Microbiota in Transplantation

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Renal Conference
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Outline

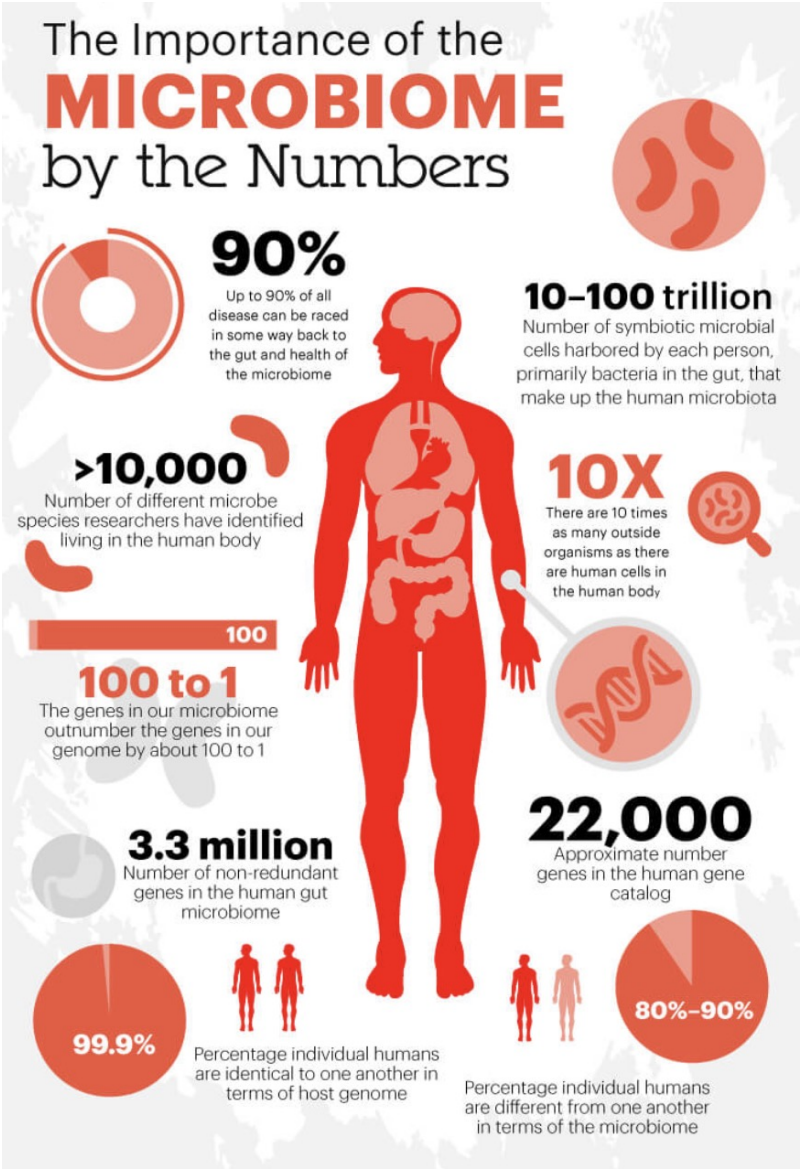
1. Short overview of the influence of the microbiome on drug disposition -- “pharmacomicrobiomics”

2. Discuss the ongoing microbiome study in kidney transplant -- The MISSION Study

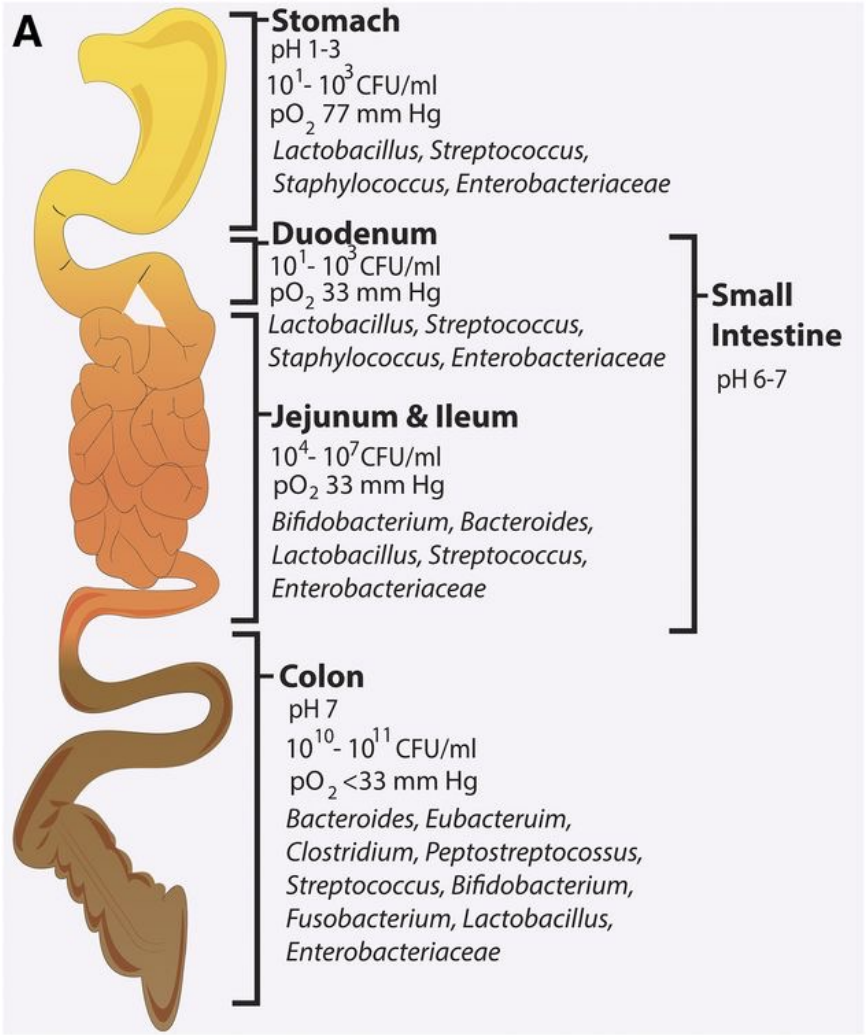
3. Quantifying Mycophenolate mofetil metabolism using an in-vitro assay

The Human Microbiome

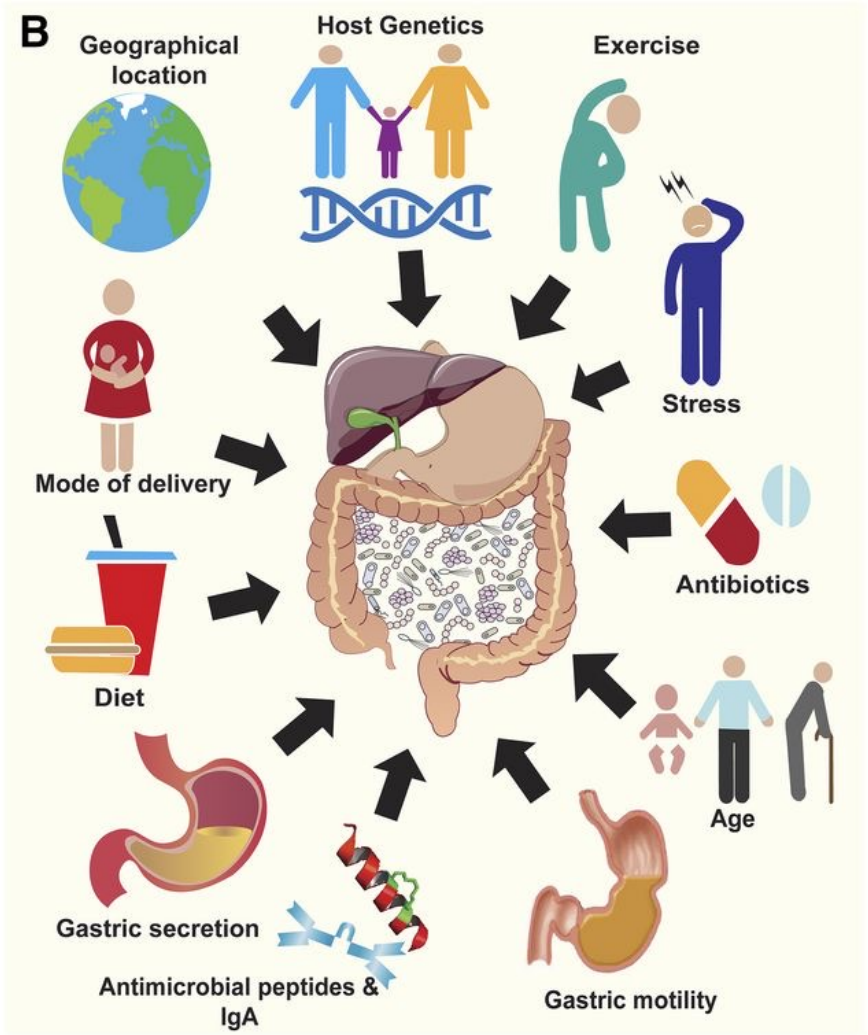
- The microbiome (>10,000 species in humans) is associated with health and disease.
- Diversity of the microbiome among individuals is high (80-90% different).
- Many factors affect the microbiome including diet and xenobiotics.
- Most of human microbiome resides in the GI tract but varies extensively between individuals.



Metabolic niches in the gut microbiome

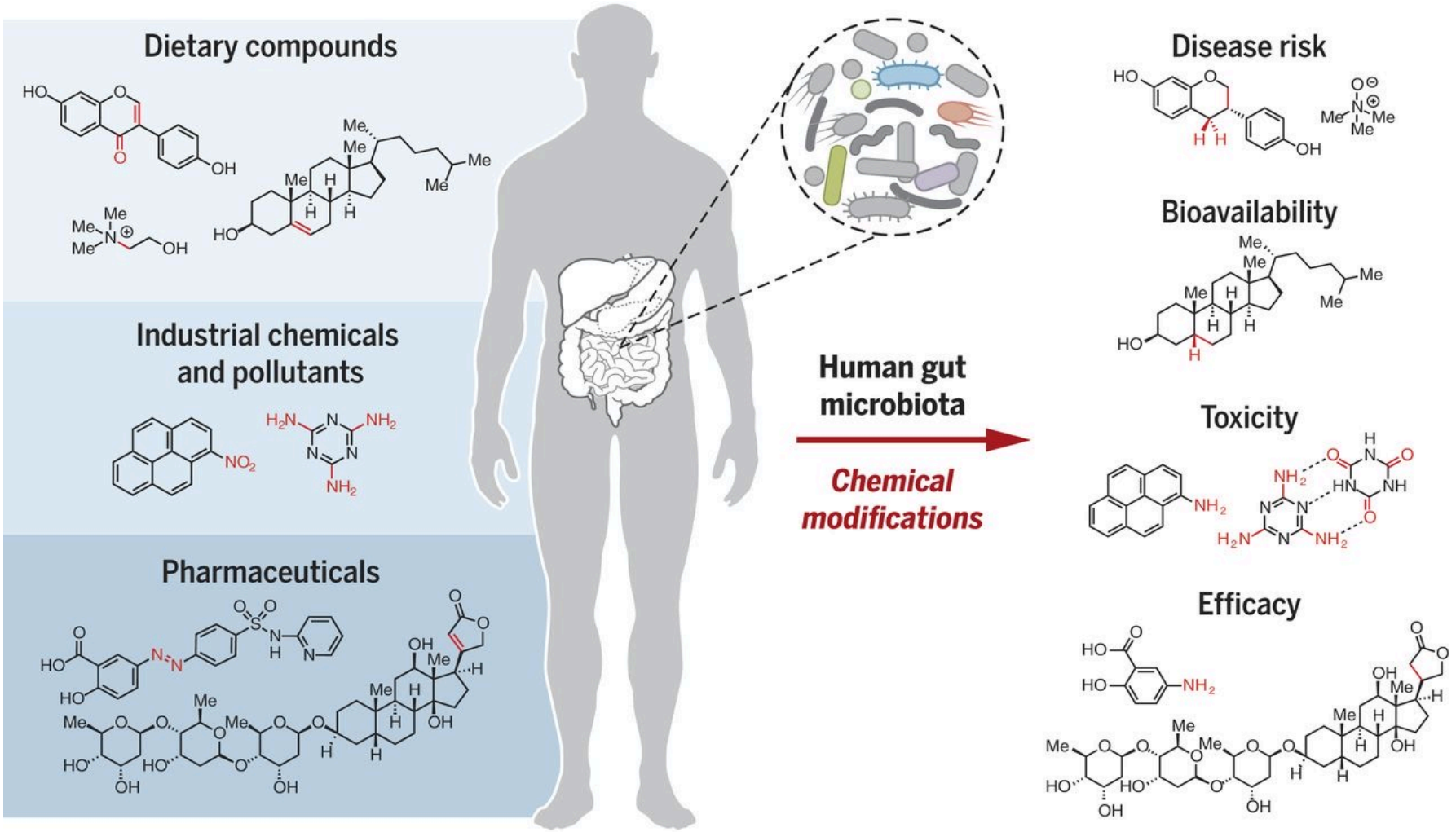


Factors affecting the composition and function of the intestine metabolic niche



Gerard Clarke et al. Pharmacol Rev 2019;71:198-224

Human gut microbes metabolize xenobiotics

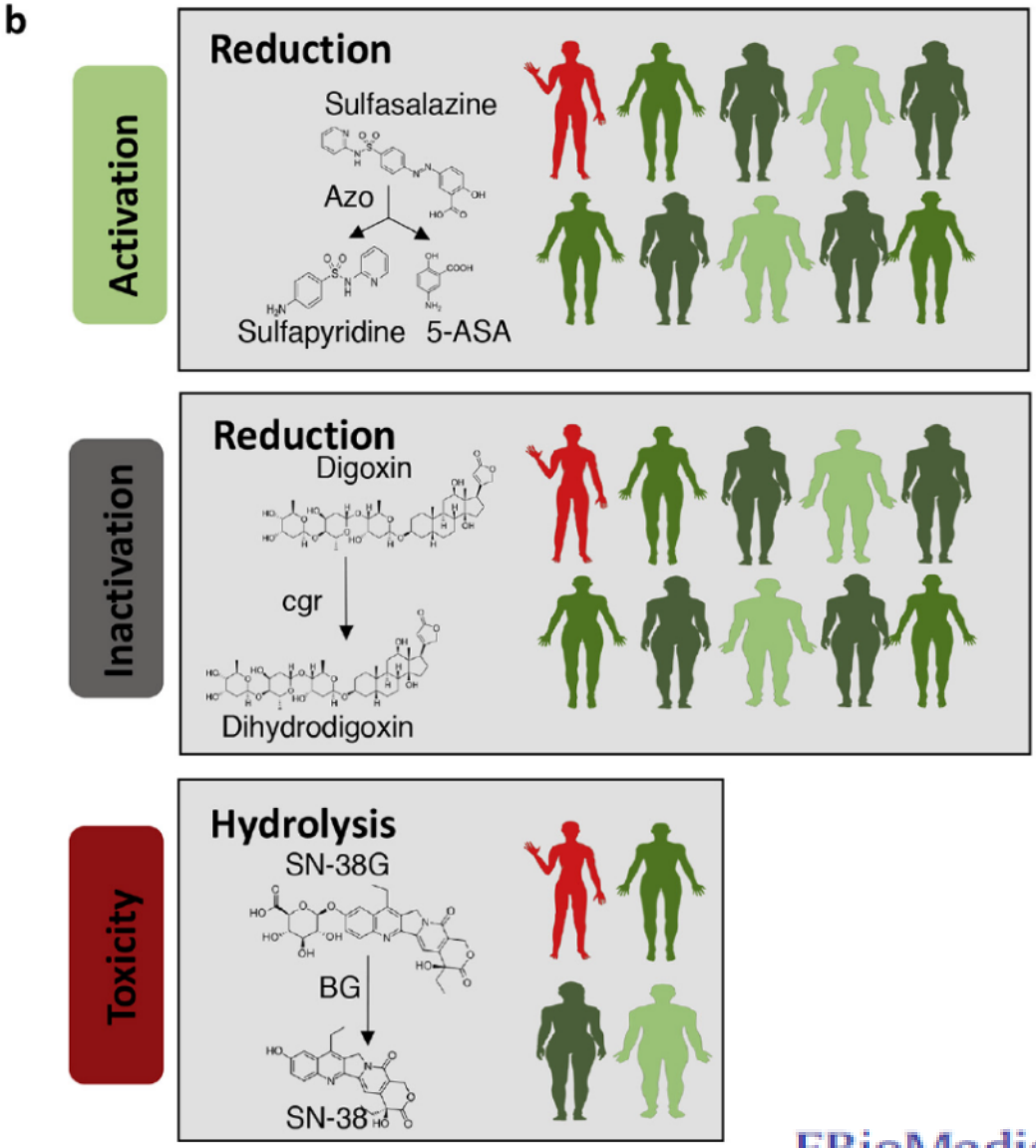


Nitzan Koppel et al. Science 2017;356:eaag2770

Microbiome Effects Drug Metabolism, PK and Drug Outcomes – Possible Mechanisms

- Gut microbiome may express enzymes that activate or inactivate drugs (↑or↓drug clearance).
- A drug may be sequestered by direct binding to the bacterial organism (inactivate).
- A drug may be metabolically reactivated by enzymes made by the bacteria.
- An altered microbiota may generate alternative drug metabolites (active or inactive).
- Gut microbiome may alter drug transport (↑or↓drug movement across membranes and absorption).

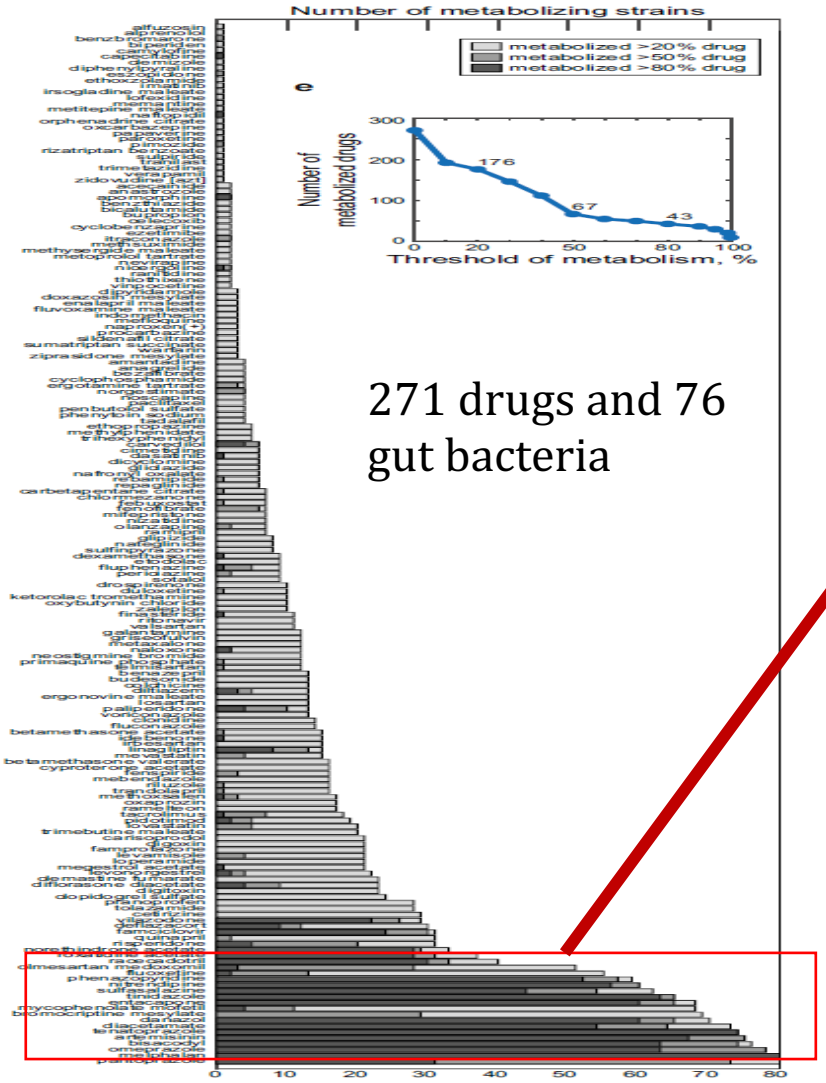
Gut microbiota-host liver metabolic interactions drive variability in drug response



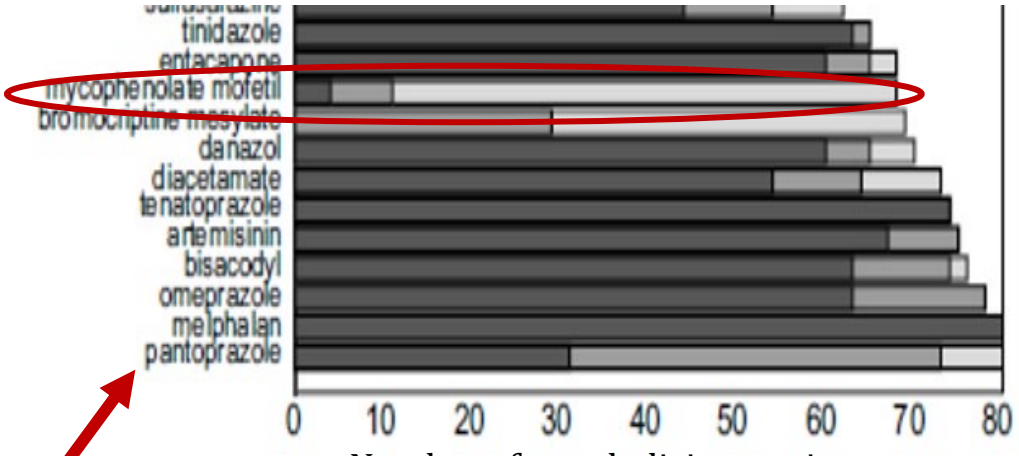
EBioMedicine 44 (2019) 708–715

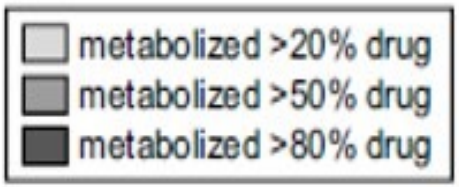
Mapping human microbiome drug metabolism by gut bacteria and their genes

Michael Zimmermann[1,3], Maria Zimmermann-Kogadeeva[1,3], Rebekka Wegmann[1,2] & Andrew L. Goodman[1*]

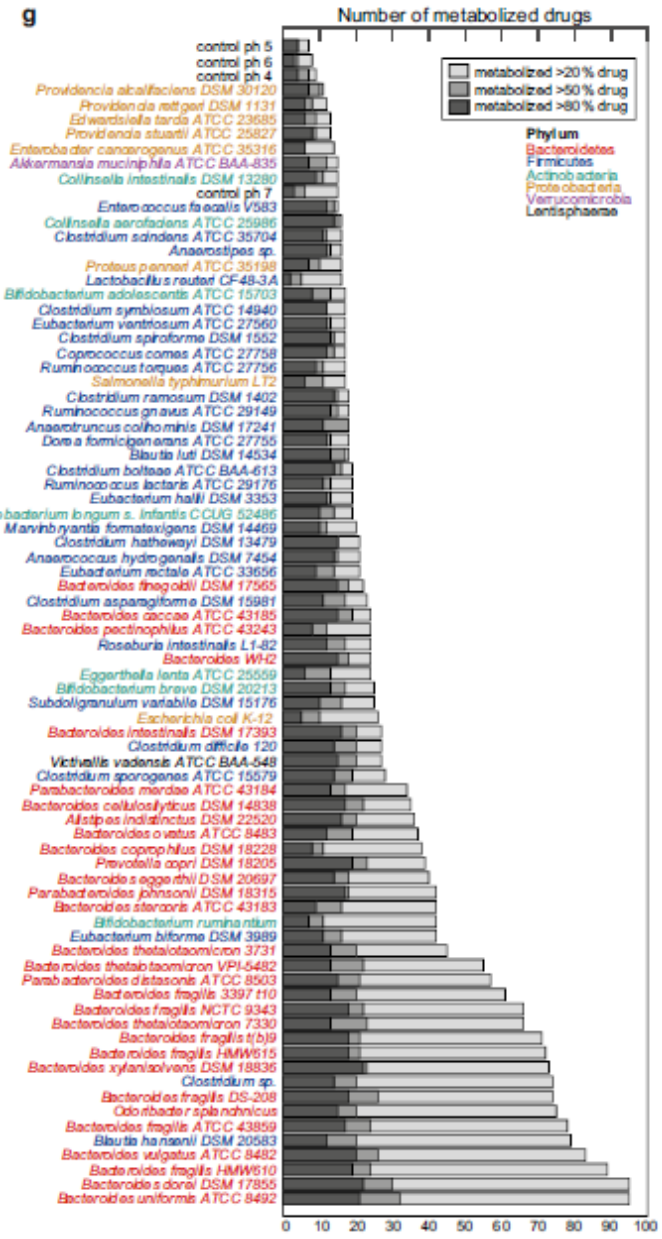


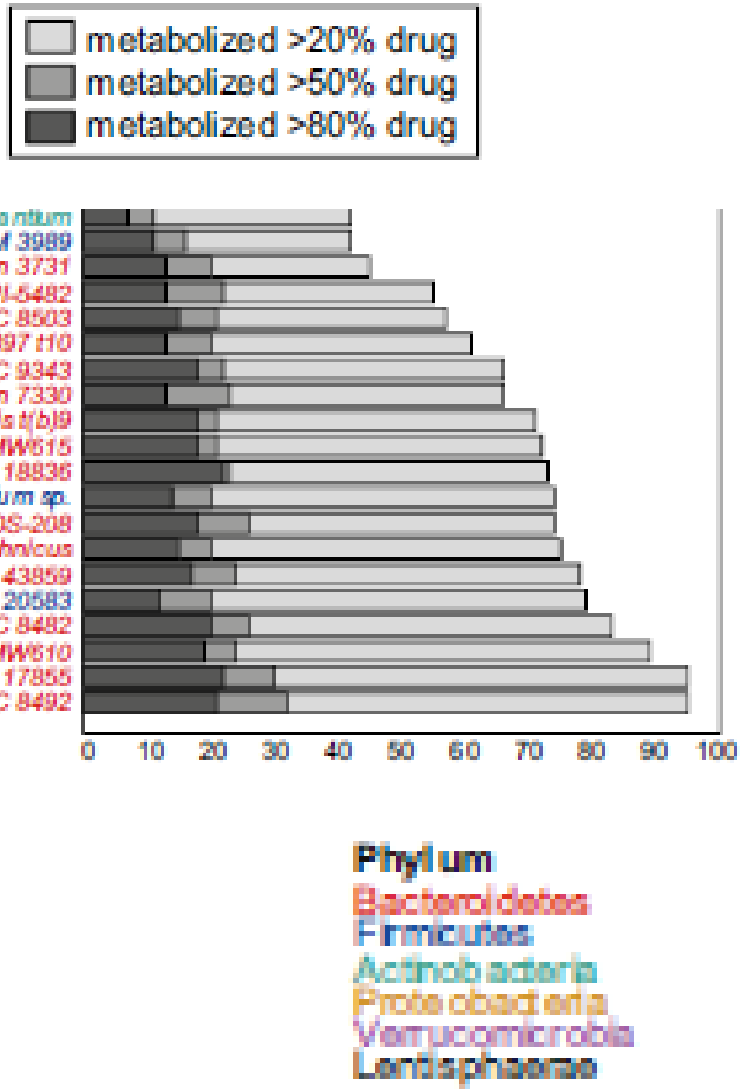
271 drugs and 76 gut bacteria





2/3 of drugs were reduced by 20% or more by one or more bacteria strains


g
Number of metabolized drugs
metabolized >20% drug
metabolized >50% drug
metabolized >80% drug
Phylum
Bacteroidetes
Firmicutes
Actinobacteria
Proteobacteria
Verrucomicrobia
Lentisphaerae
control ph 5
control ph 6
control ph 4
Providencia alcalifaciens DSM 30120
Providencia rettgeri DSM 1131
Edwardsiella tarda ATCC 23685
Providencia stuartii ATCC 25827
Enterobacter cancerogenus ATCC 35316
Akkermansia muciniphila ATCC BAA-835
Collinsella intestinalis DSM 13280
control ph 7
Enterococcus faecalis V583
Collinsella aerofaciens ATCC 25986
Clostridium scindens ATCC 35704
Anaerostipes sp.
Proteus penneri ATCC 35198
Lactobacillus reuteri CF48-3A
Bifidobacterium adolescentis ATCC 15703
Clostridium symbiosum ATCC 14940
Eubacterium ventriosum ATCC 27560
Clostridium spiroforme DSM 1552
Coprococcus comes ATCC 27758
Ruminococcus torques ATCC 27756
Salmonella typhimurium LT2
Clostridium ramosum DSM 1402
Ruminococcus gnavus ATCC 29149
Anaerotruncus colihominis DSM 17241
Dorea formicigenerans ATCC 27755
Blautia luti DSM 14534
Clostridium bolteae ATCC BAA-613
Ruminococcus lactaris ATCC 29176
Eubacterium hallii DSM 3353
bacterium longum s. infantis CCUG 52486
Marvinbryantia formatexigens DSM 14469
Clostridium hathewayi DSM 13479
Anaerococcus hydrogenalis DSM 7454
Eubacterium rectale ATCC 33656
Bacteroides finegoldii DSM 17565
Clostridium asparagiforme DSM 15981
Bacteroides caccae ATCC 43185
Bacteroides pectinophilus ATCC 43243
Roseburia intestinalis L1-82
Bacteroides WH2
Eggerthella lenta ATCC 25559
Bifidobacterium breve DSM 20213
Subdoligranulum variabile DSM 15176
Escherichia coli K-12
Bacteroides intestinalis DSM 17393
Clostridium difficile 120
Victivallis vadensis ATCC BAA-548
Clostridium sporogenes ATCC 15579
Parabacteroides merdae ATCC 43184
Bacteroides cellulosilyticus DSM 14838
Alistipes indistinctus DSM 22520
Bacteroides ovatus ATCC 8483
Bacteroides coprophilus DSM 18228
Prevotella copri DSM 18205
Bacteroides eggerthii DSM 20697
Parabacteroides johnsonii DSM 18315
Bacteroides stercoris ATCC 43183
Bifidobacterium ruminantium
Eubacterium biforme DSM 3989
Bacteroides thetaiotaomicron 3731
Bacteroides thetaiotaomicron VPI-5482
Parabacteroides distasonis ATCC 8503
Bacteroides fragilis 3397 t10
Bacteroides fragilis NCTC 9343
Bacteroides thetaiotaomicron 7330
Bacteroides fragilis t(b)9
Bacteroides fragilis HMW615
Bacteroides xylanisolvens DSM 18836
Clostridium sp.
Bacteroides fragilis DS-208
Odoribacter splanchnicus
Bacteroides fragilis ATCC 43859
Blautia hansenii DSM 20583
Bacteroides vulgatus ATCC 8482
Bacteroides fragilis HMW610
Bacteroides dorei DSM 17855
Bacteroides uniformis ATCC 8492
0 10 20 30 40 50 60 70 80 90 100


metabolized >20% drug
metabolized >50% drug
metabolized >80% drug
Bifidobacterium ruminantium
Eubacterium biforme DSM 3989
Bacteroides thetaiotaomicron 3731
Bacteroides thetaiotaomicron VPI-5482
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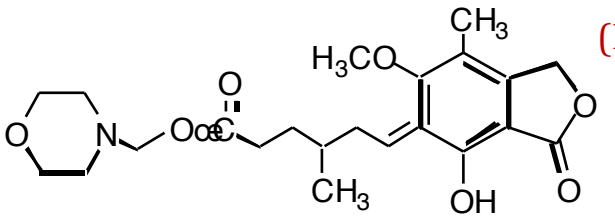
0 10 20 30 40 50 60 70 80 90 100
Phylum
Bacteroidetes
Firmicutes
Actinobacteria
Proteobacteria
Verrucomicrobia
Lentisphaerae

What is the effect of the Microbiome on Immunosuppressant Drugs?

- Mycophenolate Mofetil

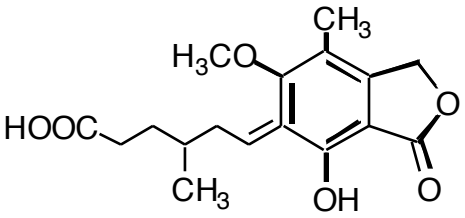
Major Metabolic Pathway of Mycophenolate



(MMF)

Mycophenolate mofetil

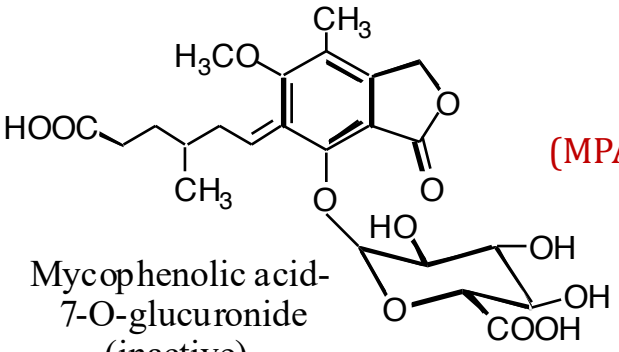
Carboxylesterase



Mycophenolic Acid (active) (MPA)

UDP-glucuronosyl-transferase

Liver and kidney



(MPAG)

Mycophenolic acid-7-O-glucuronide (inactive)

Enterohepatic recycling (EHR) (10-40%)

Beta-glucuronidase (from bacteria expressing beta-glucuronidase [GUS])

GUS is expressed by Bacteroides, Firmicutes, Proteobacteria, Acinobacteria phyla

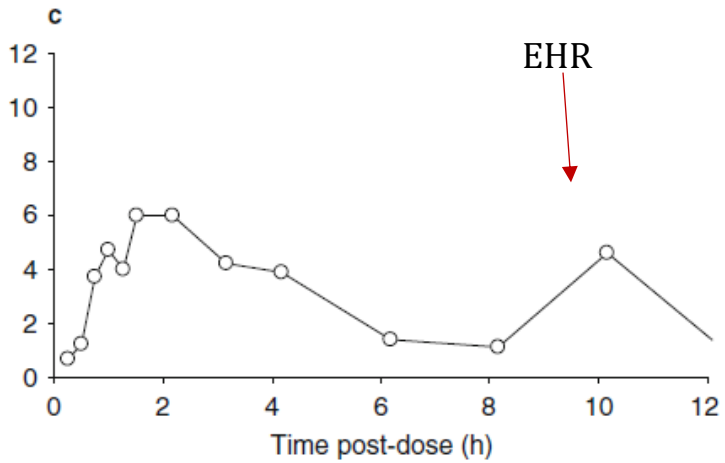


Figure 1. MPA Plasma concentration time profile showing enterohepatic recirculation

GI Adverse Events of Immunosuppressive Agents

	Oral ulcers, stomatitis	Gingival hyperplasia	Nausea, vomiting, dyspepsia	Gastro-paresis	Pan-creatitis	Diarrhea	Constipation
Tacrolimus	+	0	++	0/+	0/+	++	+
Cyclosporine	0	+	+	+	0/+	+	0
mTOR inhibitors	++	0	++	0	0/+	++	++
Steroids	0	0	+	0	+	0	0
Azathioprine	0	0	+	0	+	0	0
Mycophenolate	+	0/+	++	0	0/+	++	+

Undesirable effects are listed using the following categories: ++ very common (≥1/10); + common (≥1/100 to <1/10); 0/+ uncommon (≥1/1000 to <1/100); 0 rare (<1/1000).

GI complications frequently necessitate dose reduction, interruption, or even discontinuation, conversion to a less potent agent, increasing the risk of acute rejection or graft loss and add healthcare costs

M.M. Tielemans et al. / Transplantation Reviews 33 (2019) 55-63

Mycophenolate Hematological Toxicity

- Incidence of MPA related anemia high occurring in 10-13% of kidney transplant recipients.
- Incidence of MPA related leukopenia is high occurring in 22-30% of kidney transplant recipients.
- These toxicities result in dose reduction, conversion to less potent agents such as azathioprine and use of expensive growth factors (GCSF and erythropoeitin), increased risk of rejection.

Supporting Evidence that Mycophenolate Metabolism and PK may be Altered by the Gut Microbiome and may Drive Side Effects

Many Antibiotic Related DDI Interactions

Table 2 Pharmacokinetic alterations of some drugs by gut microbiota in vivo: recent progress

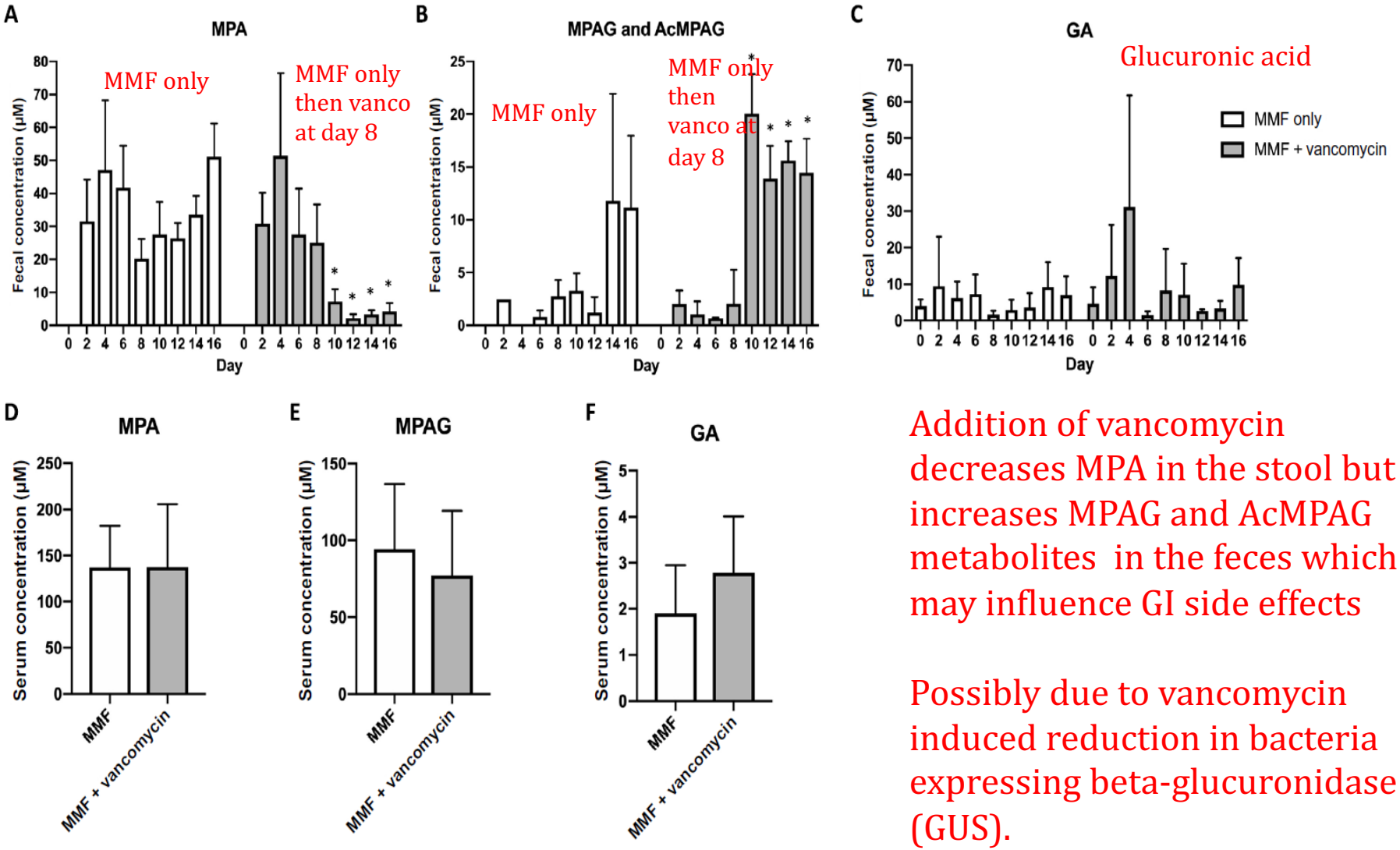
Drugs	Treatments	PK alteration of parent drugs	PK alteration of metabolite(s)
Acetaminophen	Pretreatment with antibiotic mixture of bacitracin, streptomycin and neomycin for 5 days (b.i.d.) in rats	↑ $AUC_{0-24\ h}$ (82%), ↑ AUC_{∞} (81%)	↑ $AUC_{0-24\ h}$ (83%)[a], ↑ AUC_{∞} (81%)[a]
Amiodarone	Pretreatment with a probiotic, *E. coli* strain Nissle 1917, for 7 days in rats	↓ $AUC_{0-30\ h}$ (43%)	↑ C_{max} (150%)
Amlodipine	Pretreatment with ampicillin for 2 days in rats	↑ C_{max}, ↑ AUC	NA
Aspirin	Pretreatment with ampicillin for 3 days in rats	↑ AUC (251%)	↑ AUC (361%), ↑ C_{max} (345%)
Deleobuvir	Pretreatment with streptomycin and neomycin for 6 days (b.i.d) in rats	No change	↓ C_{max} (86%) , ↓ AUC (89%)
Mycophenolic acid	Pretreatment with amoxicillin and clavulanate 5 times (every 12 h) in rats	↓ $AUC_{0-12\ h}$ (18 ~ 25%), ↑ CL (38 ~ 80%)	NA
Lovastatin	Pretreatment with either ampicillin or antibiotic mixture of cefadroxil, oxytetracycline and erythromycin in rats	NA	↓ C_{max} (39 ~ 50%), ↓ AUC (35 ~ 51%)

Arch. Pharm. Res. (2017) 40:1345-1355

Vancomycin relieves mycophenolate mofetil–induced gastrointestinal toxicity by eliminating gut bacterial β-glucuronidase activity

Michael R. Taylor[1,2,3], Kyle L. Flannigan[4,5], Hannah Rahim[1,2,3], Amina Mohamud[1,2,3], Ian A. Lewis[6], Simon A. Hirota[4,5,7], Steven C. Greenway[1,2,3,8]*

Mycophenolate mofetil (MMF) is commonly prescribed and has proven advantages over other immunosuppressive drugs. However, frequent gastrointestinal side effects through an unknown mechanism limit its use. We have found that consumption of MMF alters the composition of the gut microbiota, selecting for bacteria expressing the enzyme β-glucuronidase (GUS) and leading to an up-regulation of GUS activity in the gut of mice and symptomatic humans. In the mouse, vancomycin eliminated GUS-expressing bacteria and prevented MMF-induced weight loss and colonic inflammation. Our work provides a mechanism for the toxicity associated with MMF and a future direction for the development of therapeutics.

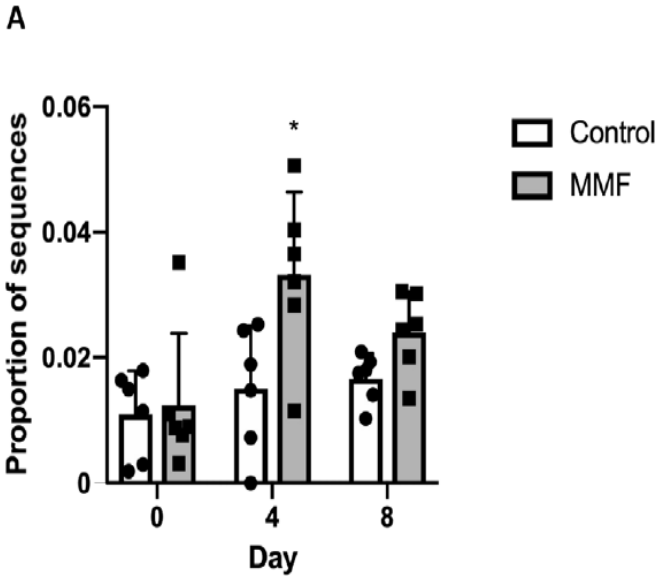


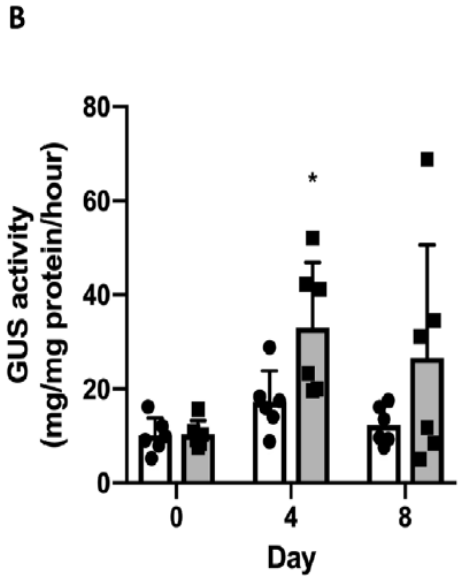
Addition of vancomycin decreases MPA in the stool but increases MPAG and AcMPAG metabolites in the feces which may influence GI side effects

Possibly due to vancomycin induced reduction in bacteria expressing beta-glucuronidase (GUS).

Fig. 5. Changes in MMF metabolites. The concentrations of MMF metabolites were measured in mouse fecal pellets and serum. (**A** to **C**) Comparison of MPA, MPAG, and GA levels in mice exposed to MMF only for up to 16 days and mice initially exposed to MMF only for 8 days and then with the addition of vancomycin for 8 days. Vancomycin treatment resulted in significant increases in MPAG and significant decreases in MPA levels ($P < 0.05$). (**D** to **F**) In serum from mice consuming MMF, levels of MPA, MPAG, and GA were not affected after 8 days of treatment with vancomycin. AcMPAG was not detected in the serum. Data are means ± SEM. The mean concentration of each metabolite before (days 0 to 8) and after (days 10 to 16) the introduction of vancomycin was compared independently in each treatment group using a two-way analysis of variance (ANOVA), with $^*P < 0.0005$.

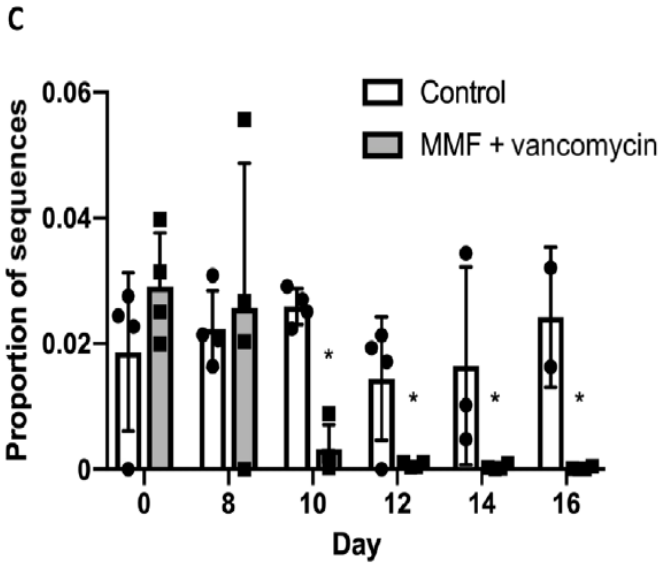
MMF associated with increased GUS expr sequence abundance

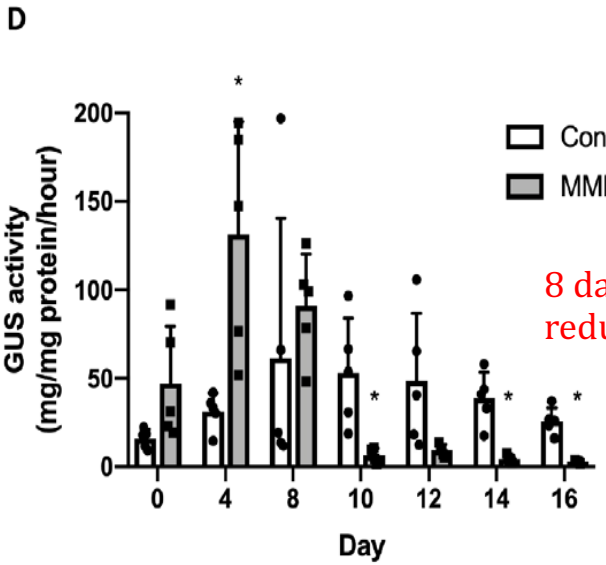




MMF associated with increased GUS activity

8 days of vanco reduces GUS expres abundance





8 days of vanco reduces GUS activity

Fig. 3. MMF–up-regulated GUS expression and activity are prevented by vancomycin. PICRUSt and an enzyme activity assay were used to assess gene expression and GUS activity in mouse fecal pellets. (**A**) The proportional abundance of KEGG ortholog K01195 increased significantly (*$P = 0.006$) in the presence of MMF after 4 days. (**B**) GUS activity in fecal pellets collected from mice consuming MMF also increased significantly ($P = 0.03$) after 4 days. (**C**) The introduction of vancomycin on day 8 rapidly eliminated the expression of K01195. (**D**) Vancomycin, introduced after day 8, abolished GUS activity in fecal pellets (*$P < 0.01$). All data are plotted as means and SDs with data points for individual samples represented.

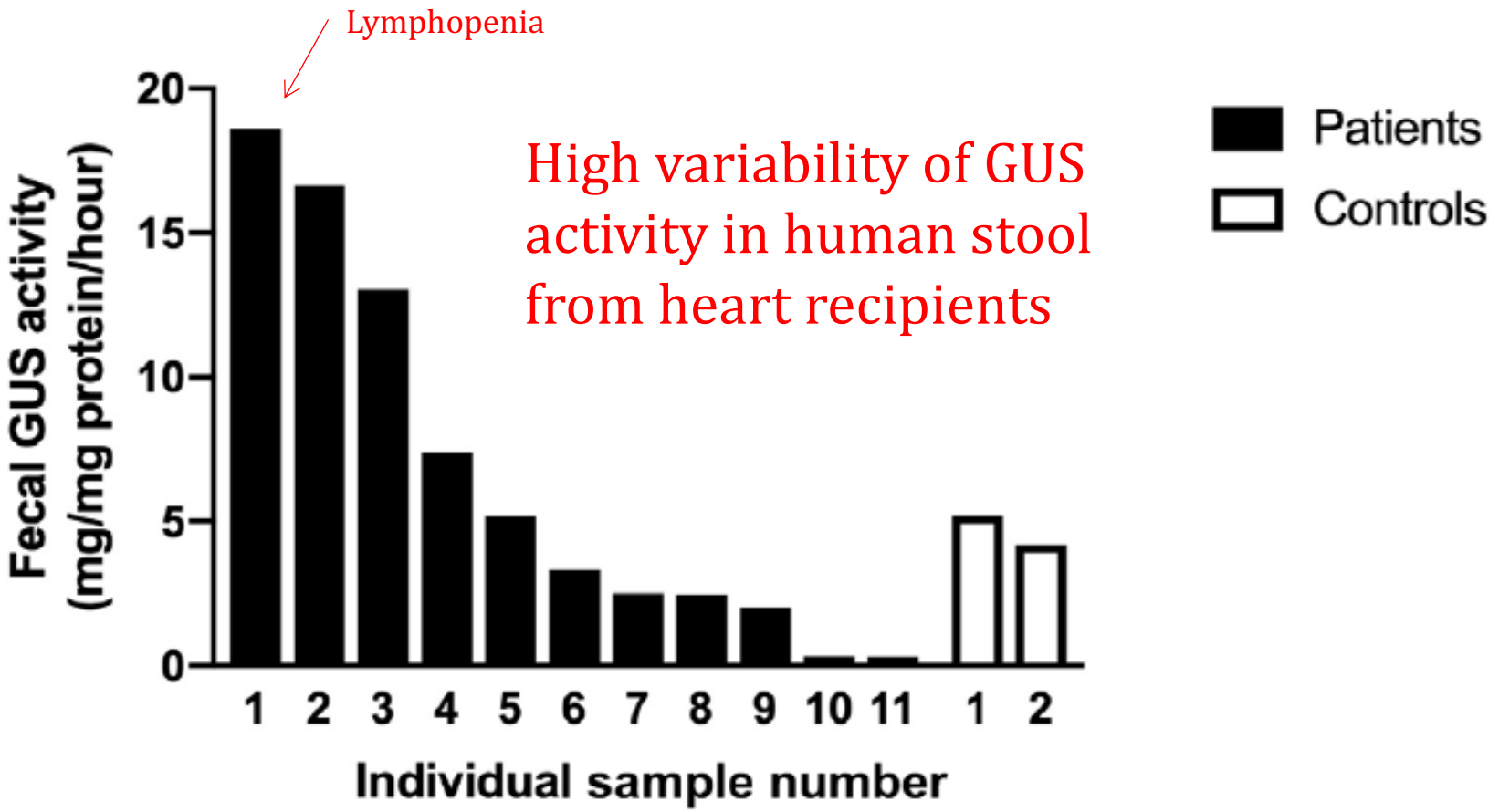


Fig. 6. GUS activity in human stool. Samples were collected from 11 adult and pediatric heart transplant recipients and 2 healthy, nontransplant controls. The highest level of GUS activity seen in patient 1 correlated with lymphopenia requiring a dose reduction in her MMF. Patient 3 had developed hematochezia and colonic lymphonodular hyperplasia while taking MMF. Patients 10 and 11 had been taken off MMF before sample collection.

Microbiome and **I**mmunosuppre**SSION** in Kidney Transplantation (**MISSION) Study**

Study Aims

Aim 1: To determine the association of microbiome diversity with plasma MPA and metabolite concentrations posttx.

Aim 2: To determine the association of microbiome diversity in tx recipients with MPA-associated toxicities such as anemia and leukopenia posttx.

Aim 3: To determine the association of microbiome diversity with diarrhea in tx recipients on MMF posttx.

Study Design

Aim 1 & 2: Mycophenolate PK between days 30-90 posttx in research unit

- Collect two stool microbiome samples beginning at 1 week post-tx and another at PK visit and month 4 and 6.
- Follow subjects for mycophenolate related anemia, and leukopenia, for 12 months.
- Assess stool for ***beta-glucuronidase*** transcripts.
- New cross-sectional cohort also planned*

Aim 3: Assess subjects for diarrhea from time of tx until 6 months post-tx.

- Collect two microbiome samples beginning at beginning at 1 week post-tx and another at PK visit and month 4 and 6.
- Record acute rejection and eGFR posttransplant

Study Endpoints

- Pharmacokinetics – AUCs of MPA, MPAG, acyl MPAG and enterohepatic recirculation
- Tacrolimus troughs, measured clinically
- MPA related anemia
- MPA related leukopenia
- Diarrhea as a patient reported outcome

Criteria to Determine Study Eligibility

INCLUSION CRITERIA

1. Undergoing kidney transplantation
2. Male or female at least 18 years of age at time of enrollment
3. Receiving oral tacrolimus and MMF (Cellcept or generic) for maintenance immunosuppression. MMF must be given orally in equal doses on a every 12hr schedule, at least 48 hr before PK visit to assure steady state
4. Gives written informed consent
5. Able and willing to complete all study-related procedures and visits
6. Living or Deceased Donor Kidney tx recipient
7. Able and willing to complete all study-related visits

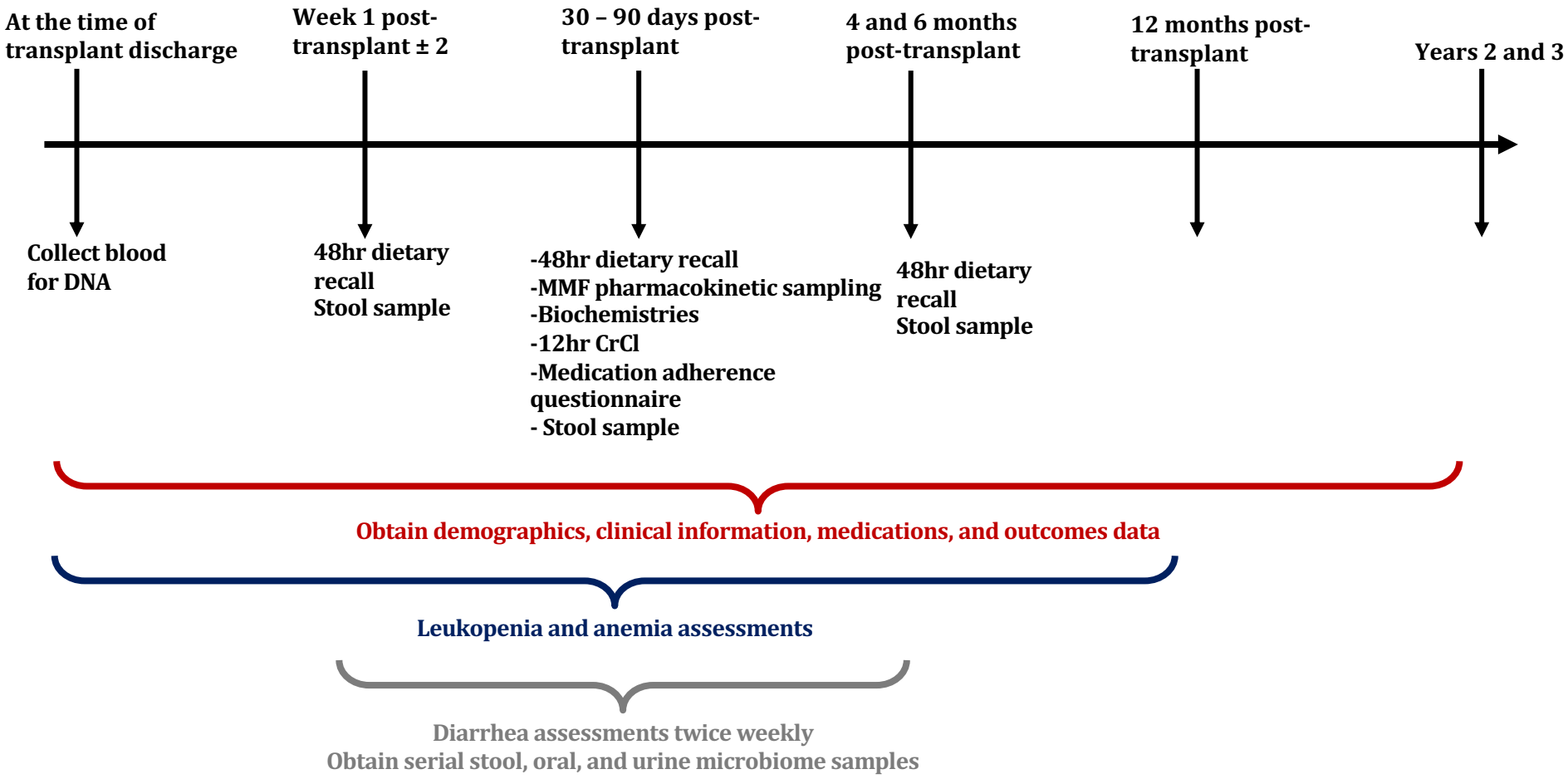
EXCLUSION CRITERIA

1. Recipient of a previous non-kidney transplant
2. Patient is a multi-organ transplant recipient
3. Patients that take medications that significantly impact (inhibit or induce) UGT enzymes
4. Presence of active gastroparesis
5. Liver dysfunction (total bilirubin >2x Upper limit of normal) within 2 weeks of PK visit.
6. Patients that take medications that significantly impact (inhibit or induce) the UGT enzymes
7. Patient is HIV positive
8. Pregnant or nursing (lactating) women

Important Study Details

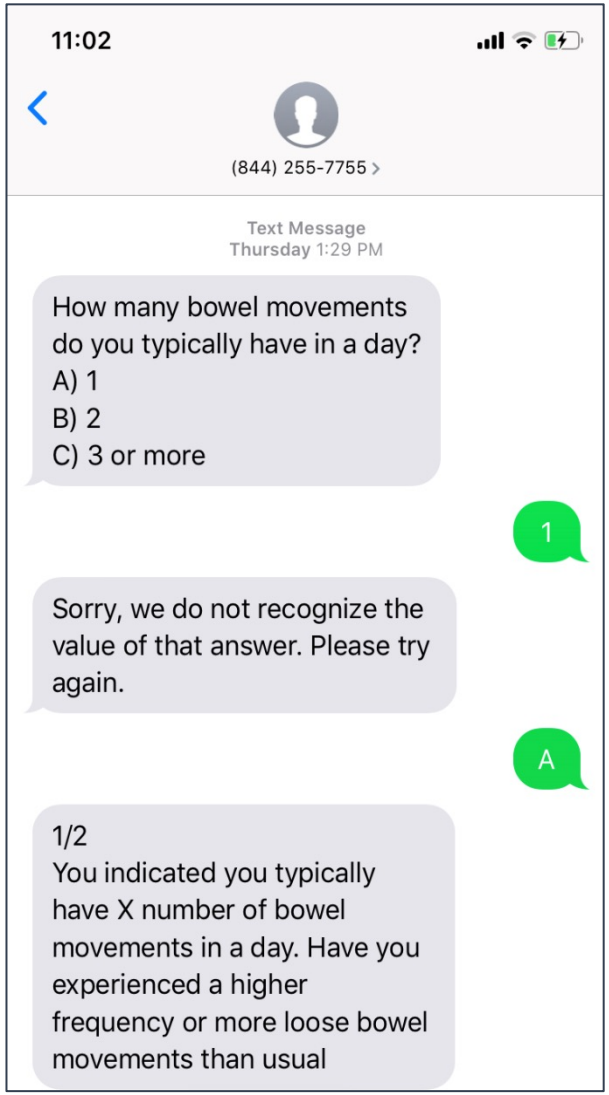
- Participants enrolled from UMN and Hennepin kidney transplant programs
- Microbiome samples will be collected 4 times in first 6 months. The participant may bring the stool samples into the clinic.
- Participants will be admitted to CRU for ~13 hours for a one time MMF pharmacokinetic study between days 30-60.* Must be on mycophenolate mofetil (not Myfortic) and tacrolimus.
- Participant must be on BID dosing of MMF (to capture EHR peak) at time of pharmacokinetics.
- Stay on MMF if possible until end of study at 12 months and dosing not changed to non BID intervals
- Text messages will be sent twice weekly with questions about diarrhea occurrence and severity for 6 months posttx
- Follow participant for 12 months for leukopenia and anemia occurrence
- Paid for participation ($200 for PK visit and additionally for text messages and more stool samples)

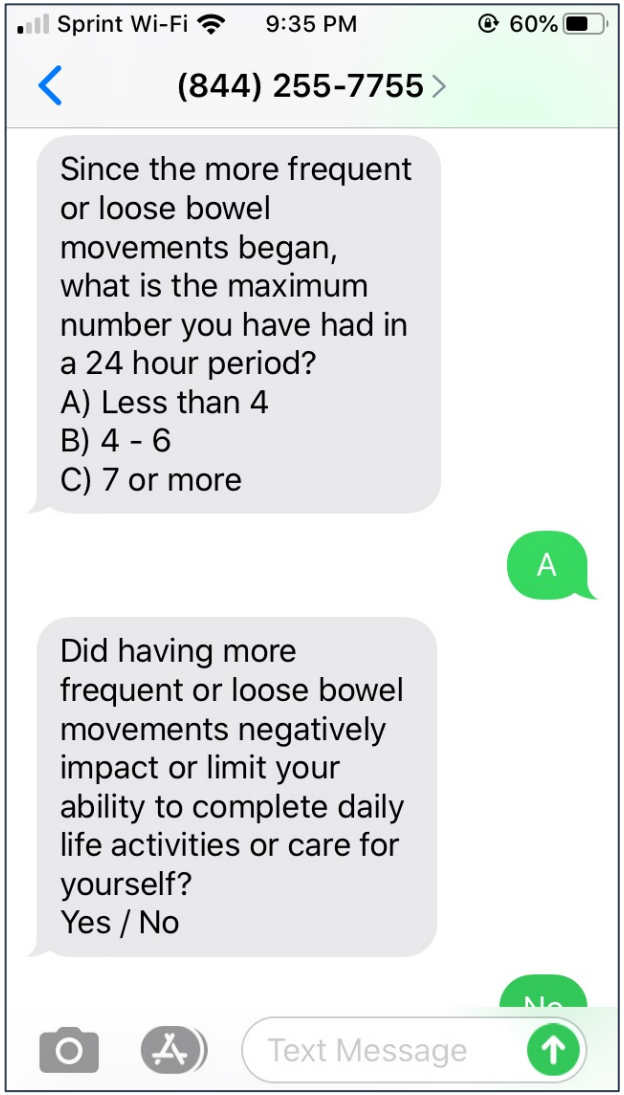
Schedule of Events for Prospective Cohort



Diarrhea Assessments

Participants will be texted twice weekly to assess frequent of loose bowel movements x 6 months





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Conclusion

- Microbiome plays a role in drug disposition in transplantation but clinical significance is unknown
- We will determine association of microbiome:
  - MMF pharmacokinetic
  - MMF related anemia
  - MMF related leukopenia
  - MMF related diarrhea
  - Accounting for diet, concomitant meds, & genetics
- Serial microbiome specimens, and clinical information for ancillary studies

Quantifying Mycophenolate mofetil metabolism using an in-vitro assay

Glucuronidation: Sugar-driven symbiosis between microbiota and host

- Glucuronidation inactivates and detoxifies molecules by increasing their water solubility, which promotes their removal from the body via the kidneys or GI tract.
- In the GI tract, these glucuronides serve as substrates for bacterial GUS proteins
  - As a by-product of glucuronide hydrolysis, bacteria regenerate the original molecule that was eliminated by the host
- **Glucuronidated endogenous** compounds include bilirubin, hormones, neurotransmitters, bile acids, and fatty acids, all of which influence host homeostasis.

Pellock SJ, Redinbo MR. Glucuronides in the gut: Sugar-driven symbioses between microbe and host. *J Biol Chem*. 2017;292(21):8569-8576. doi:10.1074/jbc.R116.767434

Exogenous glucuronides in the gut

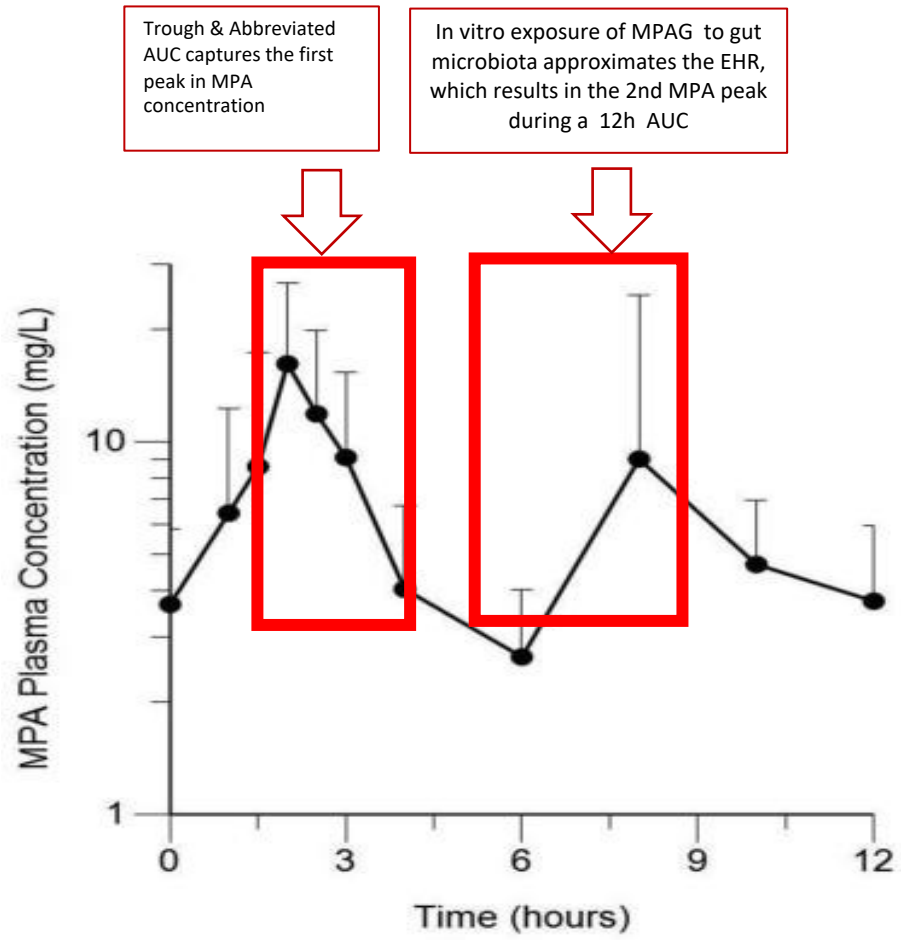
- Glucuronidation is also an important part of drug metabolism to mark compounds for excretion.

- Glucuronides of drugs such as Mycophenolate have been a primary focus of research because of their potential importance to therapeutic efficacy and tolerance.

- Exogenous glucuronides that reach the GI are diverse in chemical structure, suggesting that a proportional breadth of functional diversity may be present in the collection of microbial GUS enzymes in the GI
  - Gut microbiota composition is an important characteristic to EHR for drugs and exogenous glucuronides

Pellock SJ, Redinbo MR. Glucuronides in the gut: Sugar-driven symbioses between microbe and host. *J Biol Chem*. 2017;292(21):8569-8576. doi:10.1074/jbc.R116.767434

Potential of a new assay to assess in-vitro metabolism

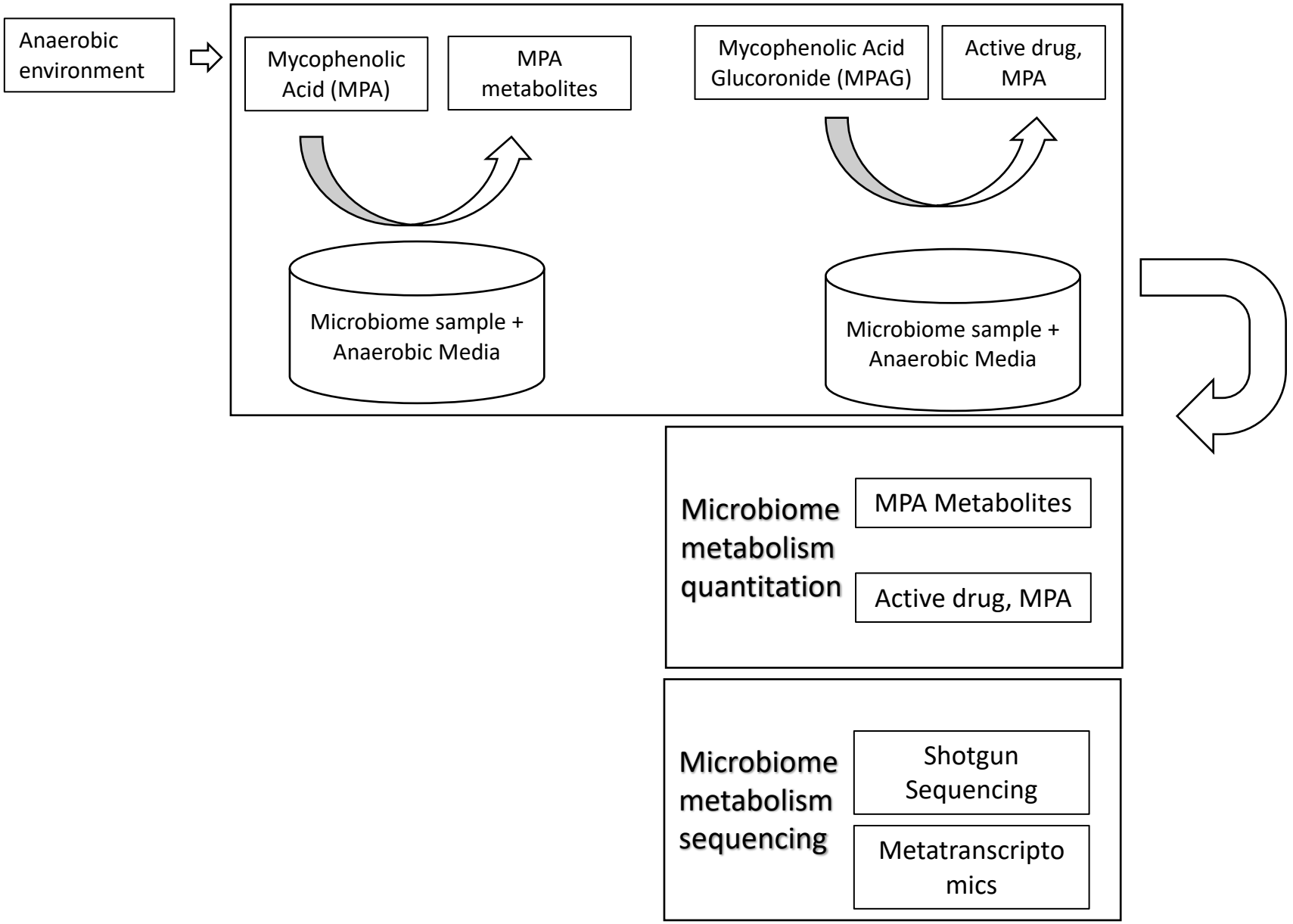


MPA 12 hour AUC curves adapted from Tornatore et. al. [1] and information captured by trough & Abbreviated AUC concentration and our in-vitro assay.

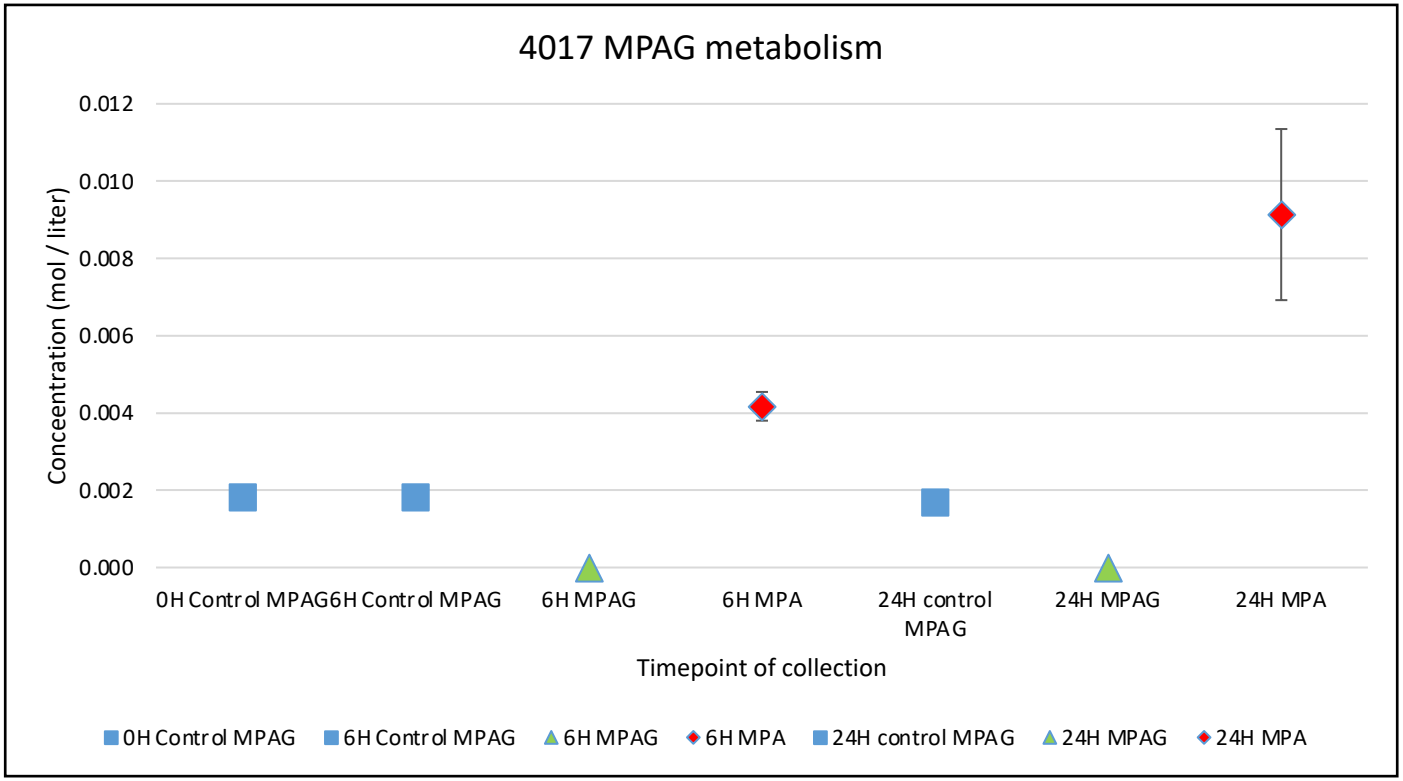
In-vitro assay Methods

- The stool microbiome samples from MISSION study patients undergoing the 12 hour AUC, were used to do pilot testing of our in-vitro assay on 4 Mission study participants.
  - These patients were on a steady state of MMF around 2 months posttx, when they underwent intensive pharmacokinetic sampling over 12 hours to carefully and accurately assess EHR.
  - A stool sample was collected with 48 hours of the pharmacokinetic sampling (most samples were collected during the PK profile)

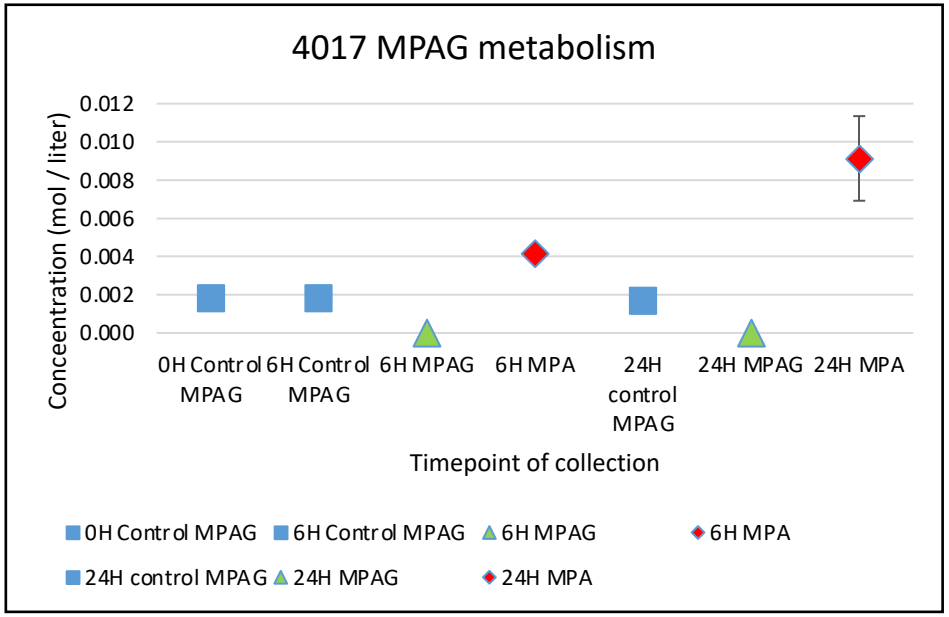
In-vitro assay Methods

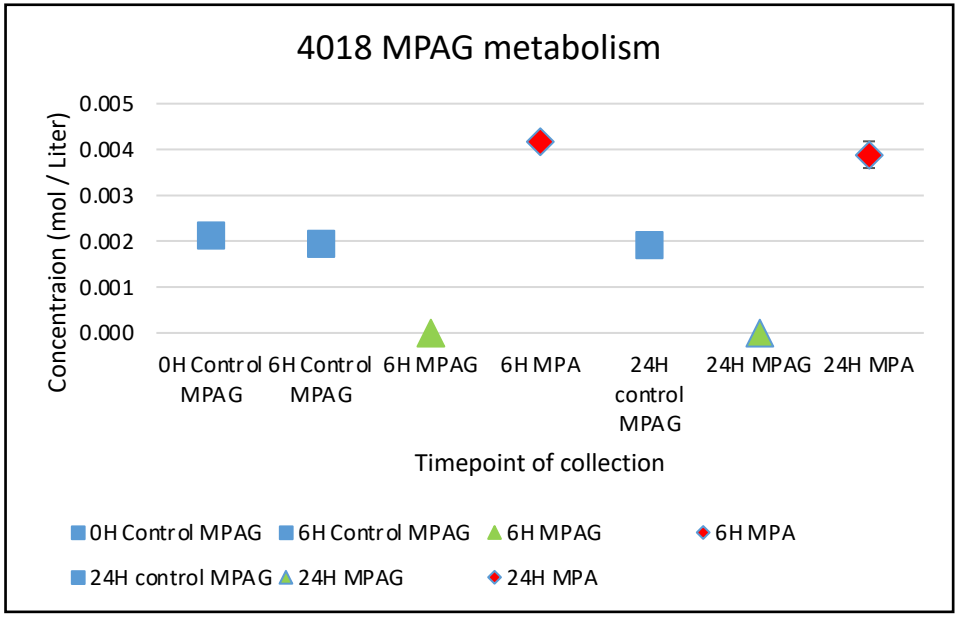


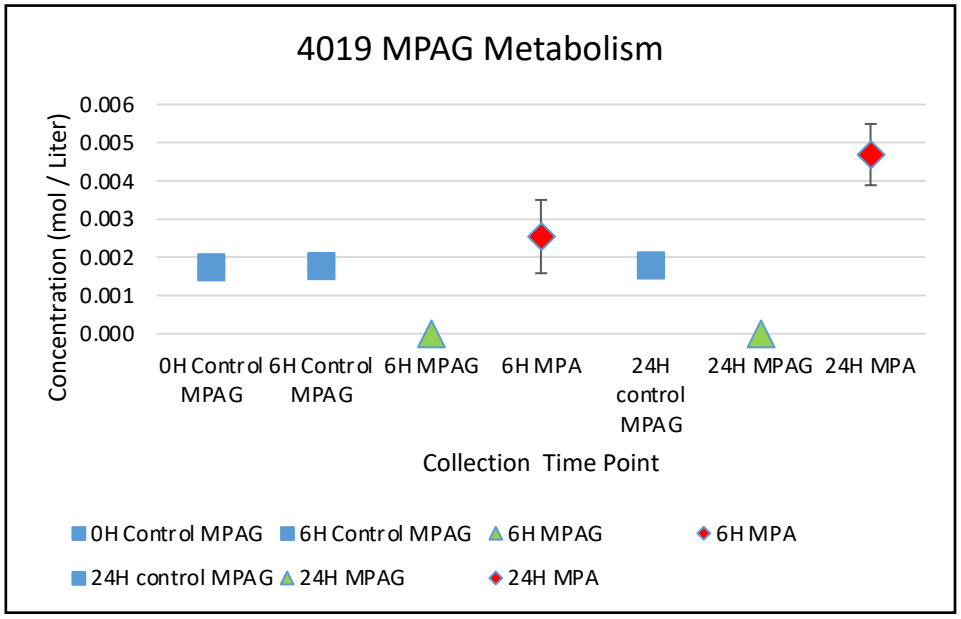
In-vitro Data

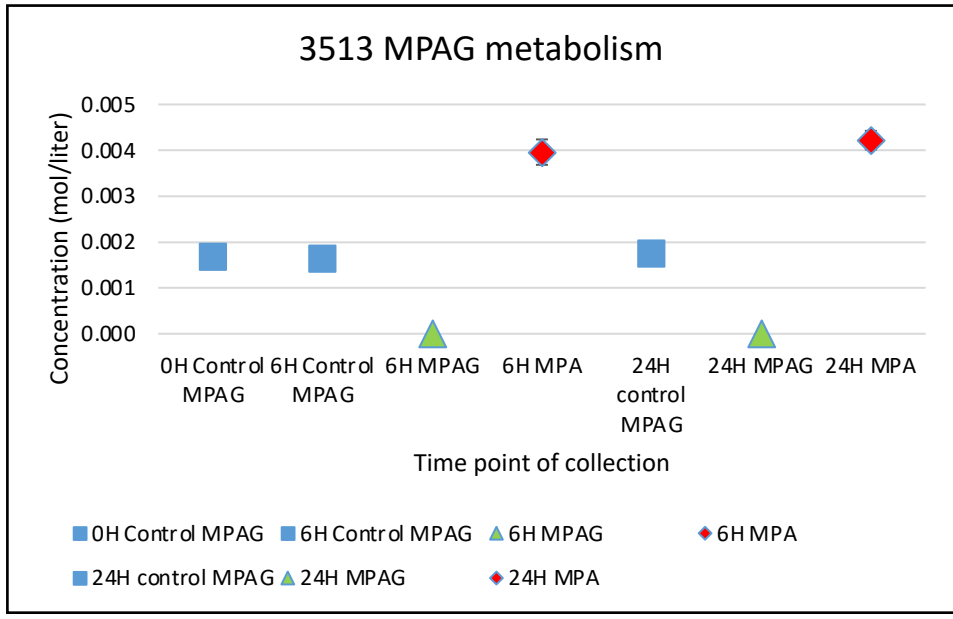


In-vitro Data

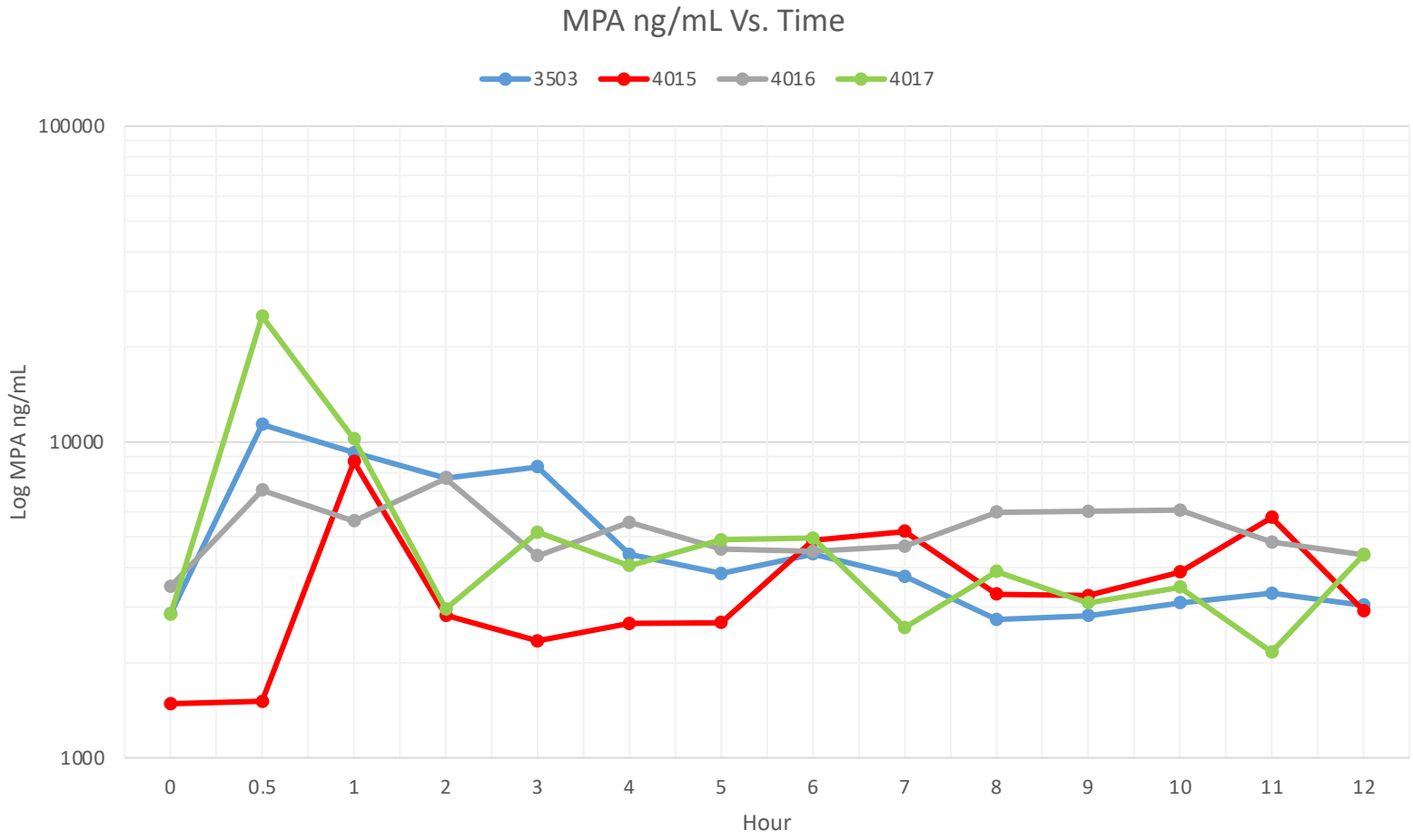




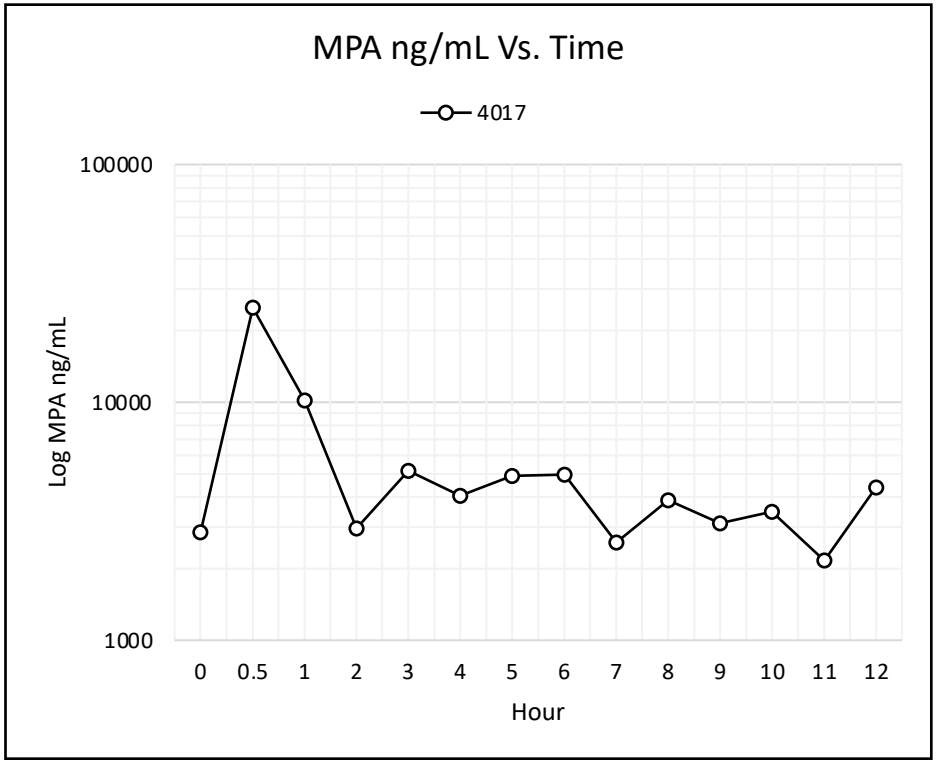


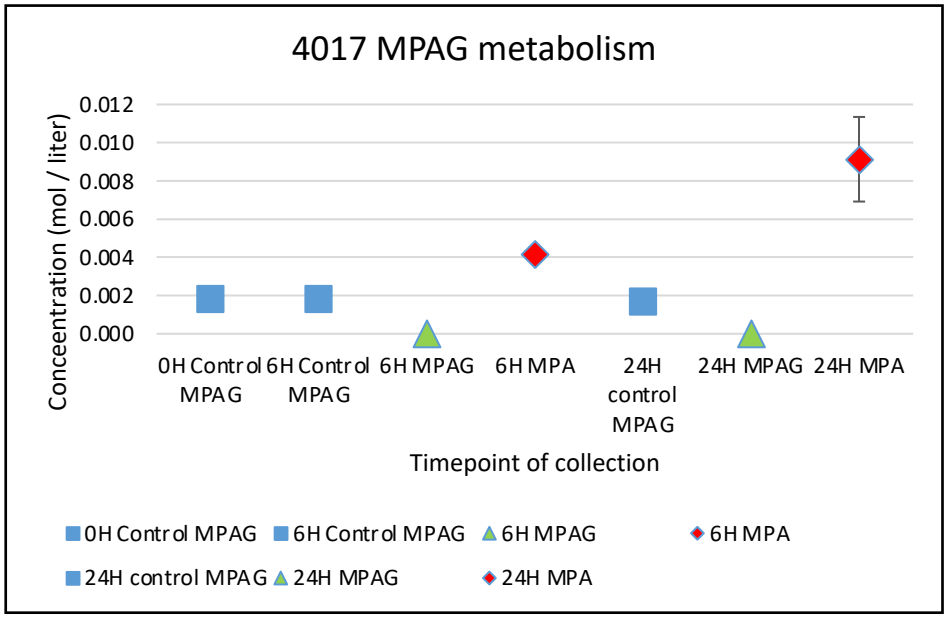


In-vivo MPA PK data



In-vivo vs in-vitro MPA PK data





Preliminary data

- MPAG concentrations decreased rapidly at 6 hours post incubation

- We detected an increase in the concentration of MPA starting at the 6 hour after incubation, with a continuing upward trend in the 24 hour aliquots.

Ongoing research

- Shotgun metatranscriptomics to quantify the amount of beta-glucuronidase enzyme transcripts present in the samples

- MPA pharmacokinetic assays to correlate with in-vitro data

Future Directions

- The methods for the in-vitro assay may determine the dose appropriate for individuals based on their microbiome
- African American patients are often prescribed higher doses of 1.5 grams twice a day.
- Further, this in-vitro approach is not limited to drugs that undergo EHR

Future Directions

Drug Name	Function	Gut Microbiota Modification	Effect of microbial modification
Irinotecan	Cancer therapy drug	De-glucuronidation of the active metabolite in the gut	Toxicity [1, 2]
Indomethacin	Anti-inflammatory drug	Beta-glucuronidase driven cleavage of NSAID glucuronide	Toxicity [2, 3]
Diclofenac	Anti-inflammatory drug	Beta-glucuronidase driven cleavage of NSAID glucuronide	Toxicity [2, 3]
Ketoprofen	Anti-inflammatory drug	Beta-glucuronidase driven cleavage of NSAID glucuronide	Toxicity [2, 3]
Mycophenolate Mofetil (MMF)	Immunosuppressive drug	Gut microbiota metabolize MPA glucuronide (MPAG) back to mycophenolic acid (MPA)	Toxicity [4-6]
Sulfasalazine	Inflammatory bowel disease (IBD) medication	Gut microbiota reduce sulfasalazine into sulfapyridine and the active anti-inflammatory agent 5-ASA	Toxicity [2, 4-6]
Sulindac	Anti-inflammatory drug	De-glucoronidation of the active metabolite in the gut	Toxicity [2, 4-6]
Loperamide	Anti-diarrheal drug	De-glucoronidation of the active metabolite in the gut	Toxicity [2, 4-6]
Levodopa	Parkinson's disease therapeutic drug	Microbial decarboxylation and p-dehydroxylation convert L-dopa to m-tyramine	Inactivation [2]
Cladribine	Cancer therapy drug	Inhibited efficacy	Inactivation[7]
Gemcitabine	Cancer therapy drug	Inhibited efficacy	Inactivation[7]
Digoxin	Congestive heart failure therapy drug	*Eggerthella lenta* metabolizes digoxin to dihydrodigoxin	Inactivation[2]
Tacrolimus	Immunosuppressive drug	Specific organisms metabolize Tacrolimus to a less effective metabolite	Inactivation [8]
Benzylpenicillin	Cancer therapy drug	Beta-lactamase inhibition	Inactivation[9]
Ipilimumab	Antil CTLA-4 human monoclonal antibody (Ab) therapeutic drug	*B. thetaiotaomicron* and *B. Fragilis* enhance T-cell response to antibodies	Activation [2, 10]
Metformin	Type 2 diabetes (T2D) therapy drug	*Akkermansia muciniphila* abundance is modulated by metformin treatment	Activation [2, 11]
Fludarabine de phosphate	Cancer therapy drug	Improved efficacy	Activation [7]
CB1954	Cancer therapy drug	Improved efficacy	Activation [7]

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- Levi Teigen, PhD

Additional Slides

78 gut bacteria were assessed to metabolize 271 drugs, including MMF, Tac and Prednisone

In vitro quantitative evidence of MMF or TAC metabolism via Liquid chromatography- mass spectrophotometry (LC-MS) after a 12-hour incubation

16 gut bacteria were associated with a decrease in MMF and TAC after a 12 hour incubation

Evidence of beta-glucuronidase activity in the gut microbiome
Evidence of TAC metabolite production

8 gut bacteria were found to produce GUS enzymes in previous in vitro studies. 1 was found to metabolize TAC but not MMF

Part of a simplified model for human gut microbiome interaction

Culturable strain available for purchase

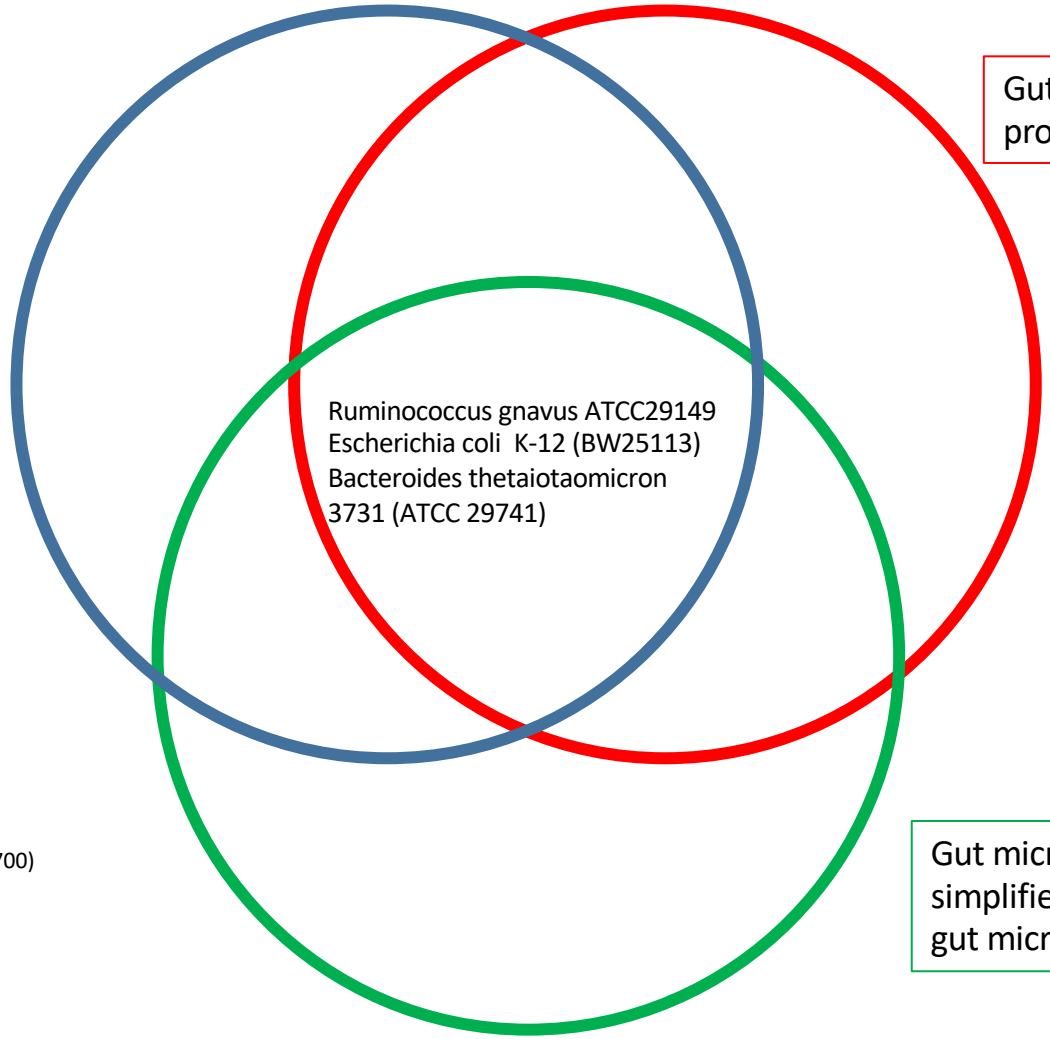
Guo Y, Crnkovic C, Won KJ, et al. Commensal gut bacteria convert the immunosuppressant tacrolimus to less potent metabolites. *Drug Metab Dispos*. 2019;47(3):194-202. doi:10.1124/dmd.118.084772

Pellock SJ, Redinbo MR. Glucuronides in the gut: Sugar-driven symbioses between microbe and host. *J Biol Chem*. 2017;292(21):8569-8576. doi:10.1074/jbc.R116.767434

Biernat KA, Pellock SJ, Bhatt AP, et al. Structure, function, and inhibition of drug reactivating human gut microbial β-glucuronidases. *Sci Rep*. 2019;9(1). doi:10.1038/s41598-018-36069-w

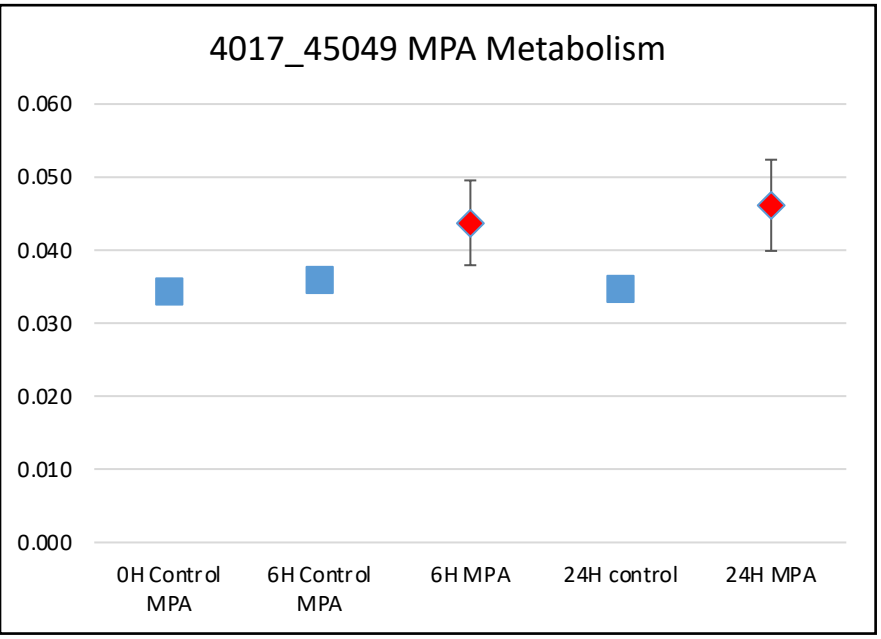
Ervin SM, Li H, Lim L, et al. Gut microbial β-glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens. *J Biol Chem*. 2019;294(49):18586-18599. doi:10.1074/jbc.RA119.010950

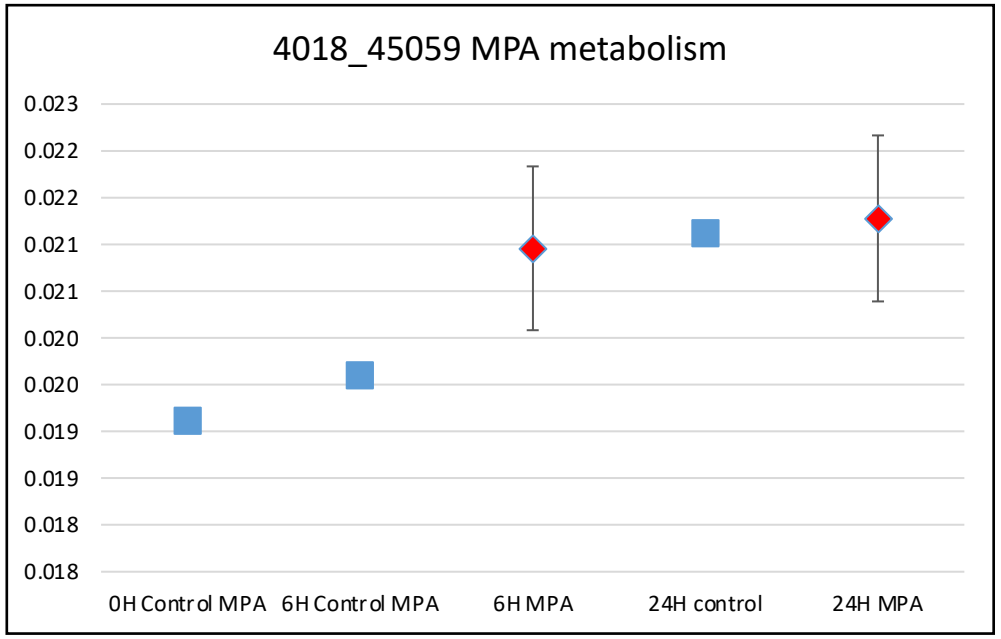
Zimmermann M, Zimmermann-Kogadeeva M, Wegmann R, Goodman AL. Mapping human microbiome drug metabolism by gut bacteria and their genes. *Nature*. 2019;570(7762):462-467. doi:10.1038/s41586-019-1291-3



*MMF specific target could include
Bifidobacterium longum subsp. infantis CCUG52486
Bifidobacterium breve DSM20213 (ATCC15700)
Bacteroides vulgatus ATCC8482
Bacteroides ovatus ATCC8483
Clostridium ramosum DSM1402
*TAC specific target could include
Faecalibacterium Prausnitzii ATCC 27766

MPA metabolism by gut bacteria (Concentrations are presented in moles / liter)





MMF metabolism by gut bacteria (Concentrations are presented in moles / liter)