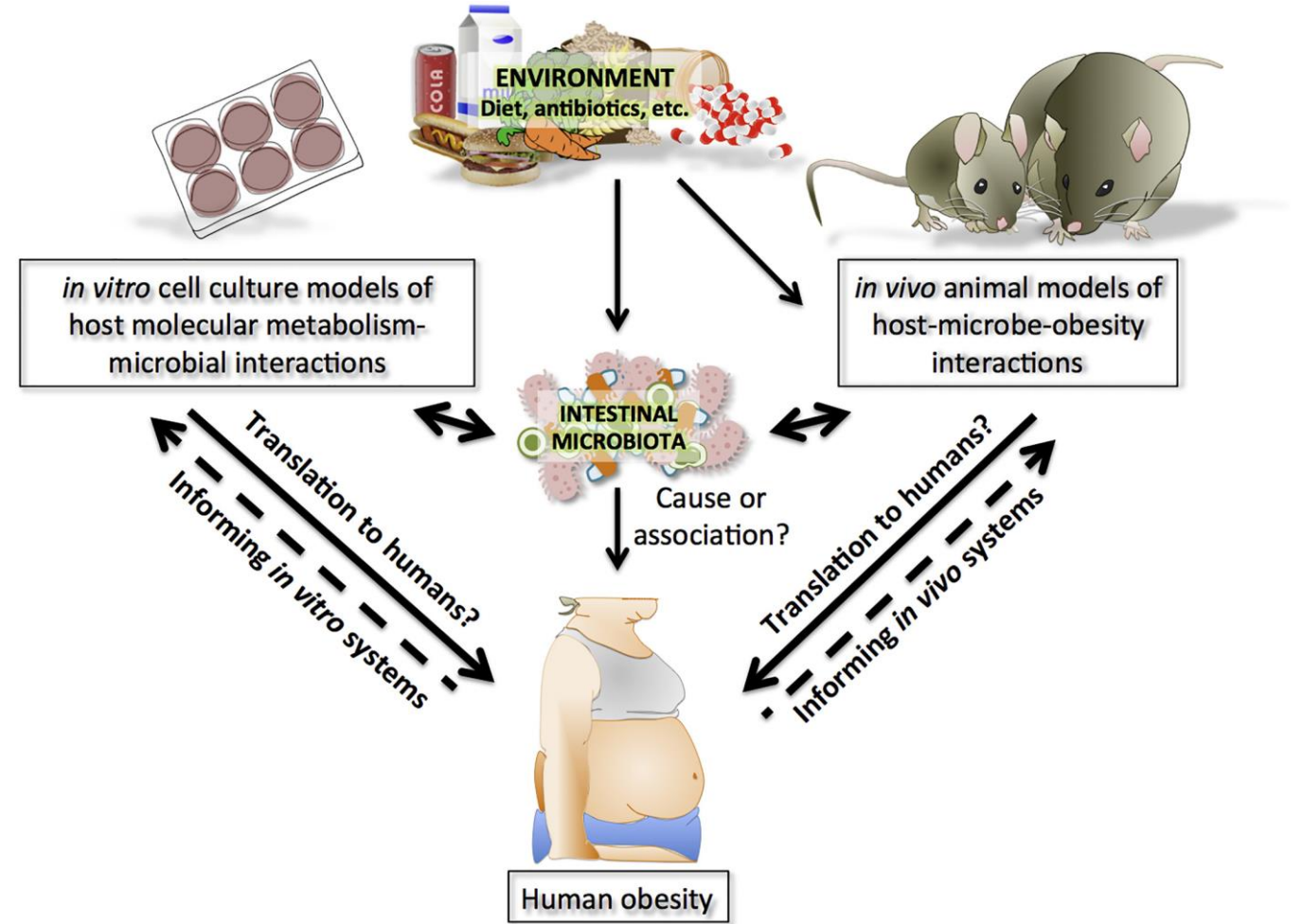
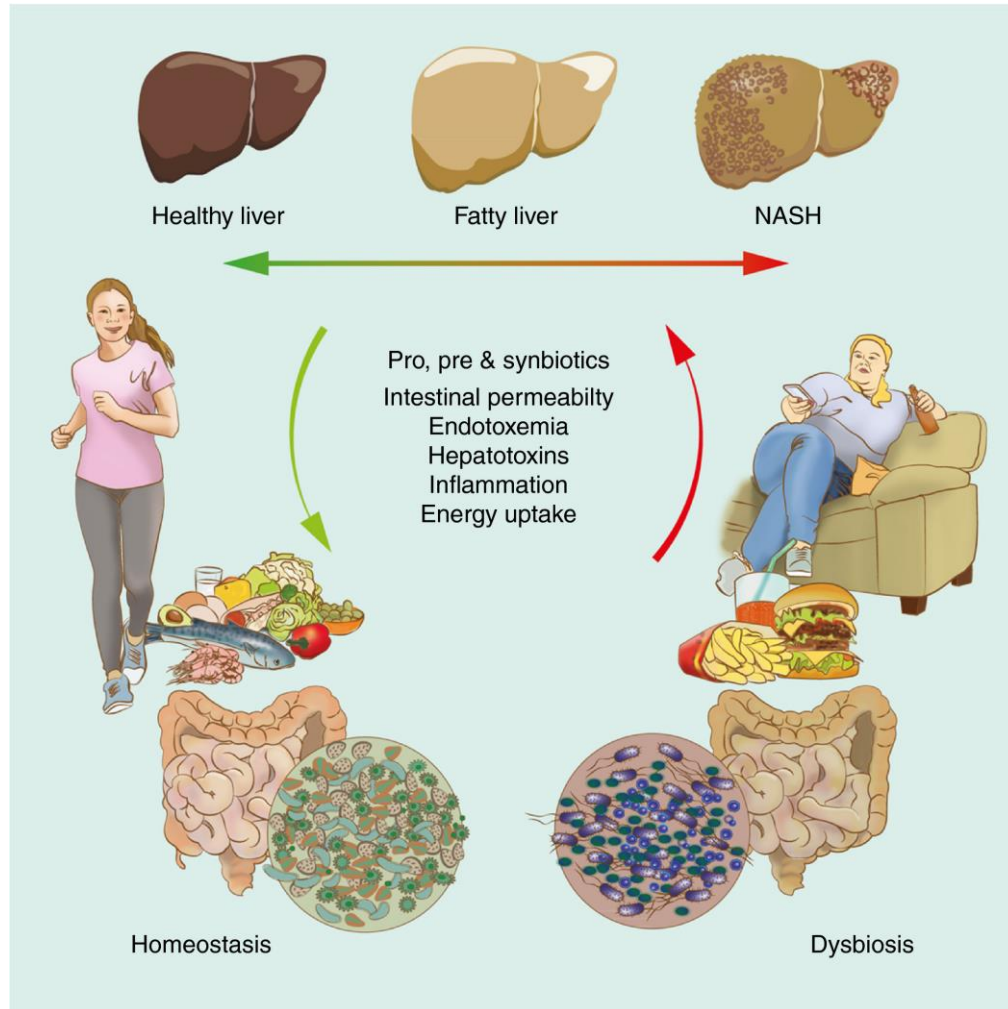
*** 22% to 33% of cirrhotics die of complications of liver failure or require a liver transplant (OLT)

Metabolic Syndrome (associated with NAFLD) and FMT: Improvement in Insulin Sensitivity Compared to Autologous FMT



However, the durability is short-term and NASH/NAFLD was not an inclusion criterion

We need to go beyond association



Summary

- NASH is a multi-faceted disorder which is associated with an altered gut-liver axis
- Bile acid perturbations and microbiota compositional changes worsen over the course of the fibrosis severity, with more extreme changes towards cirrhosis
- Treatments for NASH are increasingly focused on the gut-liver axis with modulation of bile acids
- At this time there is inconclusive evidence that using pre, pro or synbiotics can improve NASH from a regulatory standpoint although there is enough proof of concept
- FMT studies in NASH are undergoing but are also not conclusive
- Gut microbiota changes in NASH will likely be a part and parcel of an overall strategy to treat metabolic syndrome

Obrigado

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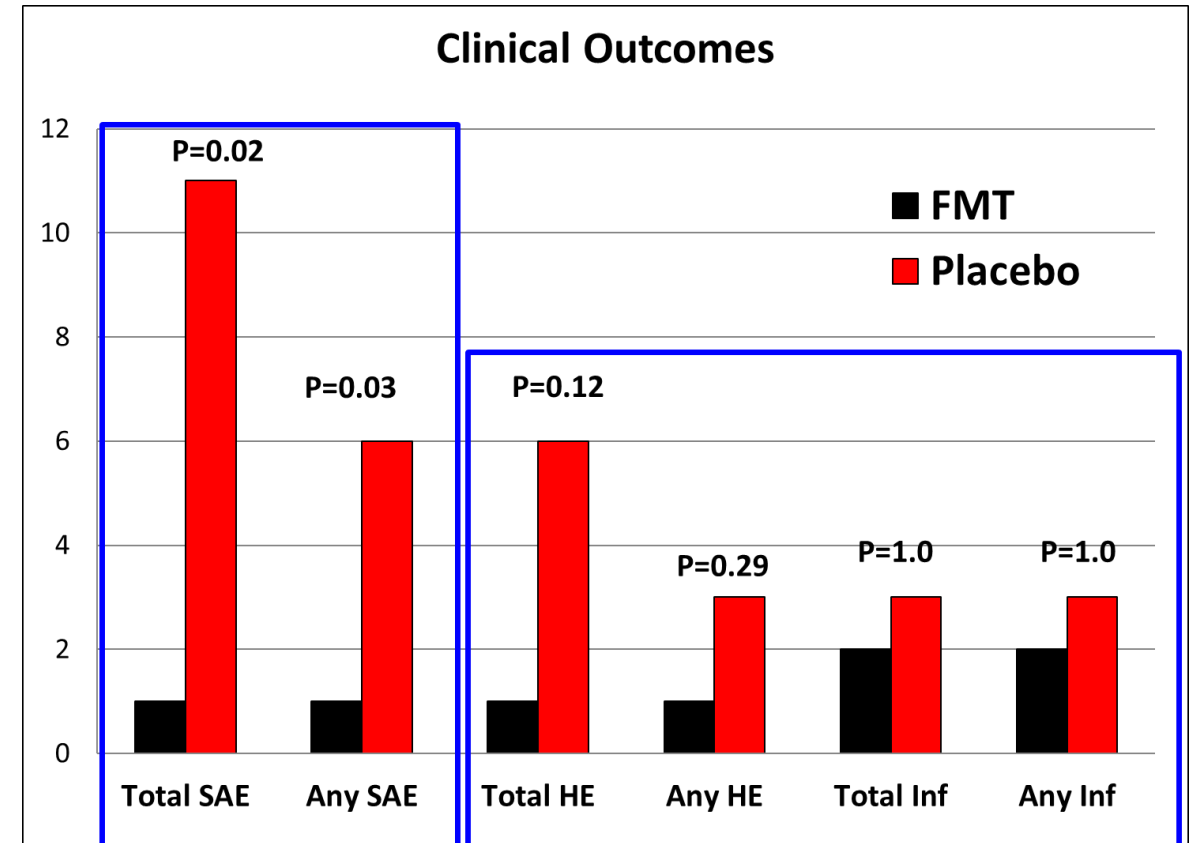
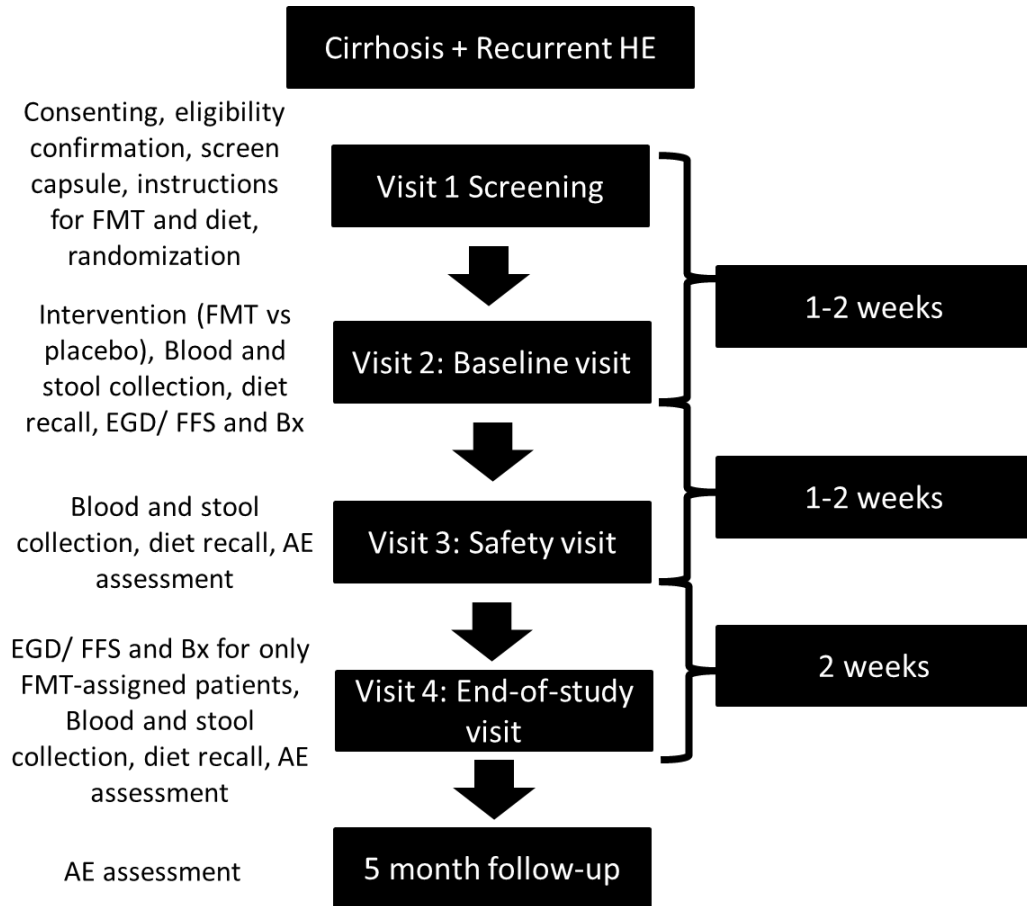
UC San Diego

B Schnabl

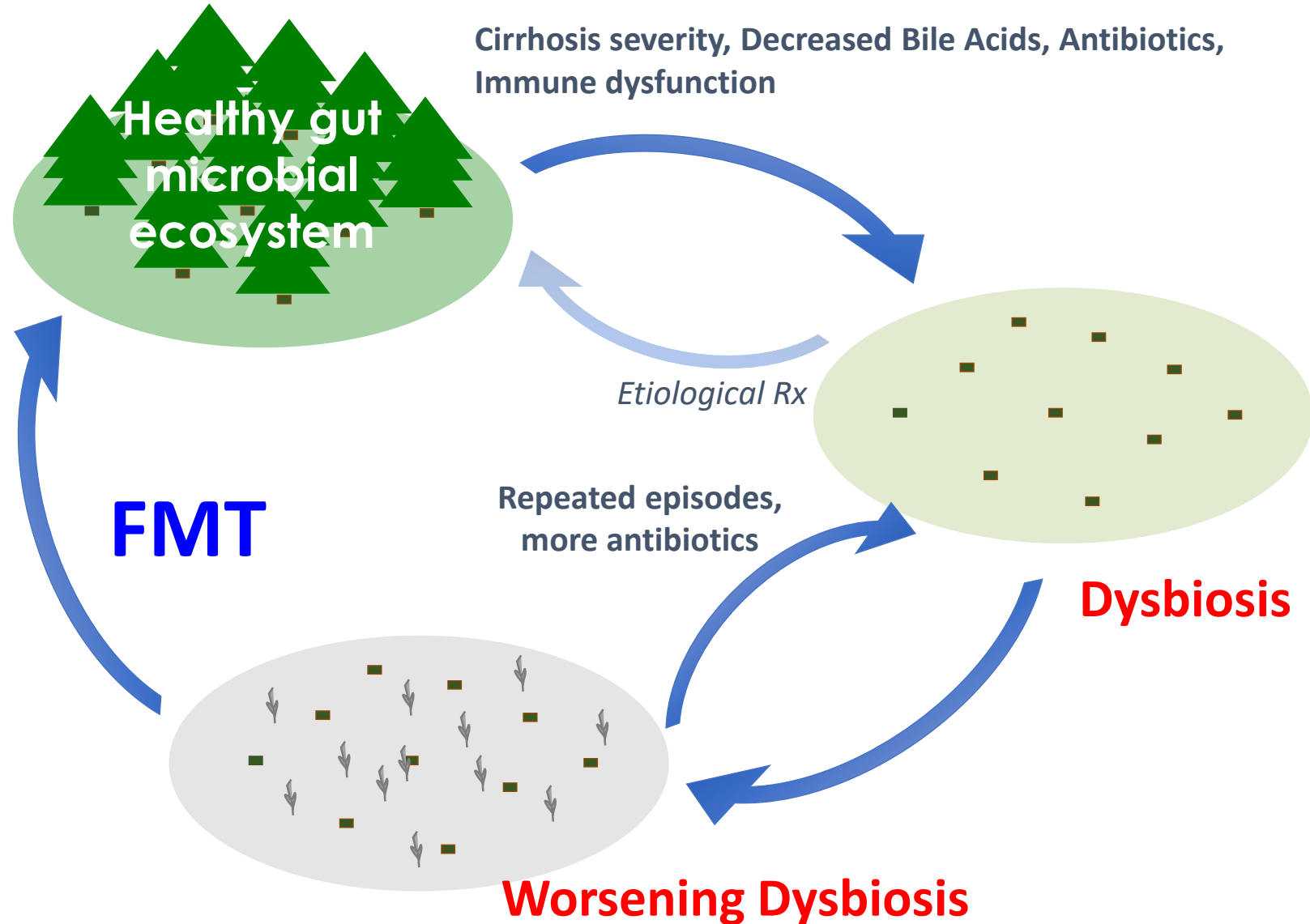
University of California, Davis

Oliver Fiehn
Sili Fan

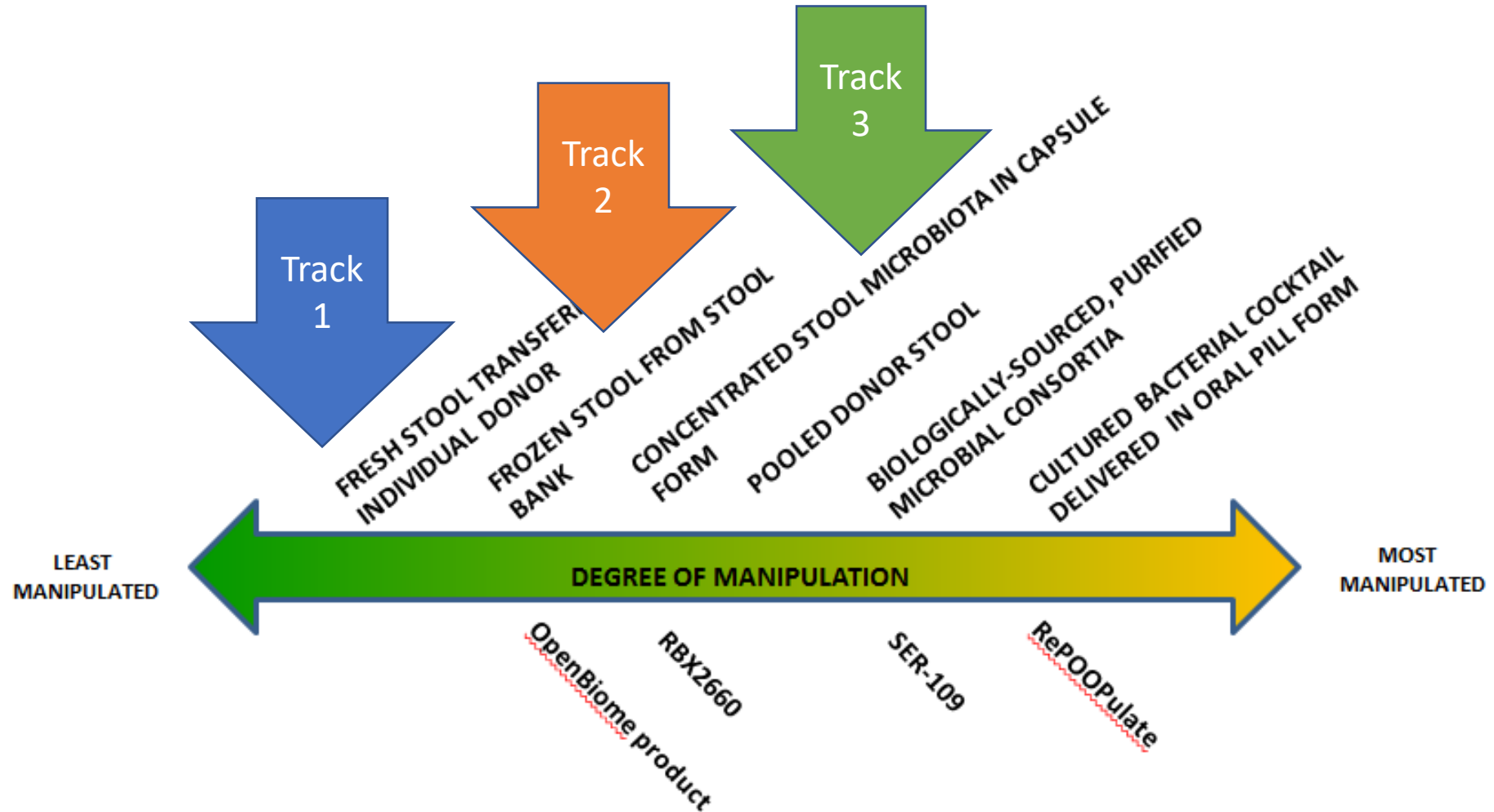
Oral capsular FMT is safe and shows benefit in HE



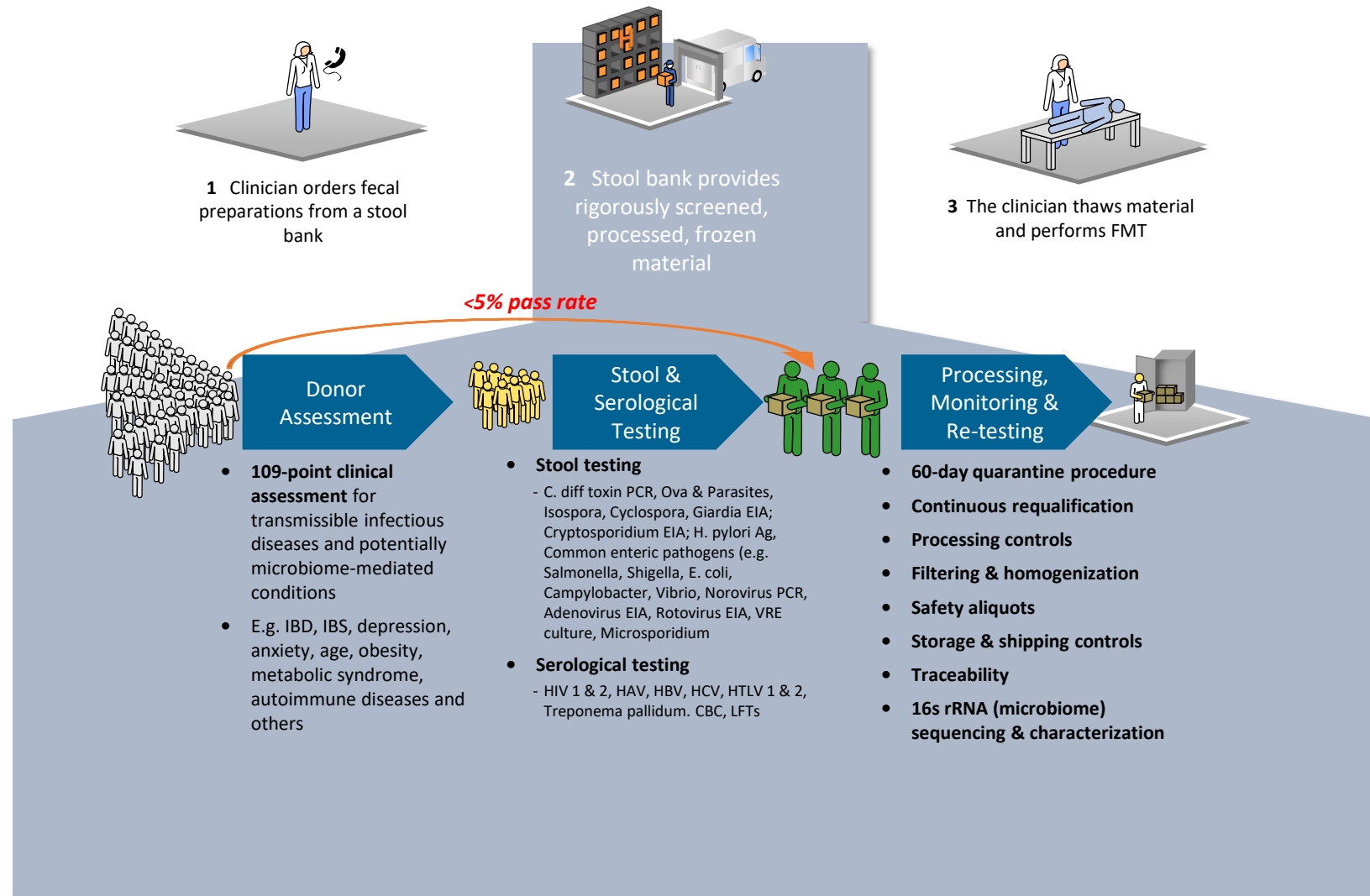
The mechanics of FMT in Cirrhosis



Spectrum of New Products

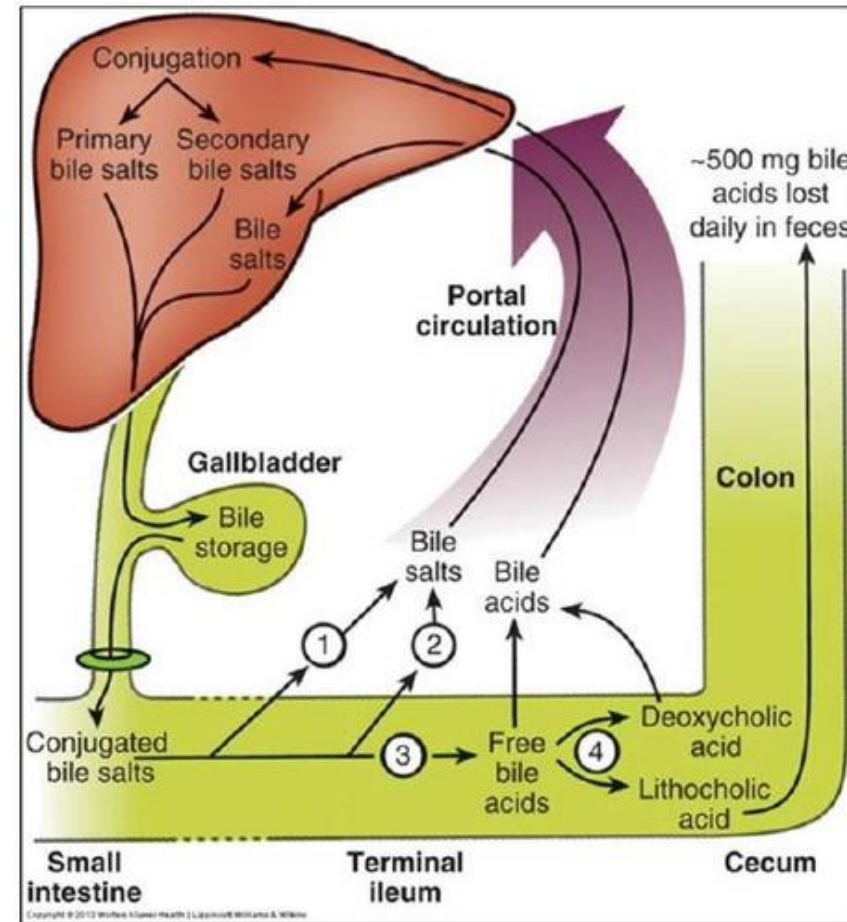


Frozen stool from a bank: OpenBiome Model



Restores Bile Acid Metabolism

- Niche exclusion: Competition for nutrients and space
- Direct suppression by antimicrobial peptides (bacteriocins)
- Activation of immune-mediated colonization resistance
- Effects on Bile-acid composition
 - Inhibition of spore germination (lithocholate)
 - Toxic to vegetative forms (deoxycholate)



Safety of FMT



- **Favorable short-term safety profile**
 - Non-severe, transient AEs common
 - Nausea, bloating, constipation, abdominal discomfort
- **Risks related to the procedure**
 - Perforation
 - Bleeding
 - Sedation-related complications
- **Risk of infection**
 - Multicenter retrospective study: No infections in 80 “high risk” immunocompromised patients
 - Peritonitis, bacteremia (E coli, Proteus, Klebsiella, Listeria)
 - CMV following home FMT (stool from unscreened infant)
 - Regurgitation of stool, fatal aspiration pneumonia with UGI

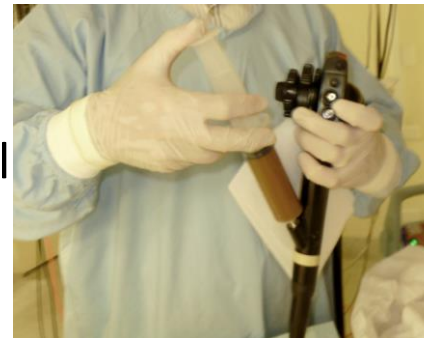
Encapsulated FMT



- Encapsulated stool
 - Kao et al JAMA 2017: As effective as FMT delivered by colonoscopy (96%)
 - Cheaper and preferable to patients
- Lyophilized, encapsulated fecal microbiota
 - 43/49 patients (88%) achieved clinical success at 8 weeks
 - As few as 2-3 capsules were effective
 - Several encapsulated products are now clinical trials

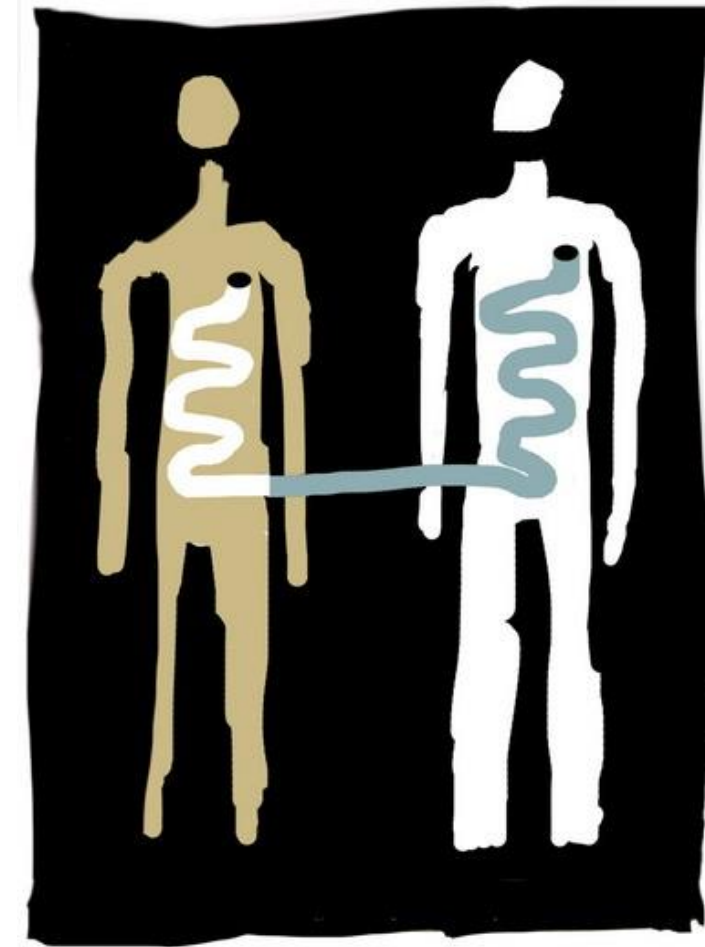
FMT 101

- Identify and screen a donor
 - Healthy, low risk, no recent antibiotics
 - Testing for infections (blood and stool)
- Collect and prepare fecal material
 - Diluents (saline, water)
 - Mixing & filter
 - Dose: 50-100 g (volume 50-500 ml)
- Administer the donor material to the patient
 - Nasogastric/nasointestinal tube
 - Endoscopic (upper or lower)
 - Retention enema
 - Capsules



Fecal Microbiota Transplantation

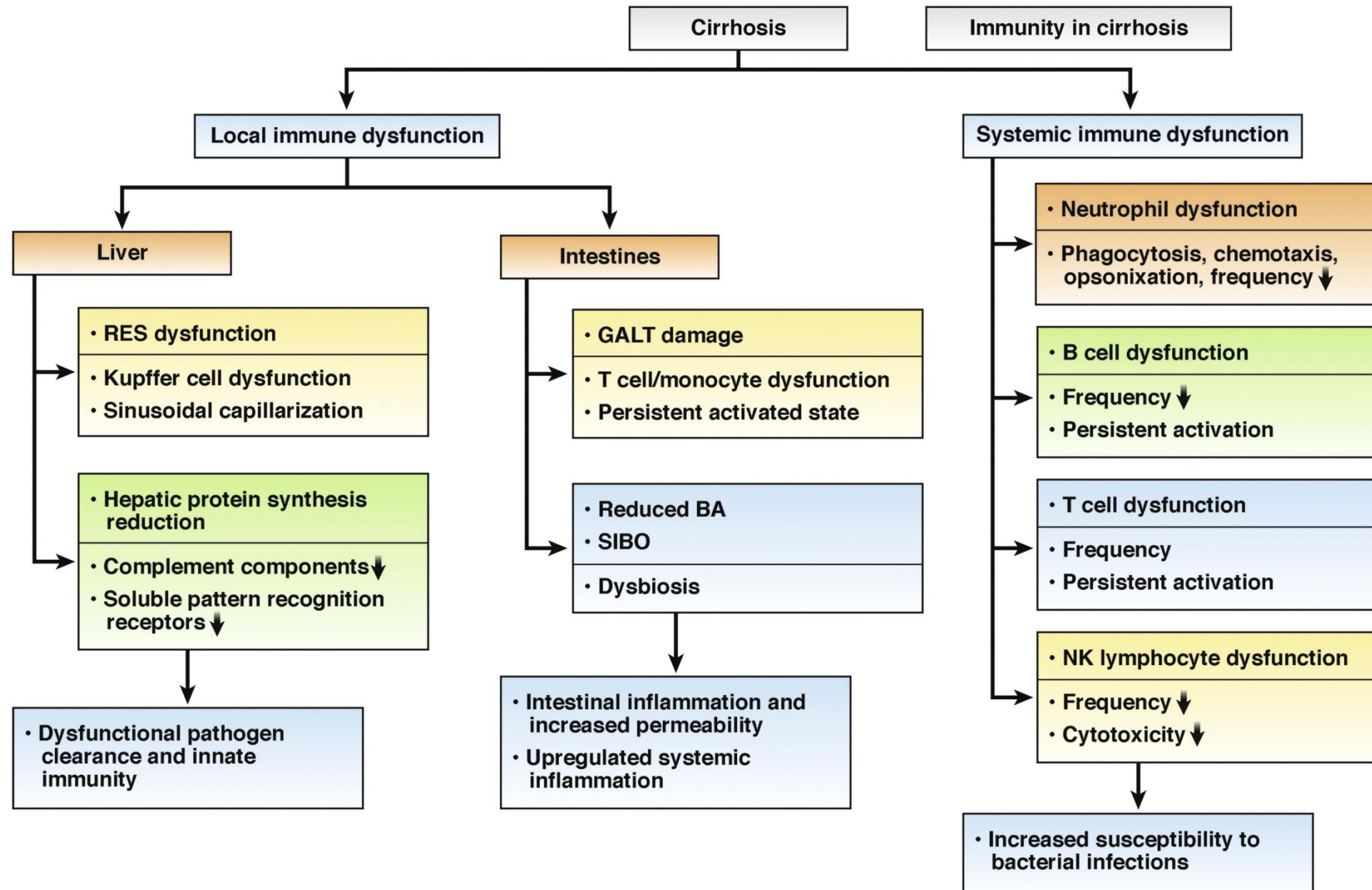
- Administration of feces (containing entire gut microbial community) from a human donor to affect the microbiota of the recipient.
- Restore diversity of microorganisms, beneficial anaerobes and butyrate-producing bacteria.
 - Engraftment
 - Augmentation
- Published guidance
 - Bakken et al 2011
 - Cammarota et al 2017



Andrea Levy, The Cleveland Plain Dealer

Hoffman D, et al 2016
Bakken et al. Clin Gastro Hep 2011
Cammarota et al. Gut 2017

Multi-layered immune dysfunction in Cirrhosis



FMT: Mechanisms of Action

Growth inhibition

- Bacteriocins
- Block adhesion and toxicity to epithelial cells

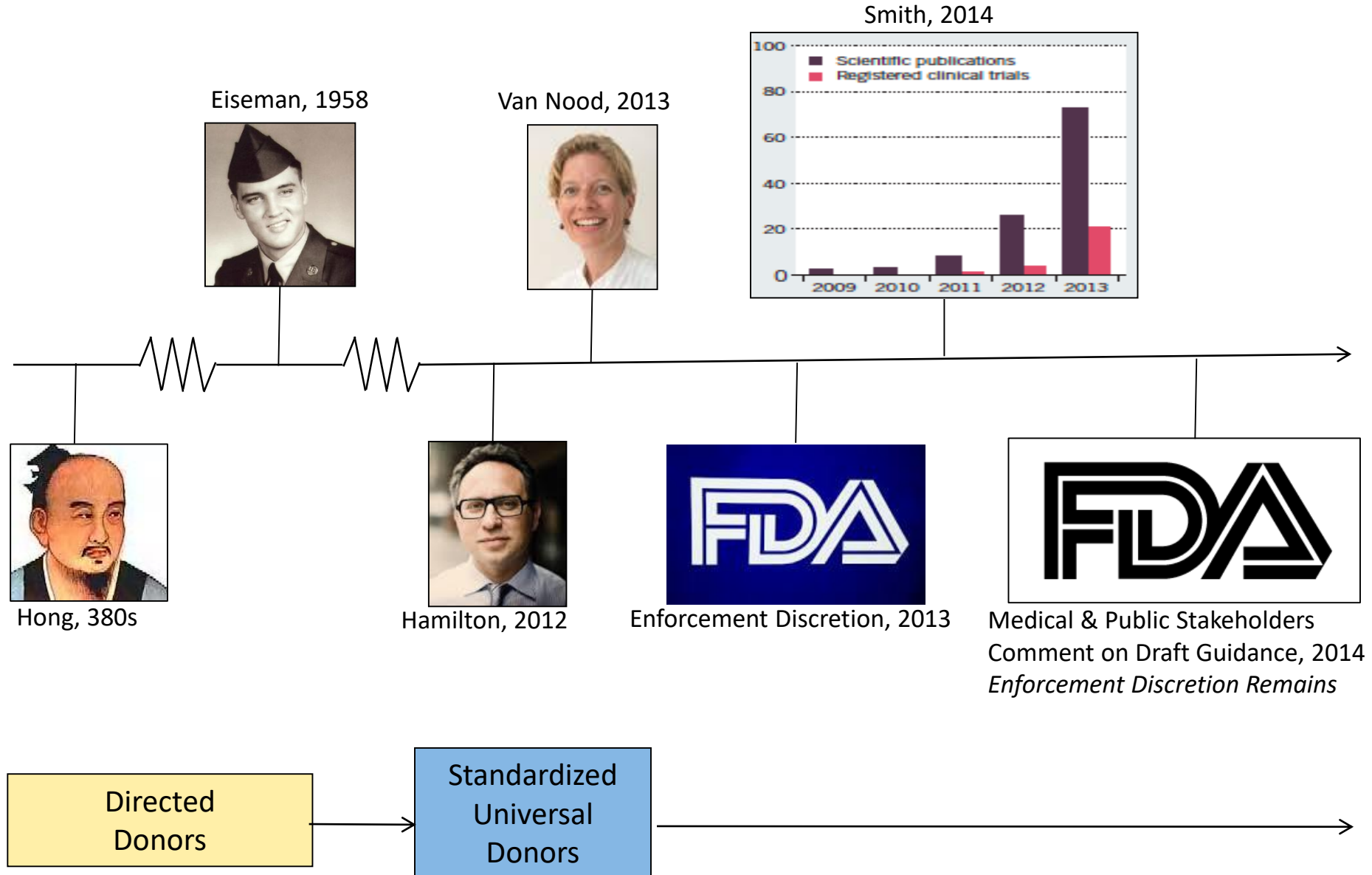
Competitive niche exclusion

- Organisms compete for limited amounts of nutrients

Immune-mediated

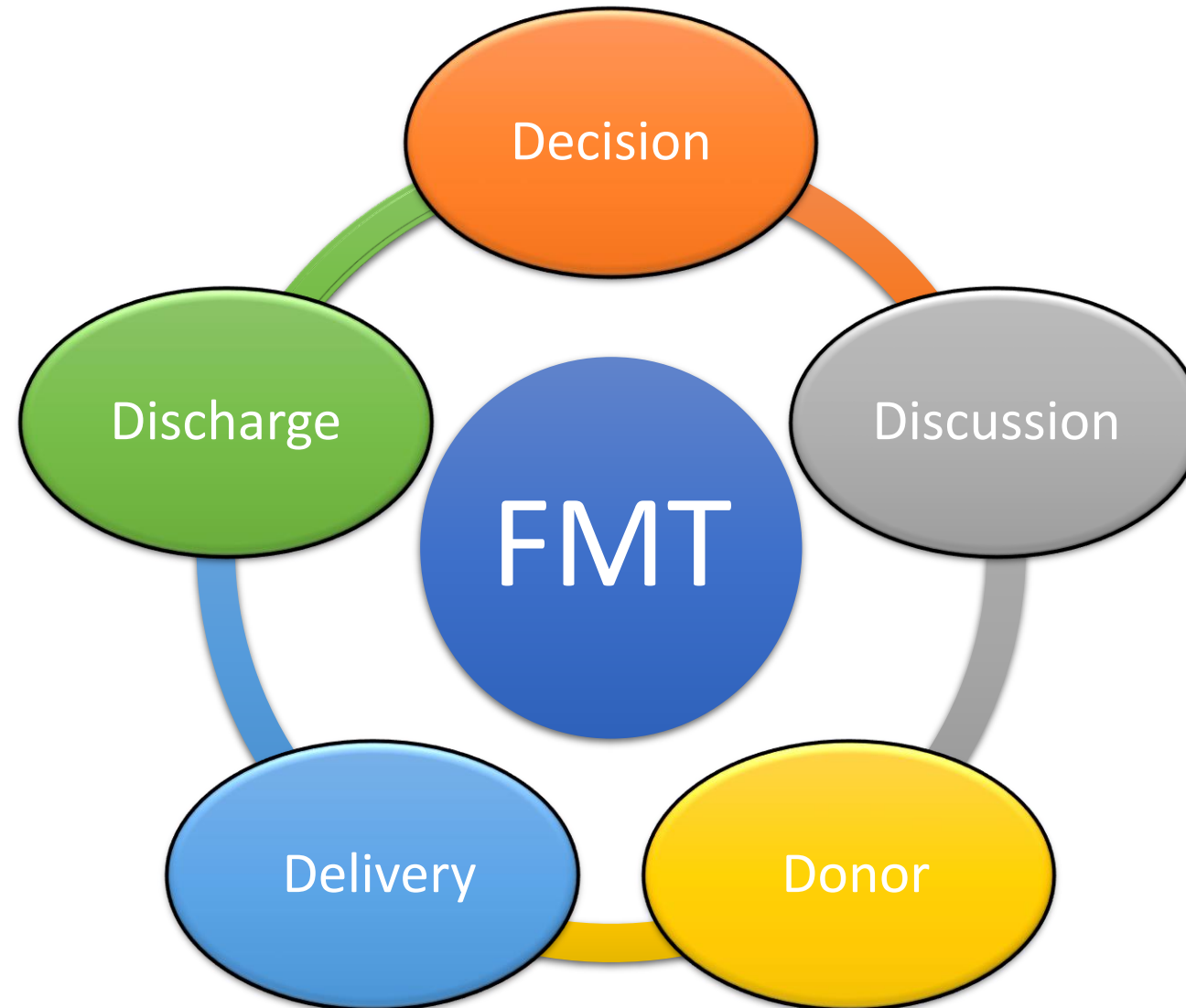
- TLR stimulation by microbiota protects against colitis

A brief history of FMT





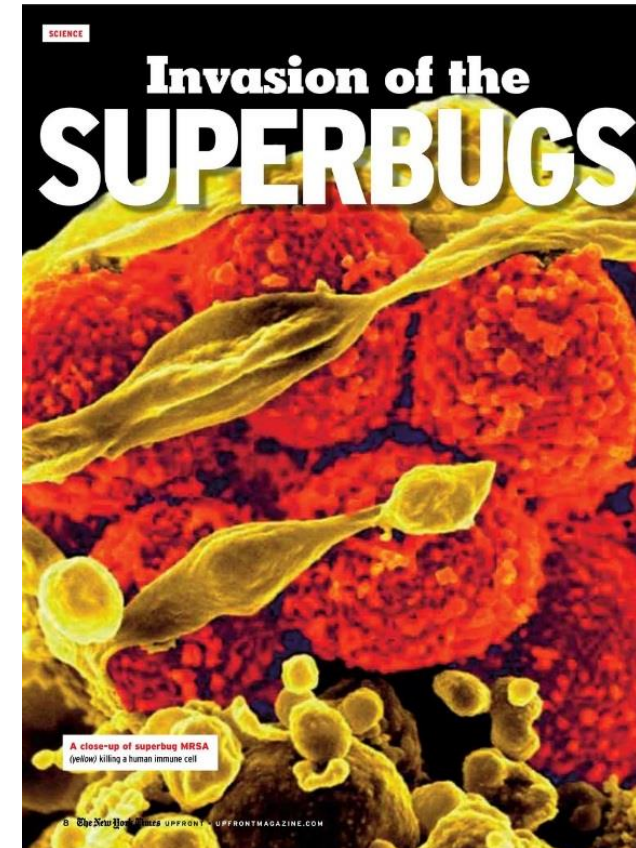
FMT 101: “The 5 D Approach”



FMT vs. Antibiotic Resistant Bacteria

Multi-drug resistant organisms (MCRO)
eradicated after FMT

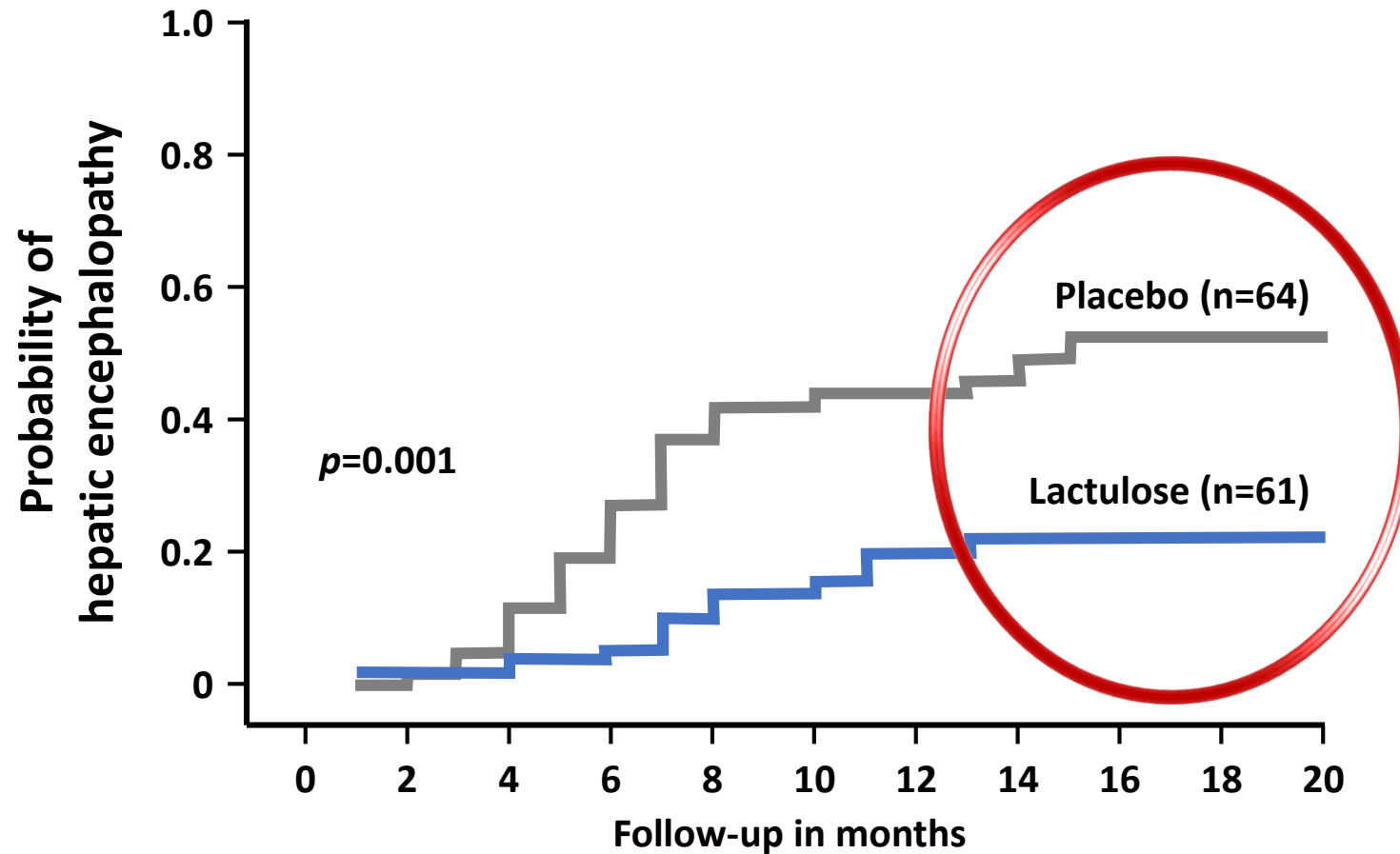
- VRE (vancomycin resistant enterococcus)
- MRSA
- Carbapenum-resistant Enterobacteriaceae
- Extended spectrum beta lactimase producing gram negatives
- CDC sponsored clinical trials
 - FMT for VRE colonization
 - Autologous FMT to prevent MDRO colonization post-antibiotics



Stripling J, et al Open Form Infect Dis 2015
Crum-Cianflone NF, et al. J Clin Microbiol 2015
Singh R, Clin Microbiol Infect 2014

How do HE therapies affect
HE recurrence outcomes?

Reduction of Overt HE Recurrence: Lactulose

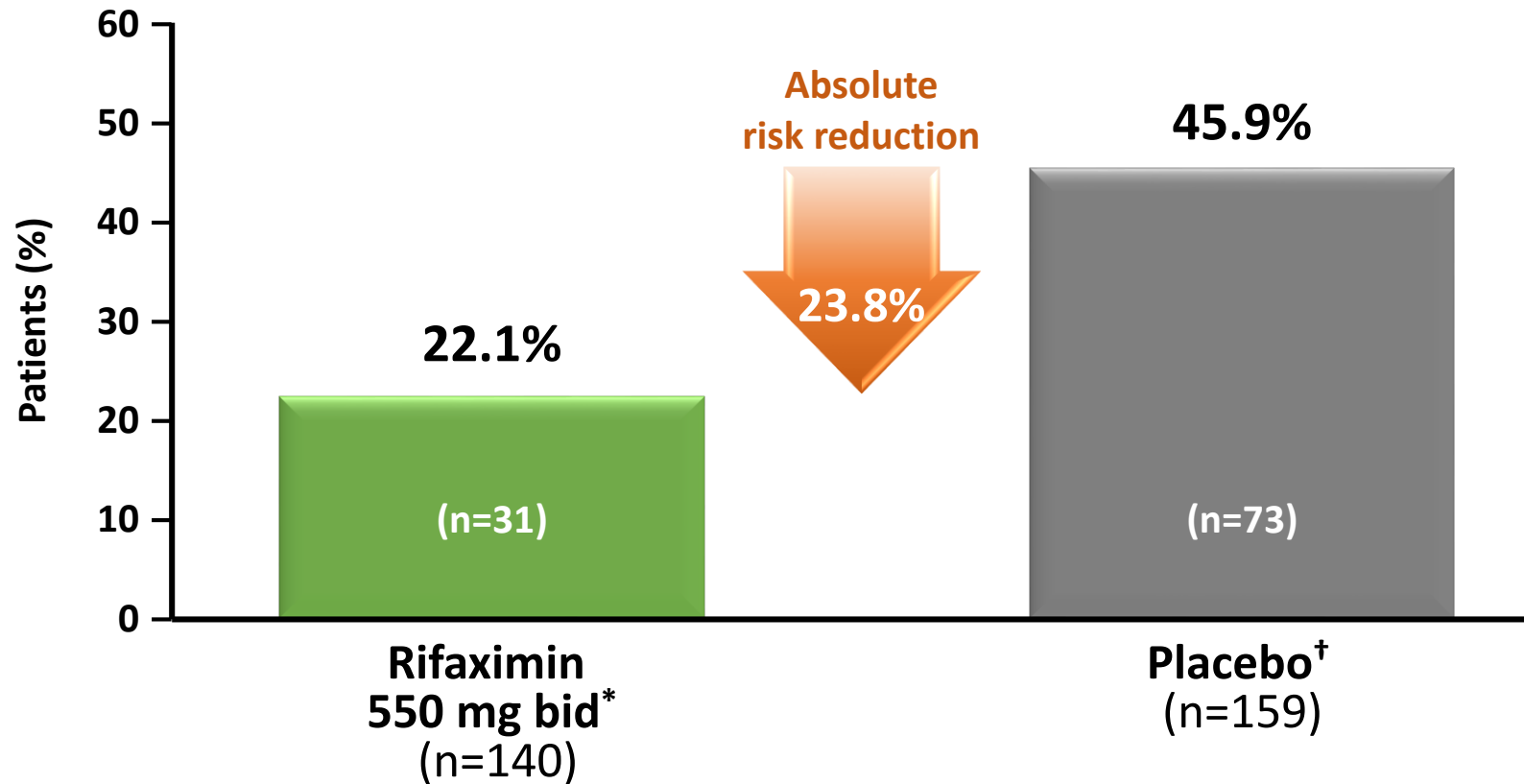


Patients at risk

Lactulose	61	60(1)	59(2)	58(3)	51(8)	45(9)	38(11)	18(12)	10(12)	7(12)	1(12)
Placebo	64	62(1)	59(4)	50(13)	37(24)	33(27)	28(27)	19(29)	13(30)	8(30)	4(30)

Reduction of Overt HE Recurrence: Rifaximin

HR for risk of a breakthrough episode in rifaximin group vs. placebo:
0.42 (95% CI, 0.28–0.64; $p<0.001$)



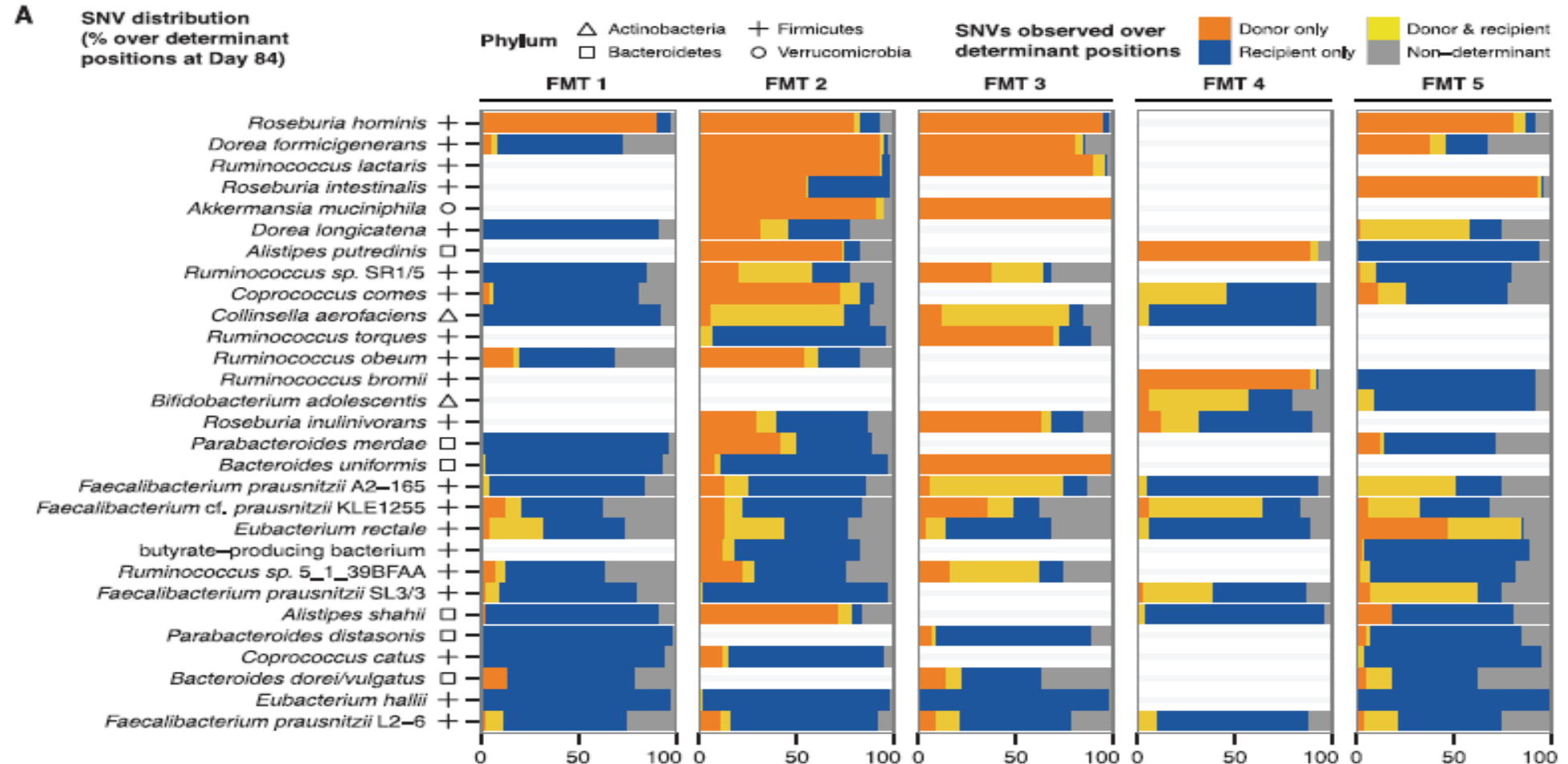
Patients who had ≥ 2 episodes of HE within 6 months prior to screening and who were in remission at trial start

*91.4% on concomitant lactulose

†91.2% on concomitant lactulose

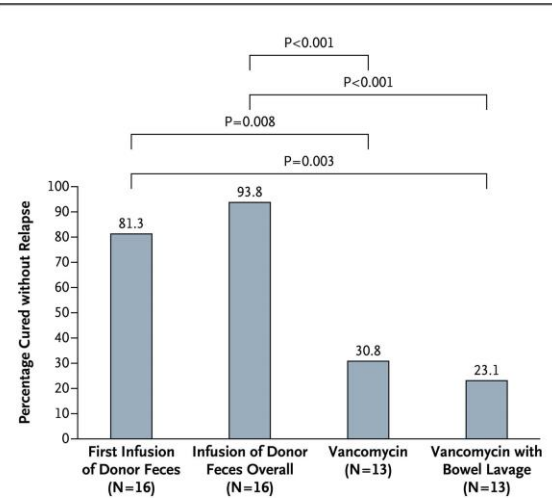
There remains a population of patients who experience recurrent HE, a leading cause of readmissions, despite being on standard of care

Engraftment is durable and Restores Bile Acid Metabolism



FMT: established role for multiply recurrent *C. difficile*

FMT vs vancomycin- duodenal infusion



Van Nood NEJM 2013

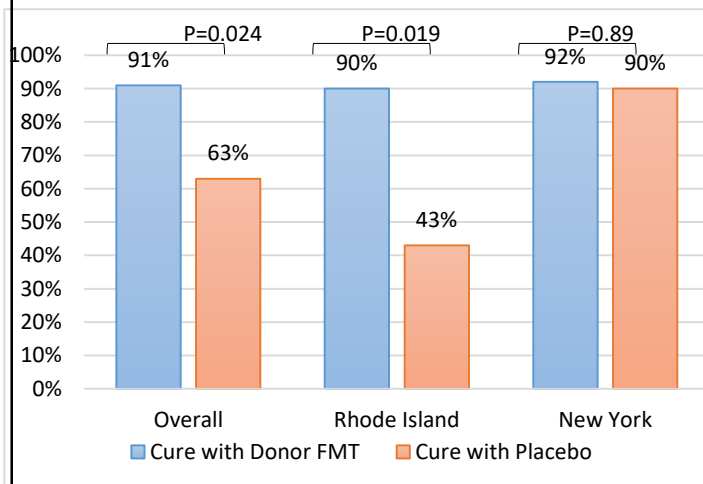
Fresh vs Frozen FMT- enema

No. of FMTs	Per-Protocol Population	
	Frozen (n = 91)	Fresh (n = 87)
1	57 (62.7)	54 (62.1)
2	19 (83.5)	20 (85.1)
3-5	9 (93.4)	9 (95.4)
>5	2 (95.6)	1 (96.6)
Total	87/91 (95.6)	84/87 (96.6)



Lee JAMA 2016

FMT vs placebo by colonoscopy



Kelly C. Ann Intern Med 2016

Encapsulated FMT

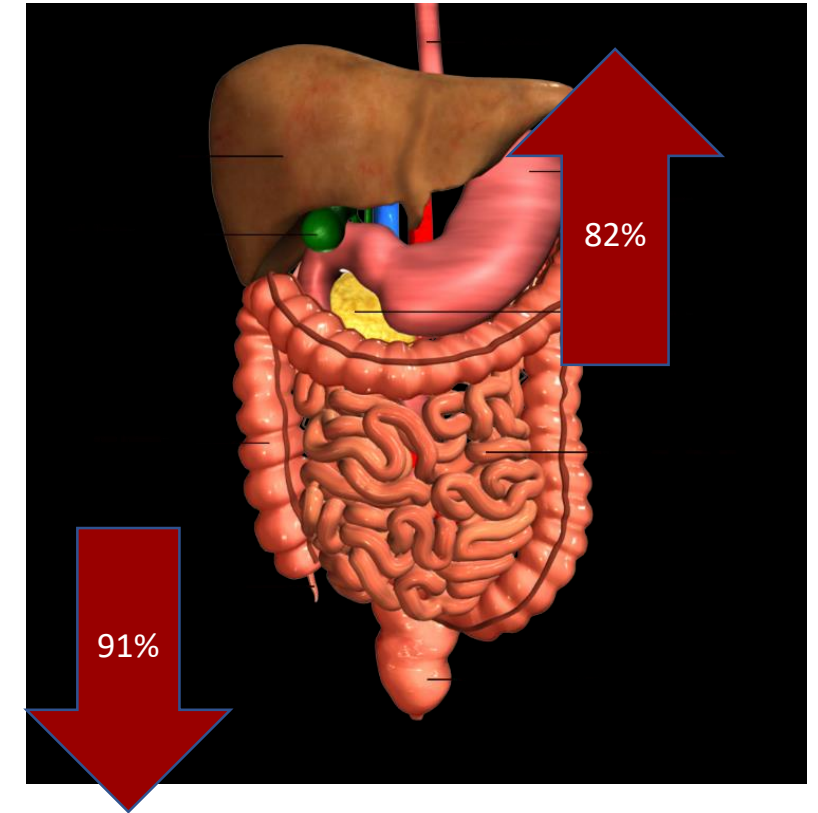
70-94% cure rate



- Fresh (Louie 2013)
- Frozen (Youngster 2014)
- Freeze-dried (Khoruts 2017)
- Capsule vs colonoscopic (Kao 2017)

How Effective is FMT in *C.difficile*?

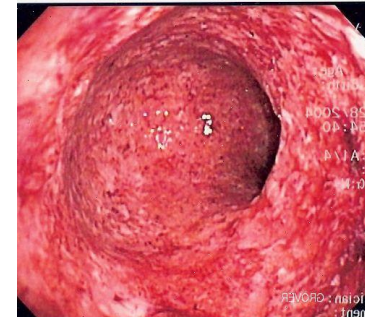
- 23%-62.5% cure after course of vancomycin
- Randomized Controlled Trials
 - 80-91% success with single FMT
 - 84-95% after 2 FMT
 - RARE to not respond to FMT
- Long term success
 - 10% chance of recurrence at 1 year
 - Usually after antibiotics



Cammarota G. J Clin Gastroenterol 2014
Kassam Z, Am J Gastroenterol 2013
Van Nood NEJM 2013; Cammarota AP&T 2015;
Lee JAMA 2016; Kelly Annals Int Med 2016;
Fischer DDW 2017

Known Risks of FMT

- Risk of infection (appears low)
 - Multicenter retrospective study: No infections in 80 “high risk” immunocompromised patients
 - Peritonitis, bacteremia (E coli, Proteus, Klebsiella, Listeria)
 - CMV following home FMT (stool from unscreened infant)
 - Regurgitation of stool, fatal aspiration pneumonia with UGI delivery
- Risks related to the procedure
 - Perforation
 - Bleeding
 - Sedation-related complications
- Other
 - Warn IBD patients: Risk of disease flares (14-25%)



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