

Long Lyme: Opportunities For Prevention And Treatment

Kim Lewis

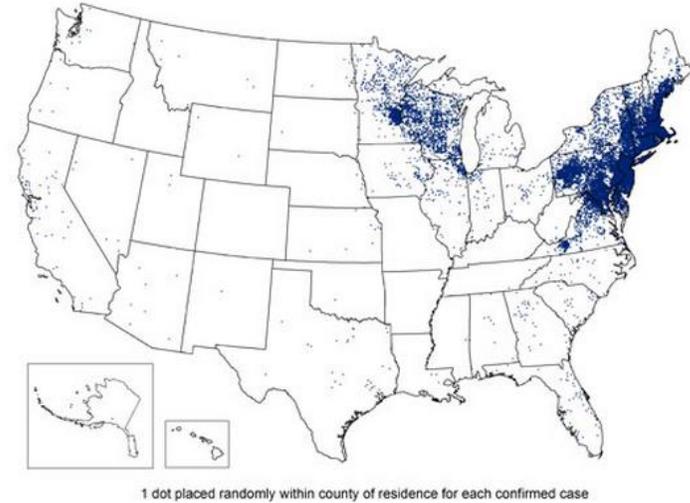


Northeastern University
Antimicrobial Discovery Center

Lyme Disease

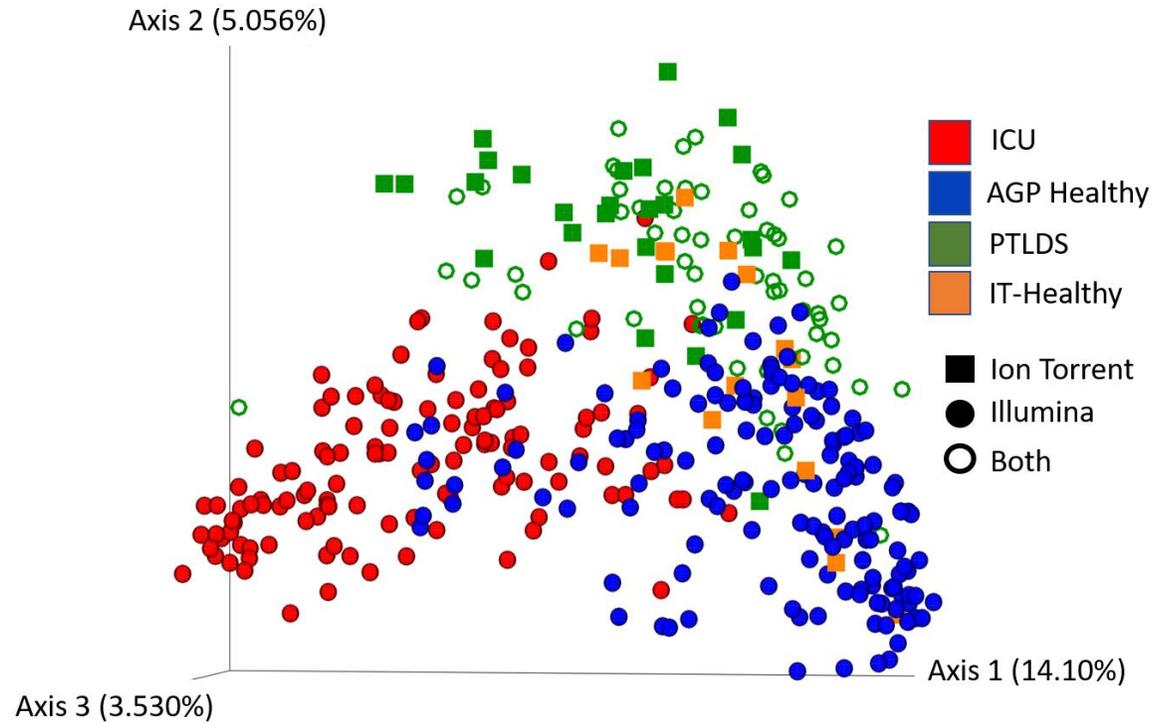
- Transmitted by *Ixodes* carrying *Borrelia burgdorferi*
- *Estimated number of cases 500,000 (CDC)*
- Treatment: Doxycycline, Amoxicillin, Cefuroxime Axetil, Ceftriaxone
- If untreated, Lyme arthritis and neuroborreliosis develops

10% develop PTLDS (Aucott et al., *International Journal of Infectious Diseases* 2022)



PTLDS Microbiome Signature

87 PTLDS patients

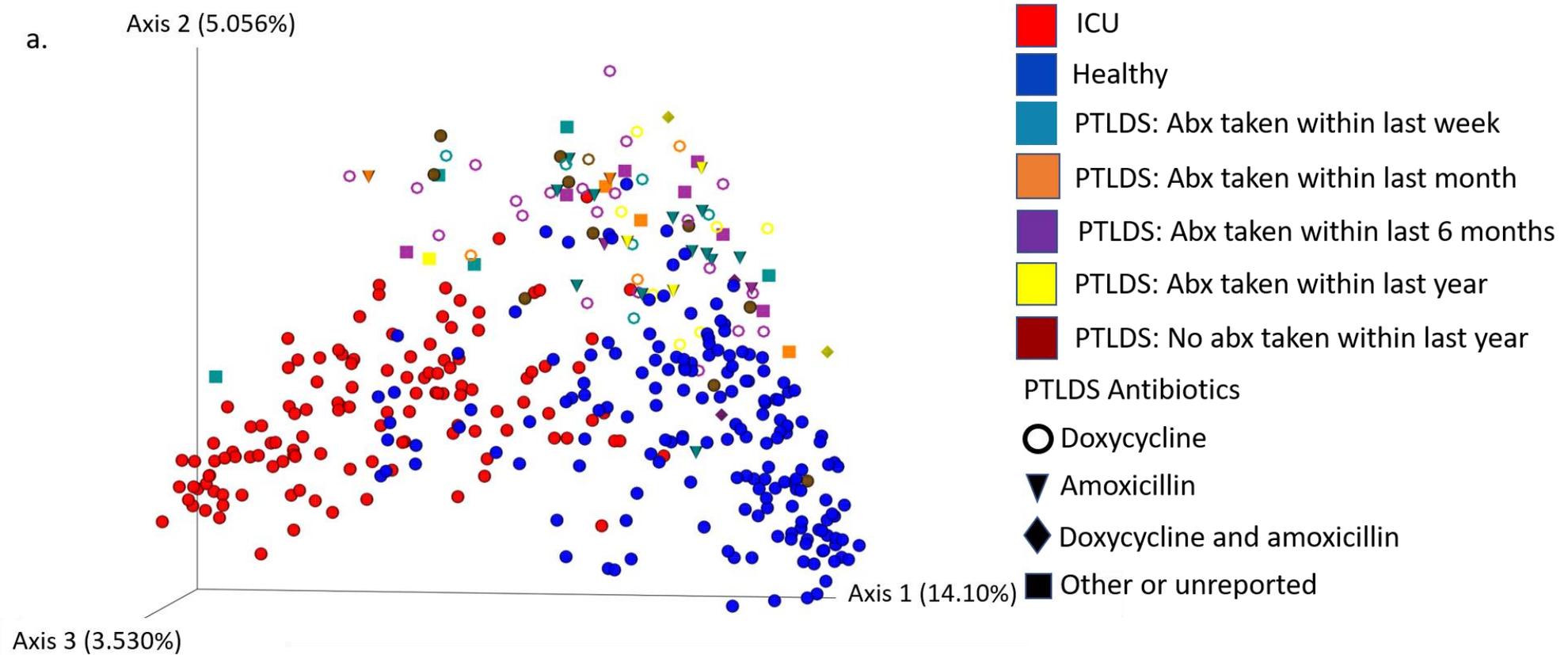


Editor's Pick Research Article | mBio 2020 11: e02310-20

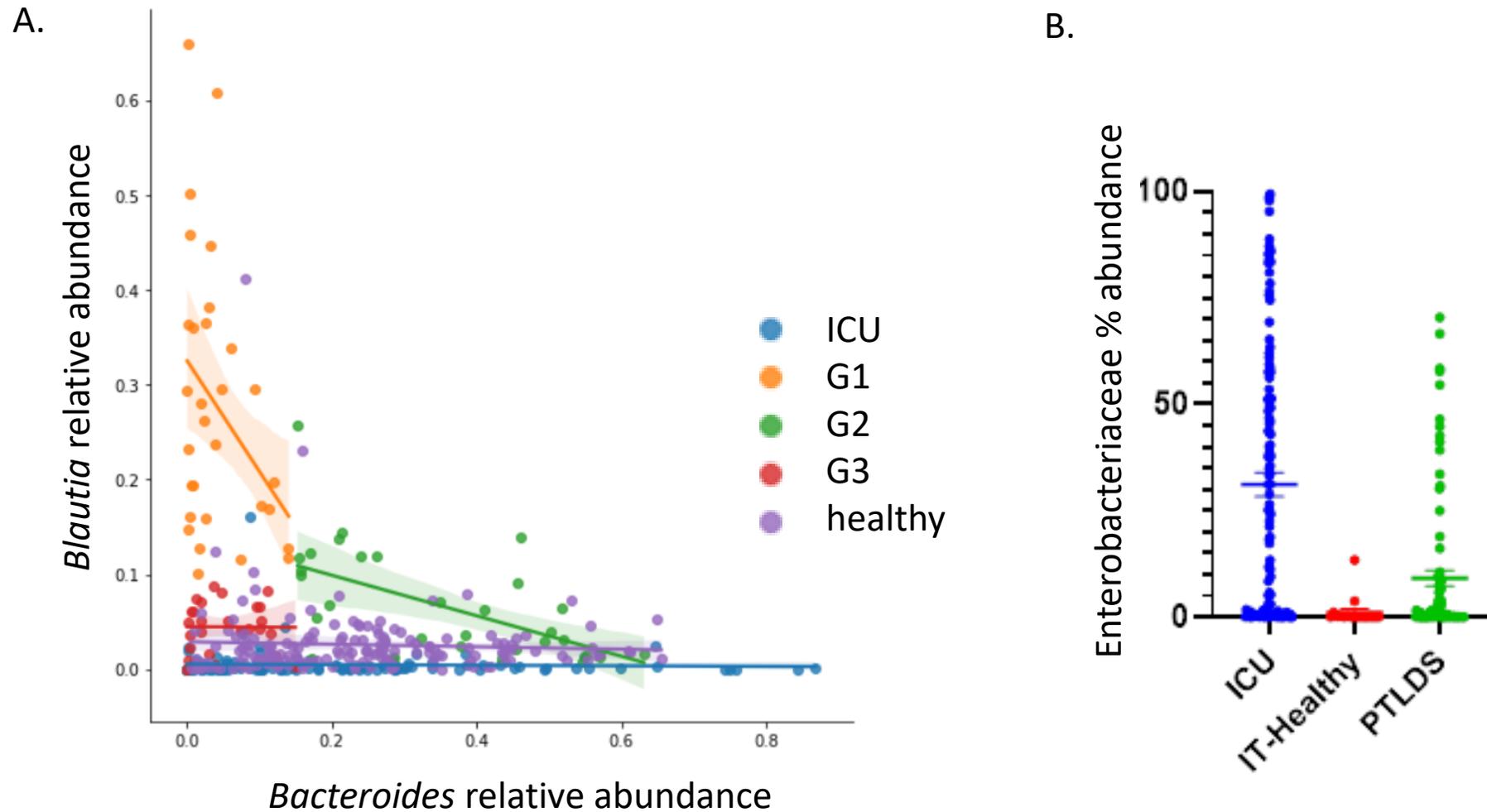
A Distinct Microbiome Signature in Posttreatment Lyme Disease Patients

Madeleine Morrissette, Norman Pitt, Antonio González, Philip Strandwitz, Mariaelena Caboni, Alison W. Rebman, Rob Knight, Anthony D'Onofrio, John N. Aucott, Mark J. Soloski, Kim Lewis

Antibiotic history alone does not explain the distinct signature observed in PTLDS

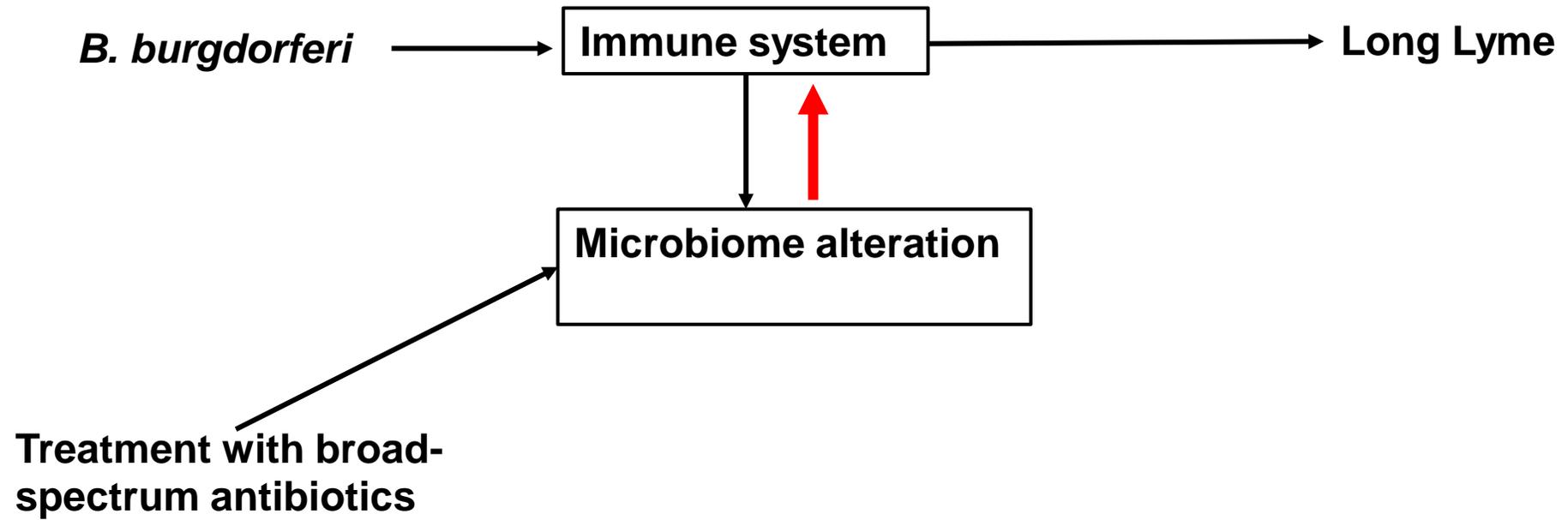


Gut Microbiome Signature – a PTLDS Diagnostic

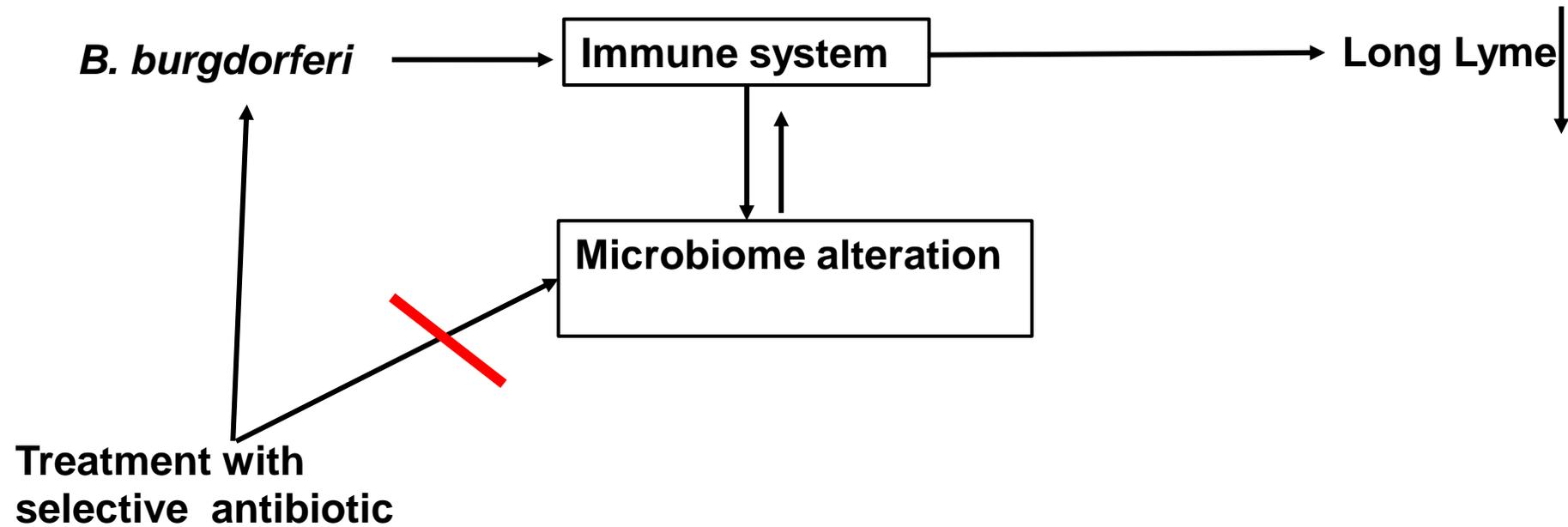


Two thirds of patients (G1 and G3) have a dysbiotic microbiome characterized primarily by high levels of *Blautia* (A) or Enterobacteriaceae (B).

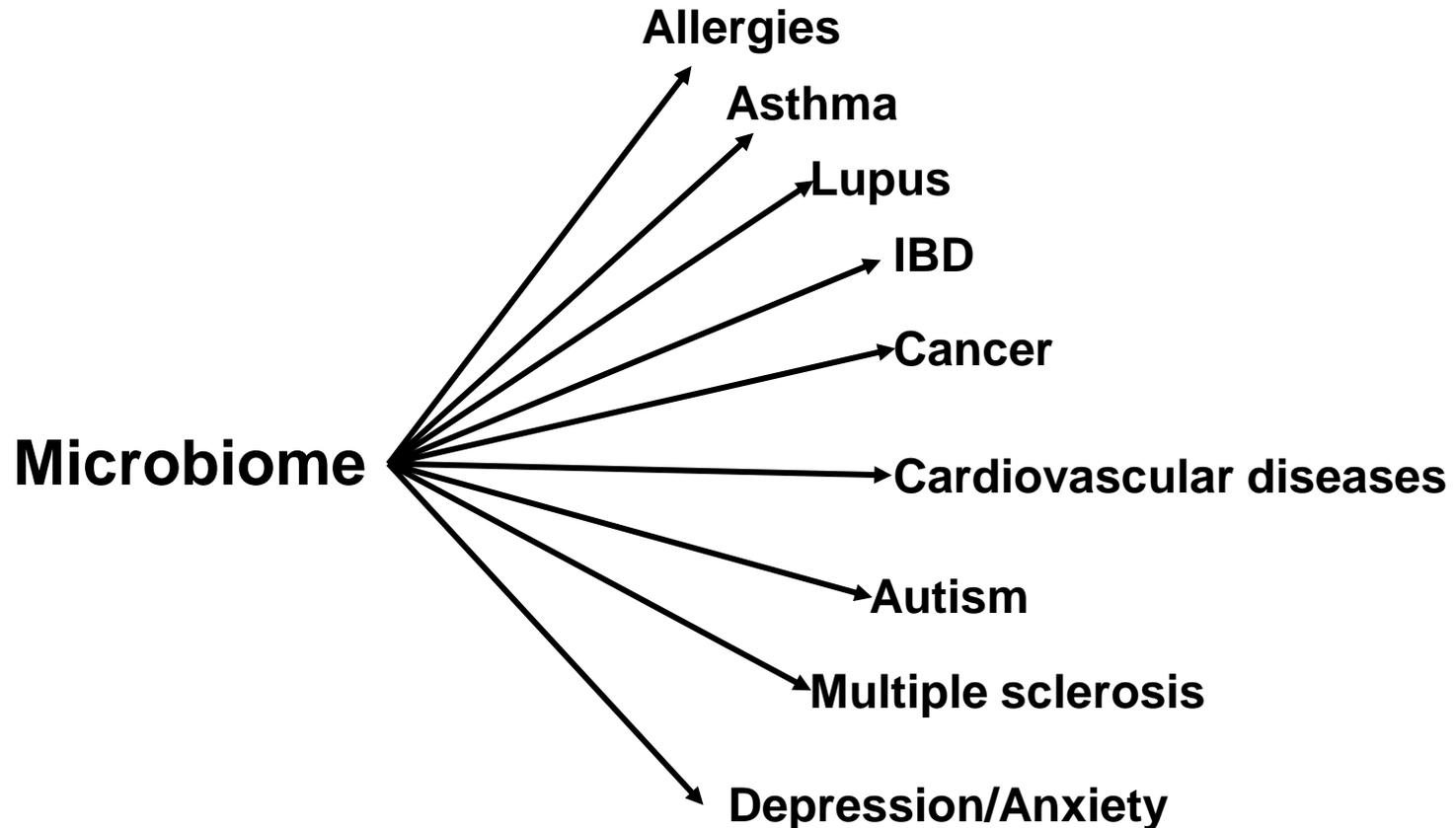
Working Hypothesis



Working Hypothesis



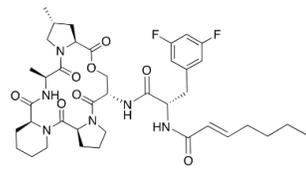
Sparing The Microbiome



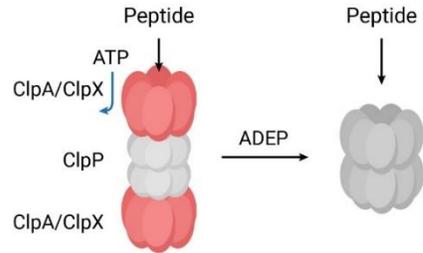
[Role of the **microbiome** in human development.](#) Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ. *Gut*. 2019 Jun;68(6):1108-1114.

[Antibiotics in the pathogenesis of diabetes and inflammatory diseases of the gastrointestinal tract.](#) Fenneman AC, Weidner M, Chen LA, Nieuwdorp M, Blaser MJ. *Nat Rev Gastroenterol Hepatol*. 2023

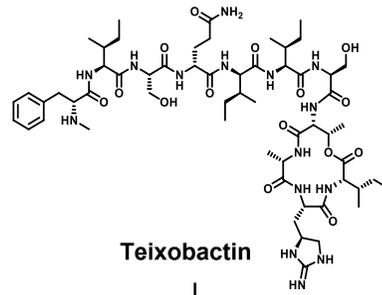
Selective Compounds to treat Lyme Disease



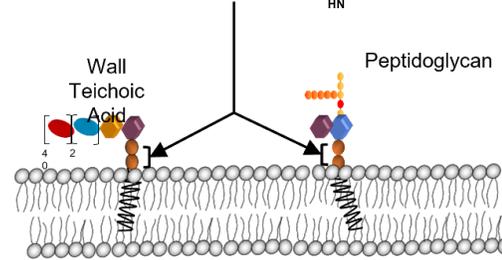
ADEP4



Conlon et al., Nature 2013

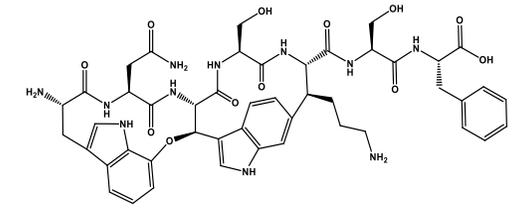


Teixobactin

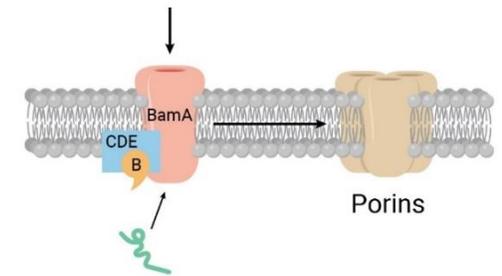


Ling et al., Nature 2015

Shukla et al., Nature 2022

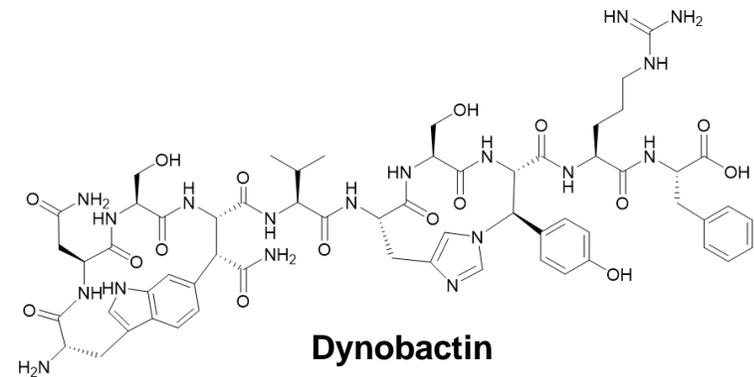


Darobactin A

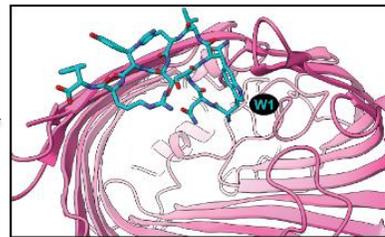


Imai et al., Nature 2019

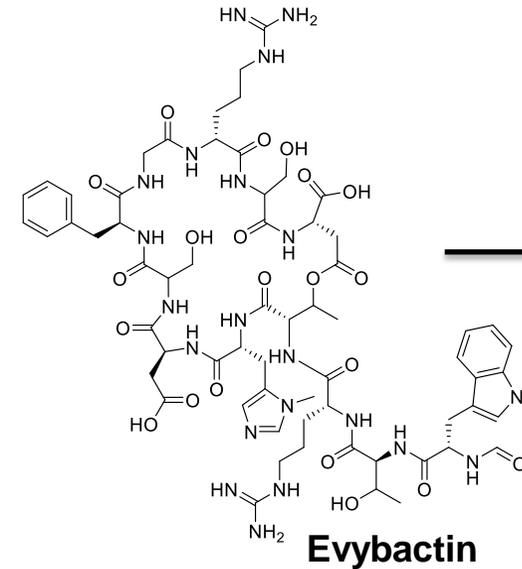
Kaur et al., Nature 2021



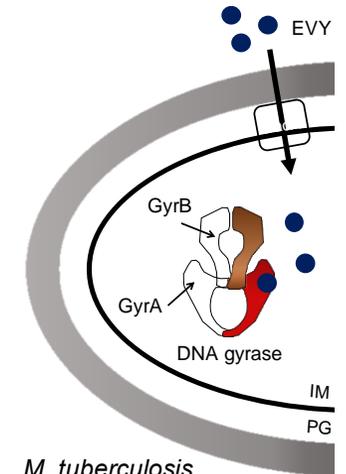
Dynobactin



Miller et al., Nature Microbiol. 2022



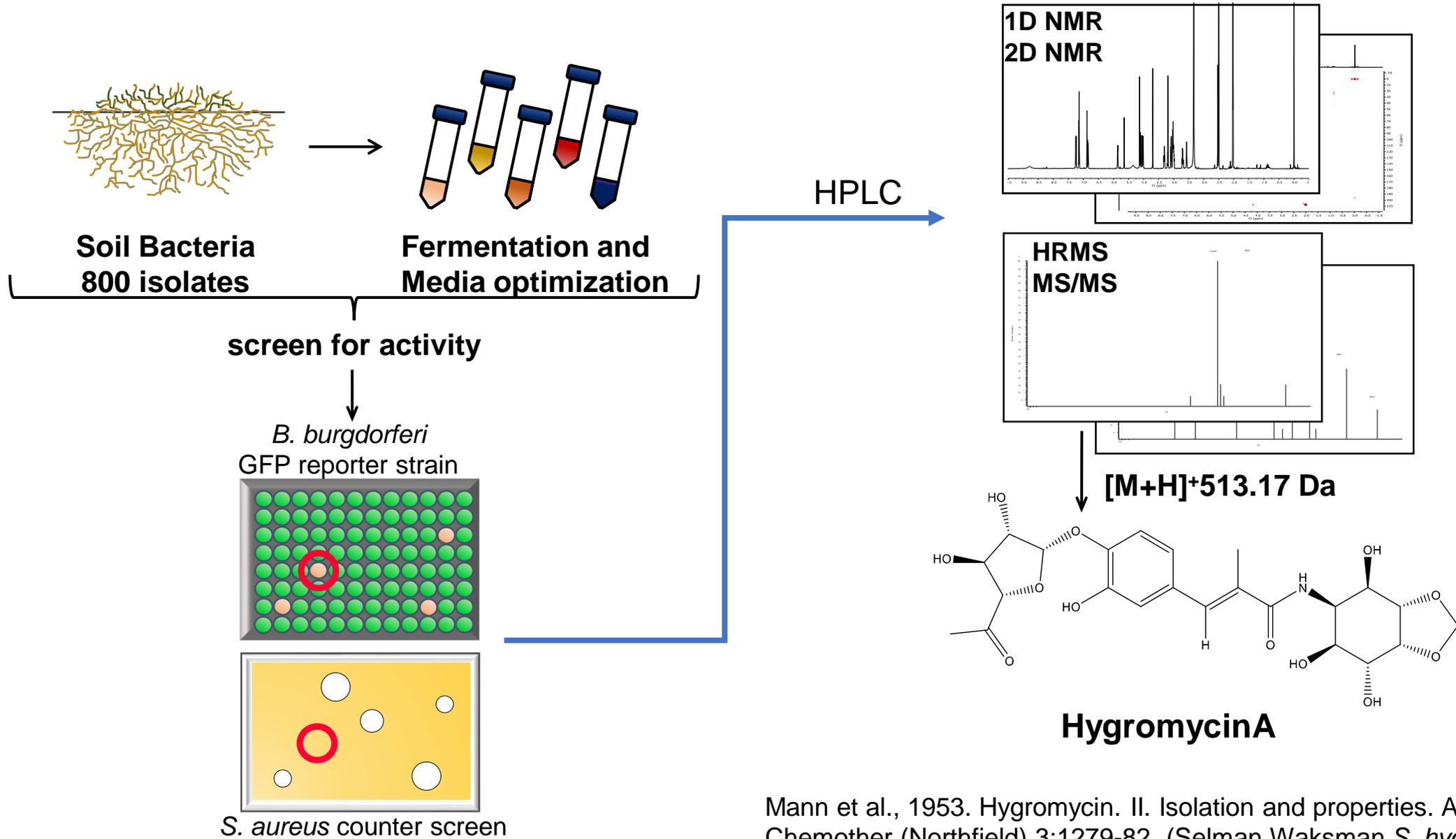
Evybactin



M. tuberculosis

Imai et al., Nature Chem. Biol. 2022

A search for selective anti- *B. burgdorferi* compounds



Mann et al., 1953. Hygromycin. II. Isolation and properties. *Antibiot Chemother (Northfield)* 3:1279-82. (Selman Waksman *S. hygroscopicus*)

Hygromycin A - Spectrum of Activity

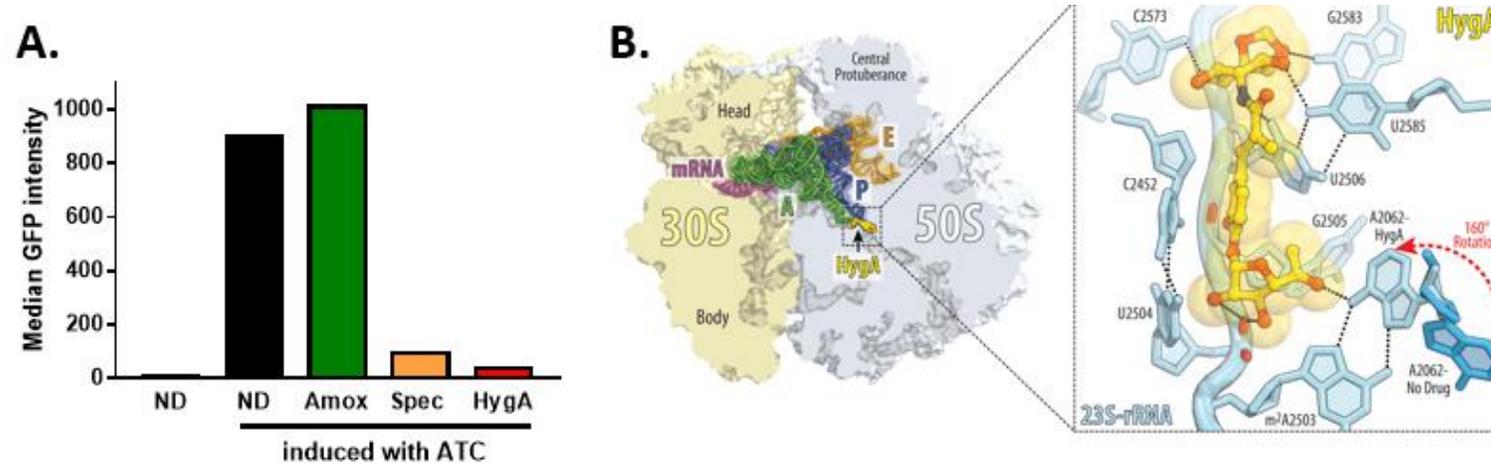
Others	MIC (µg/ml)
<i>Staphylococcus aureus</i>	32
<i>Salmonella Typhimurium</i>	>256
<i>Shigella sonnei</i>	>256
<i>Enterobacter cloacae</i>	>256
<i>Escherichia coli</i>	1000
<i>Pseudomonas aeruginosa</i>	>1000

Symbiotic Gut Bacteria	MIC (µg/ml)
<i>Bifidobacterium longum</i>	8
<i>Lactobacillus reuteri</i>	32
<i>Bacteroides fragilis</i>	128
<i>Enterococcus faecalis</i>	128

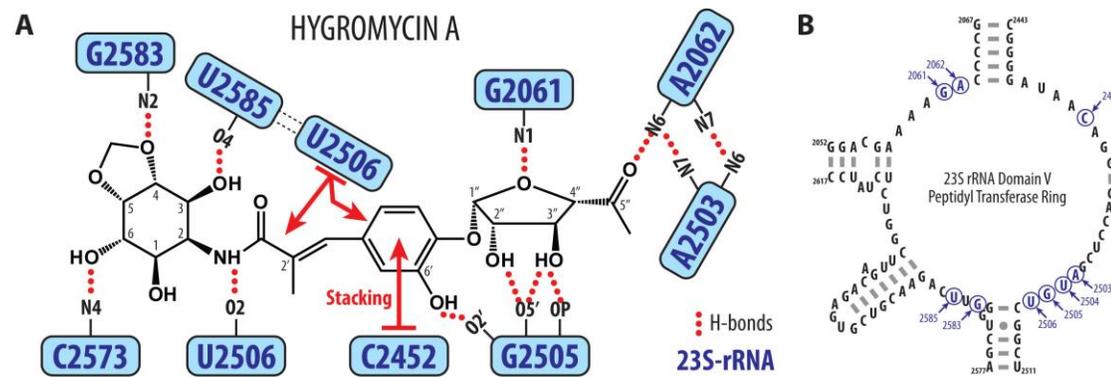
Spirochetes	MIC (µg/ml)
<i>Treponema pallidum</i>	0.03
<i>Borrelia burgdorferi</i>	0.2
<i>Borrelia afzelii</i>	0.2
<i>Borrelia garinii</i>	0.2
<i>Borrelia bavariensis</i>	0.2
<i>Borrelia turcica</i>	0.5
<i>Alkalistrochaeta americana</i>	0.5

Cytotoxicity >256 µg/ml

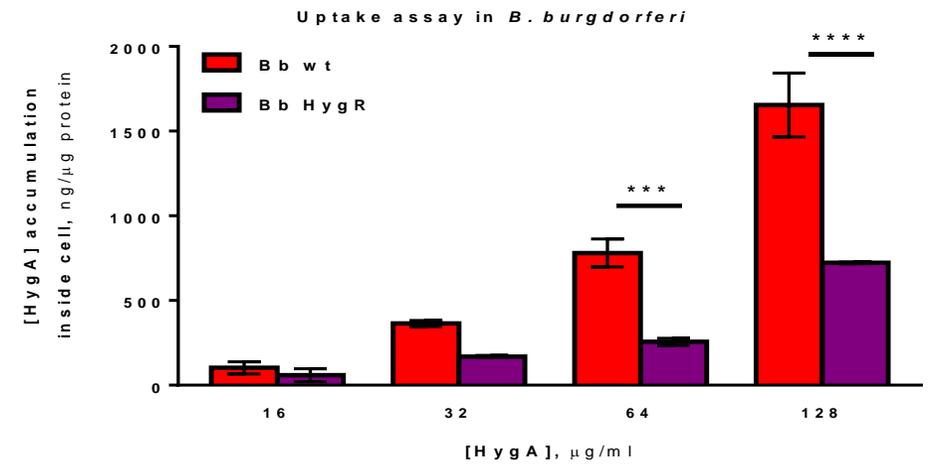
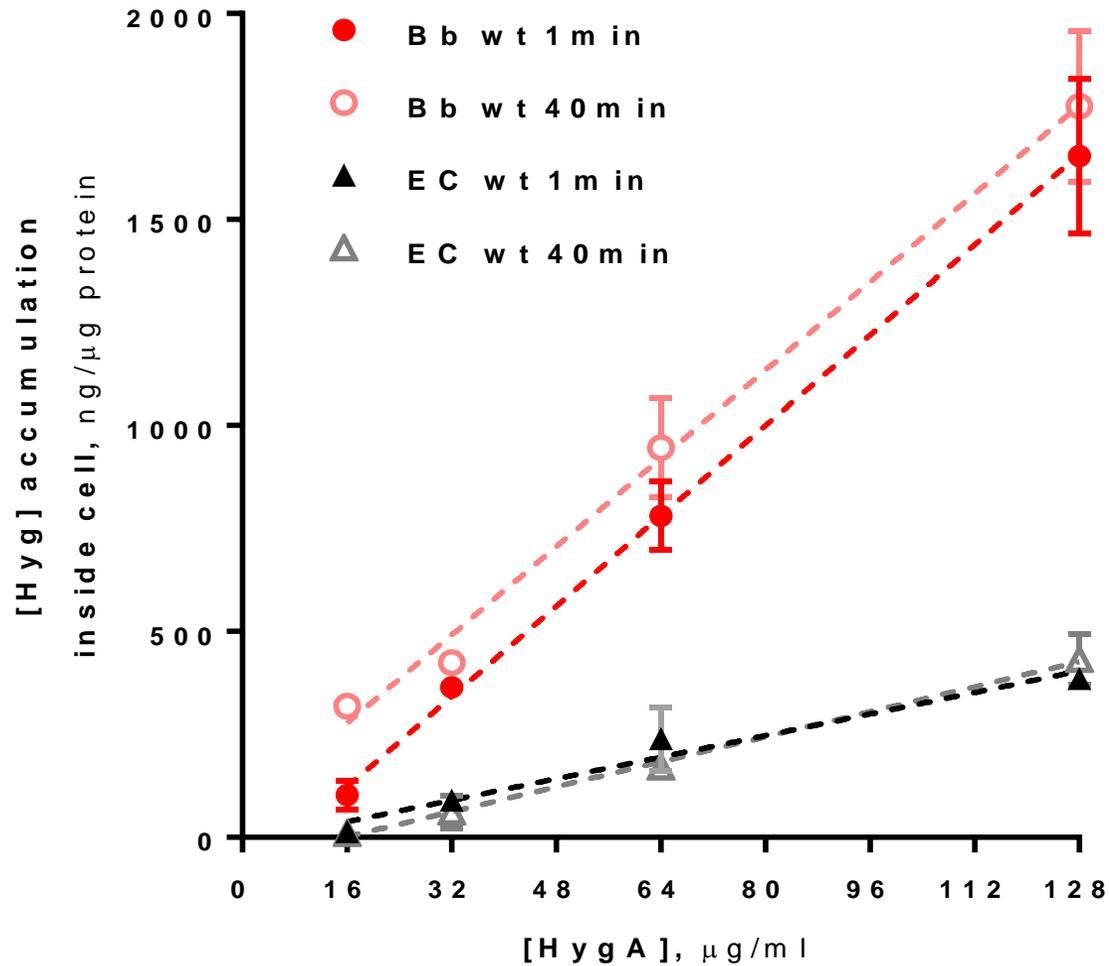
Hygromycin A Mechanism of Selectivity



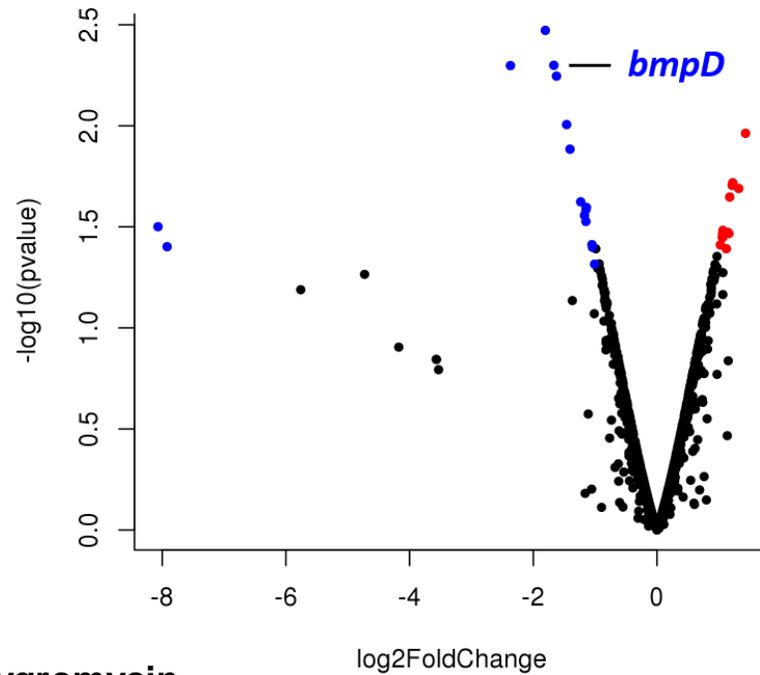
Yury S. Polikanov,^{1,2,9} Agata L. Starosta,^{3,9} Manuel F. Juetten,^{4,9} Roger B. Altman,⁴ Daniel S. Terry,⁴ Wanli Lu,⁵ Benjamin J. Burnett,⁴ George Dinos,⁶ Kevin A. Reynolds,⁵ Scott C. Blanchard,^{4,7,*} Thomas A. Steitz,^{1,2,*} and Daniel N. Wilson. *Mol. Cell* 2015.



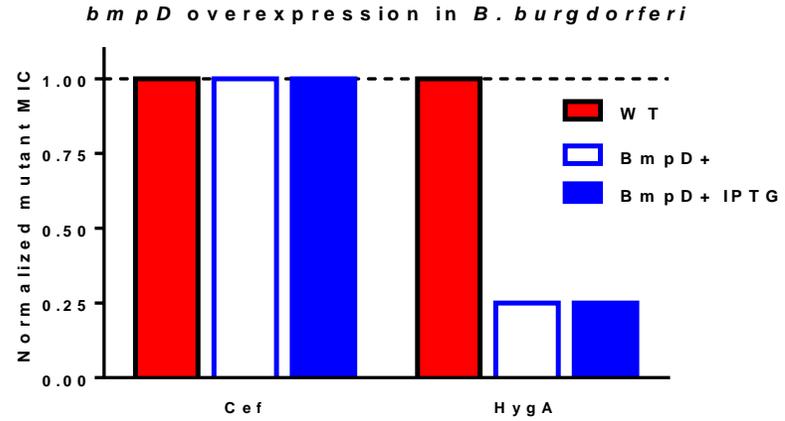
Hygromycin A Mechanism of Selectivity



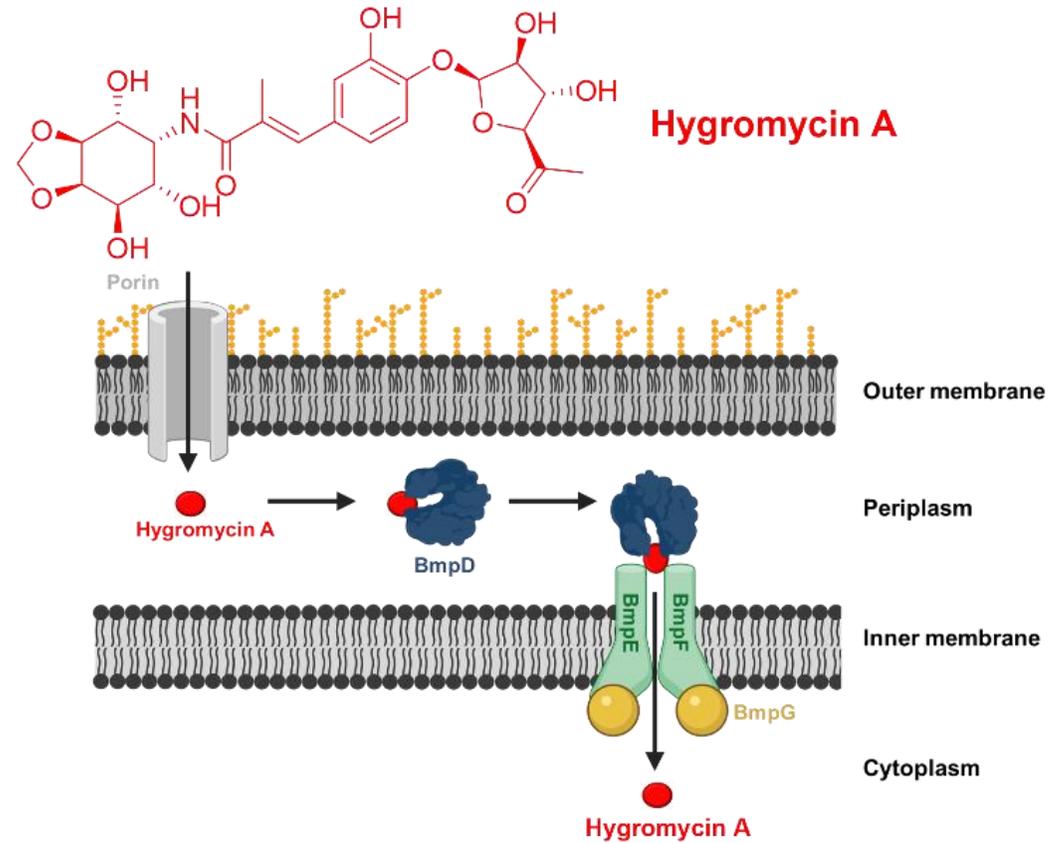
Hygromycin A Mechanism of Selectivity



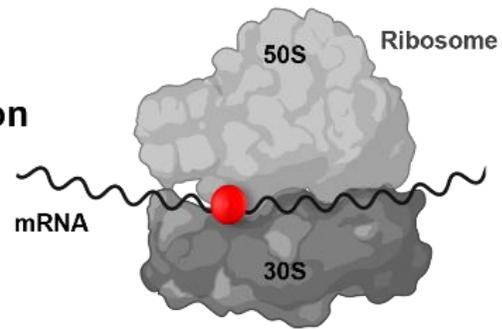
B. burgdorferi hygromycin resistant mutant, 50 SNIPS



Mechanism of hygromycin A selectivity



Hygromycin A inhibits translation



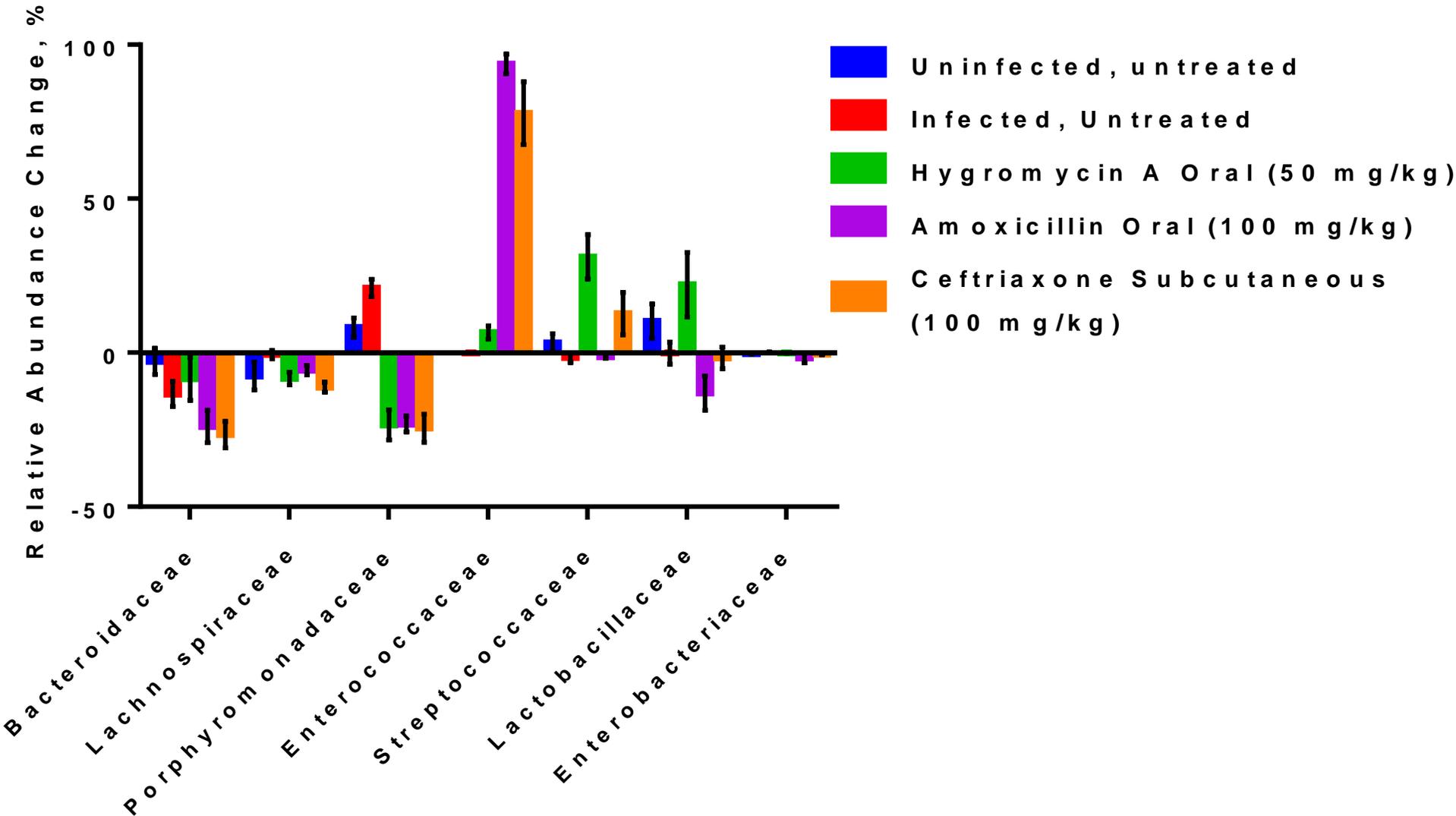
Hygromycin A Efficacy in a Mouse Model of Lyme Disease

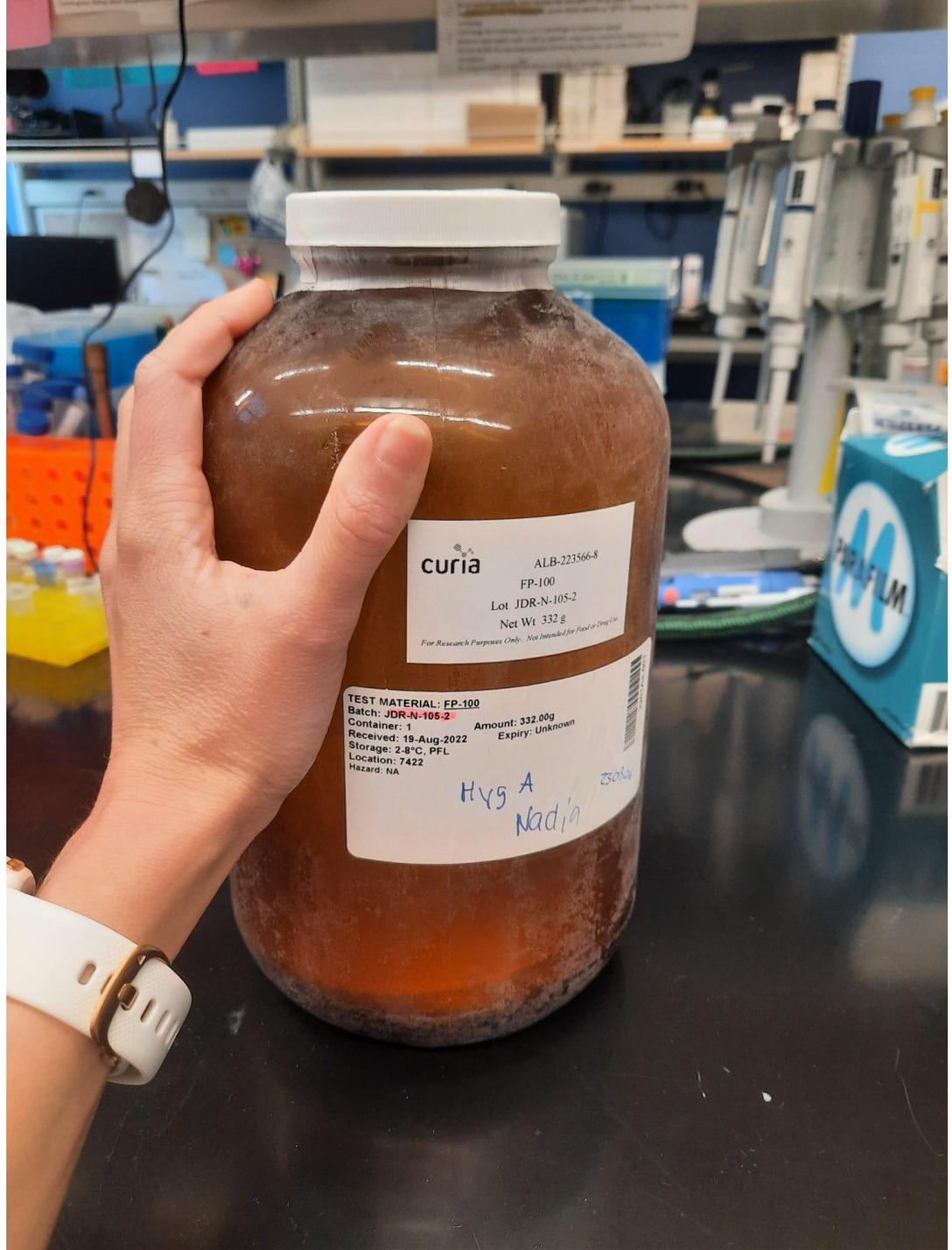
Mouse	Route of Administration	Compound	Daily Dose (mg/kg/day)	Culture positive (%)
C3H	Intraperitoneal injection	Saline	0	100
		Hygromycin A	100	0
		Ceftriaxone	312	0
C3H	Oral gavage	Hygromycin A	500	0
		Hygromycin A	140	0
		Hygromycin A	100	0
		Hygromycin A	50	50
		Amoxicillin	200	0
		Doxycycline	100	0
C3H	Bait	Hygromycin A	200	0
		Doxycycline	100	0
<i>Peromyscus leucopus</i>	Oral gavage	Hygromycin A	200	0
		Hygromycin A	100	100

A Single Dose of Hygromycin A Eradicates *B. burgdorferi* in mice

Treatment group	Culture Positive
1500 mg/kg	0/12
1200 mg/kg	5/6
800 mg/kg	5/6
400 mg/kg	6/6
0 mg/kg	8/8

Hygromycin A Does not Disrupt the Mouse Gut Microbiome





FP-100 (Hygromycin-A)

Formal IND-enabling Studies

Analytical and Bioanalytical Methods	
Rat and dog bioanalytical method and validation (complete prior to 7-day dog)	In-Process
Human bioanalytical method and validation with short-term stability prior to clinical analysis	Planned (EOY)
Dose formulation method	In-Process
In Vitro Studies	
Plasma stability, CYP inhibition, Protein binding, Caco2 permeability, CYP induction, CYP phenotyping, RBC partitioning, Hepatocyte stability, Hepatocyte MetID, Preliminary solution solubility, Preliminary cell viability, Vesicular transporter inhibition, Uptake transporter inhibition	Complete
Pharmacokinetic Studies	
Rat PK; 2 studies	Complete
Rat excretion	Complete
Dog PK	Complete
Toxicology and Safety Pharmacology Studies	
Range-finding in rat and dog	Complete
Pivotal 7-day rat	Complete
Pivotal 7-day dog	Planned (July)
hERG method development + validation & assay	In-Process
CNS rat safety pharmacology	Complete
Cardiovascular / respiratory dog safety pharmacology	Complete
Gene toxicity: Ames test and rat micronucleus	Complete
Gene toxicity: In vitro micronucleus, comet study	In-Process







**Stephanie Gao
Bryson Hawkins
Nikita Gupta
Oleg Vasilieva
Blake Caravas**

**Monique Theriault
Patil Pankaj**

**Vladimir Tikhonov
Rachel Bargabos
Nadja Leimer
Sangkeun Sun
Tahmina
Kamrunessa
Michael Gates**

**Akira Inishi
Sanika Vaidya
Norman Pitt
Raleb Taher
Kate Piyedra
Sobolevskaya
Mohini Jay**



Collaborators:

Sebastian Hiller, University of Basel

Markus Weingarh, University of Utrecht

Johan Paulsson, HMS

Richard E. Lee, St. Jude Children's Hospital

Linden Hu, Tufts

Chris Dupont, JCVI

Amy Spoering, NovoBiotic

Michael LaFleur, Arietis

Phil Strandwitz, Holobiome



SCHMIDT FUTURES