How microbes are rewriting the rules of immunity

Howard C. Hang, Ph.D., Professor

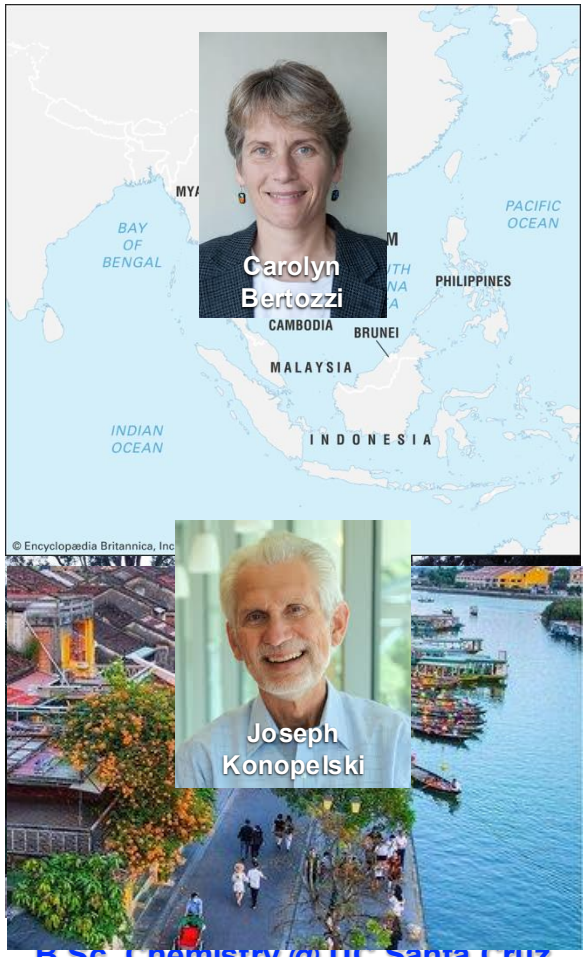
Departments of Immunology & Microbiology and Chemistry

Scripps Research, La Jolla, California

Scripps Research
Science Changing Life

Front Row Lecture 2025

Living the American Dream through Science and Amazing Mentors



B.Sc. Chemistry @ UC Santa Cruz
Hoi An, Vietnam

Professor Immunology and Microbiology, Chemistry @ Scripps Research

Postdoc @ MIT / Whitehead / Harvard

Assistant & Associate Professor
@ Rockefeller University

Fortunate to have great mentors and train / work at excellent institutions……..

Scripps Research – A small institute with a big impact

One of the premier Biomedical Research Institutes at Interface of Chemistry & Biology!

- **~150 Faculty in 6 Departments: Chemistry, Immunology & Microbiology, Structural Biology, Molecular & Cellular Biology, Neuroscience and Translational Medicine**
- **6 Nobel Laureates: 3 x Chemistry, 3 x Physiology or Medicine**
- **Founded in 1924 Scripps Metabolic Clinic**
- **Graduate Program established 1989**
- **~200 Graduate Students, ~500 postdocs**

***Nature* Index among the world's top 5 most innovative institutes, top 10 programs by U.S. News & World Report.**

Scripps Research – Department of Immunology and Microbiology

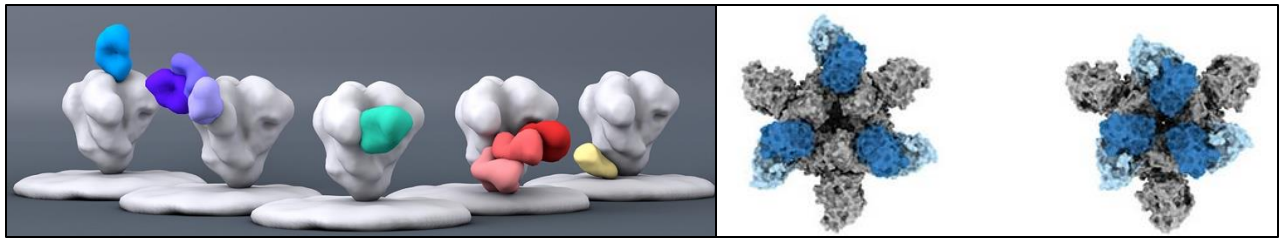
IMM Department Retreat 2025

~20 Faculty and ~10 Institute Investigators

Notable discoveries and therapies

- **1975 Albert Lasker Basic Medical Research Award: Frank J. Dixon (Scripps) with Henry G. Kunkel (Rockefeller) for Immunopathology**
- **2011 Nobel Prize in Physiology or Medicine: Bruce Beutler (Scripps) shared with Jules Hoffman (CNRS) for discovery of Toll-like receptors and Ralph Steinman (Rockefeller) for discovery of dendritic cells.**
- **Humira (anti-TNFα) (Lerner lab and others)**

- **Zeposia (S1PR agonist) (Rosen, Roberts, Oldstone labs)**
- **Viral (SARS, HIV) glycoprotein structures, antibodies & vaccines (Wilson, Ward, Burton, Schief & Irvine labs)**



At the forefront of immunology and microbiology for many decades with major impact on human health.

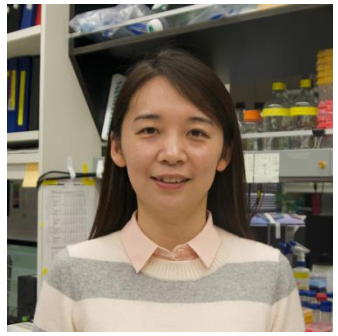
Next-Generation of Immunology and Microbiology Faculty

Bryan Briney
Associate Professor
Computational Immunology

Joseph Jardine
Assistant Professor
Synthetic Immunology

Michael Constantinides
Assistant Professor
Mucosal Immunology

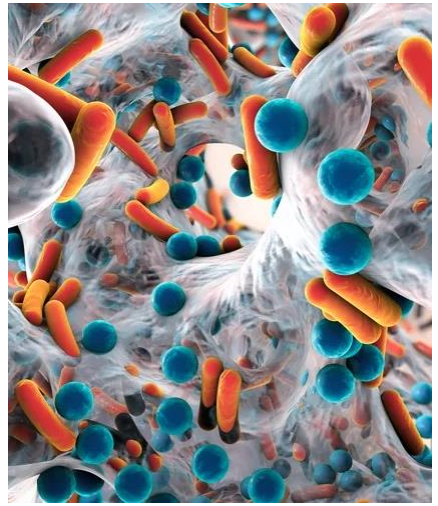
Alejandra Mendoza
Assistant Professor
Neuro-Immunology
November Front Row



Tiantian Liu
Assistant Professor
Myeloid Cell Immunology

Renan V.H. de Carvalho
Assistant Professor
Immune Longevity

- **Artificial intelligence / machine learning approaches to immunity**
- **Synthetic biology / engineering approaches to immunology**
- **Exploration and improvement of mucosal immune responses**
- **Investigation of neuronal – immune interactions**
- **Dissection of adaptive immune responses in cancer and infection**
- **Understanding and enhancement of immune longevity**



Nobel Prize in Physiology or Medicine 2025: Dr. Shimon Sakaguchi was Assistant Professor at Scripps Research.

Microbes are ubiquitous and have beneficial effects

Microbiota: microorganisms (bacteria, archaea, protists, fungi and viruses) of a particular habitat or period.

Microbiome: the combined genetic material of the microorganisms in a particular habitat or period.

Microbiota / microbiome encode unique biological activity to modulate health and disease.

DNA sequencing has been transformative for determining microbiome composition.

Hundreds of different microbiota species and trillions of microbes in each of us!

Microbiota in the News and in Museums

Could a Gut Bacteria Supplement Make Us Run Faster?

Running a marathon ramps up levels of a gut bacteria that made mice run faster, but it's unclear whether it would work in people.

39

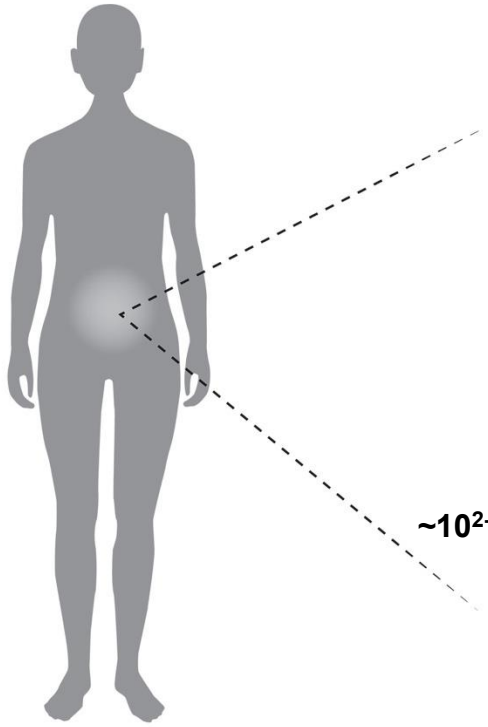
New York Times 2019

Runners at the start of the 2015 Boston Marathon. Greg M. Cooper/USA Today Sports, via Reuters

Microbiome Exhibit @ American Natural History Museum 2018

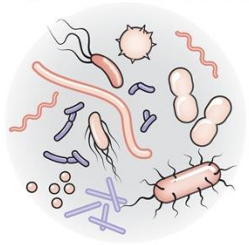
Need to understand the functions of specific microbiota species/strains on human health, disease and therapies.

What microbiota specie(s) and metabolite(s) impact health and disease?



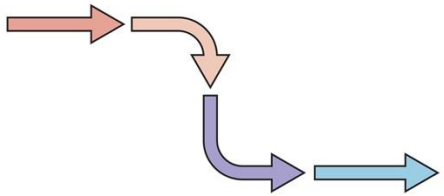
human genome (~20K genes)

Human microbiota



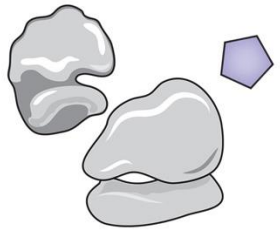
~10^{2-3} species, 10^{13} microbes

Microbial pathways



microbiome (>500K genes)

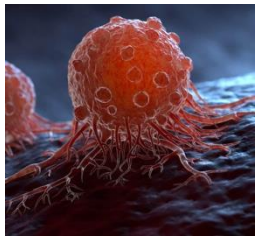
Molecules



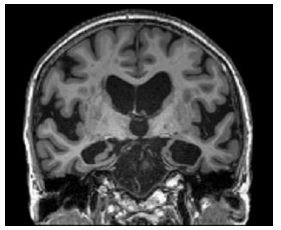
unique proteins and metabolites

Infection

Cancer



Neurodegeneration

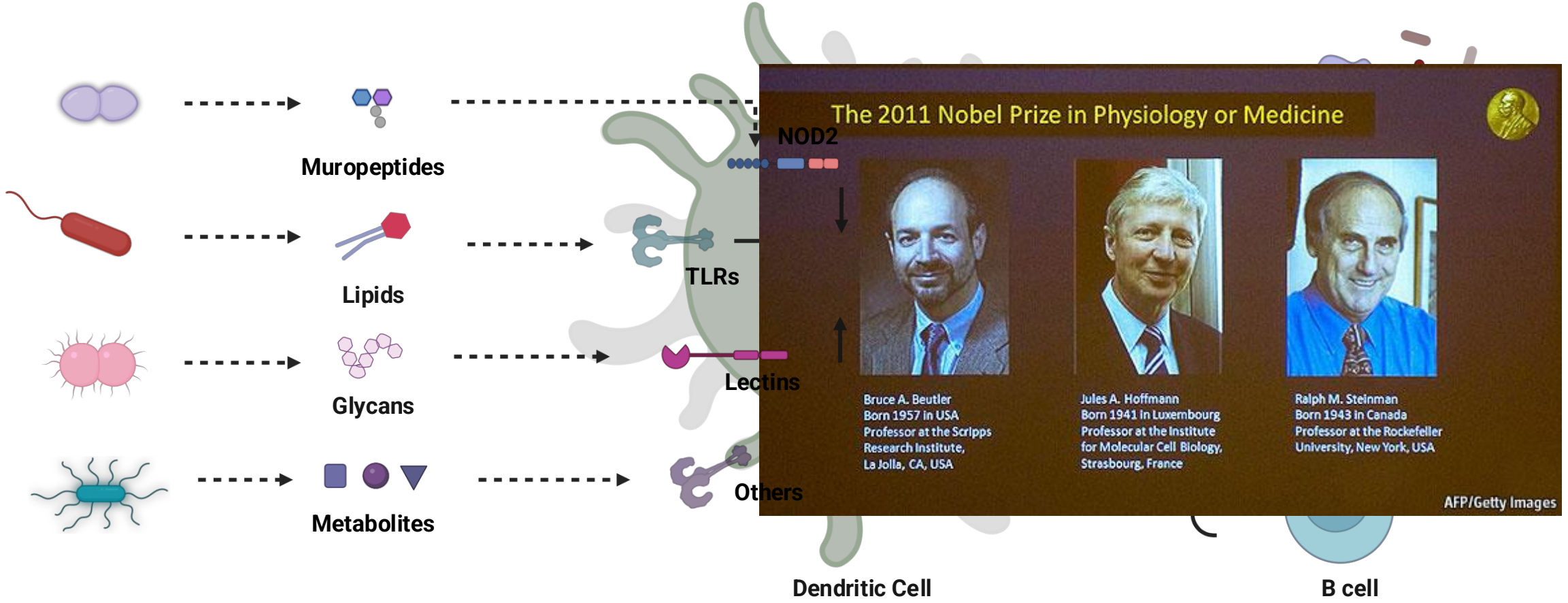


microbiota are complexity and highly variable

- location (gut, skin, oral, nasal…)
- individuals
- diet, exercise, age
- therapeutics (antibiotic and non-antibiotics)

How do different microbiota species and metabolites impact health, disease progression and therapy response?

Humans and animals have specific immune receptors for microbial metabolites

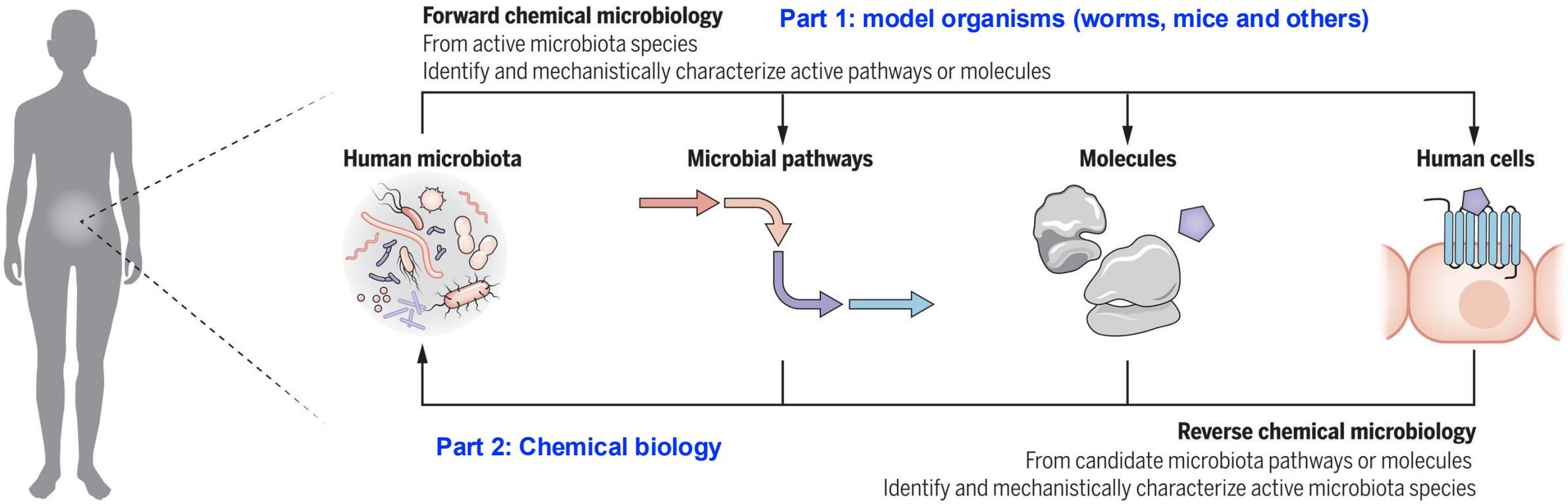


Microbiota species generate different amounts and types of metabolites that are sensed by specific receptors.

Which microbiota species and metabolites are responsible for promoting immunity in health, disease and therapy?

NLRs: Chou WC et al Nat Rev Immunol. 2023
Lectins: Reis e Sousa C, Yamasaki S, Brown GD. Immunity. 2024

Chemical genetic approaches to dissect microbiota mechanisms



Forward and reverse chemical microbiology approaches have revealed specific microbiota mechanisms of action.

Reviewed in: Yang X, Hang HC Science 2024

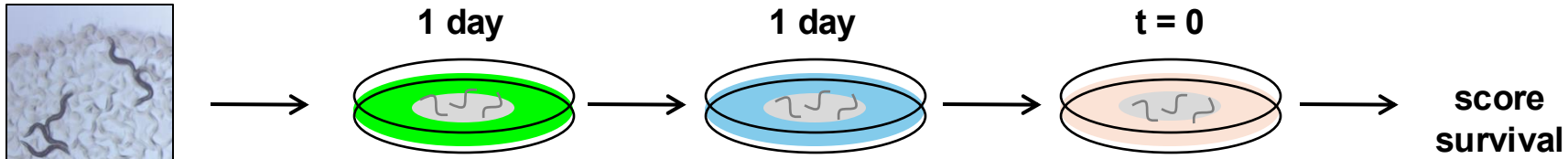
Worms can be used to explore the activity of specific microbiota species

Kavita Rangan

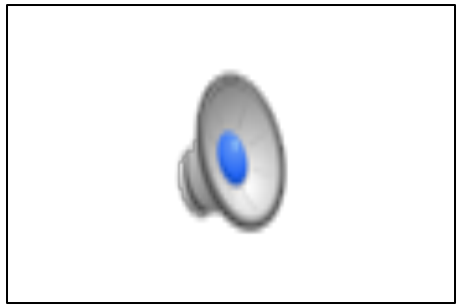
Former Graduate Student

Assistant Professor
Johns Hopkins University

Pulsed infection assay:

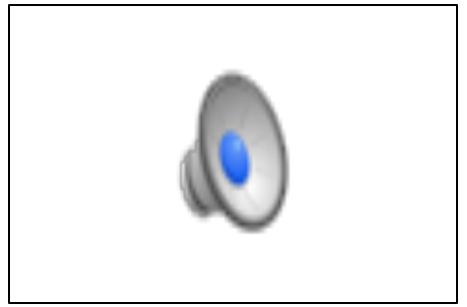


E. coli OP50; day 3



E. coli OP50 – Stm; day 3

E. faecium – Stm; day 3

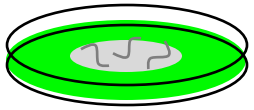


Roundworms (*C. elegans*) provides an inexpensive animal model for exploring complex biology – microbiota mechanisms.

High-throughput analysis of beneficial microbiota species (infection)

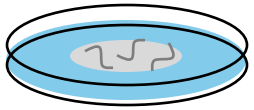
n = 30 worms
/ 96-well plate

1 day



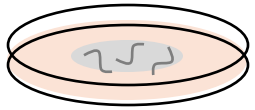
specific
microbiota

1 day

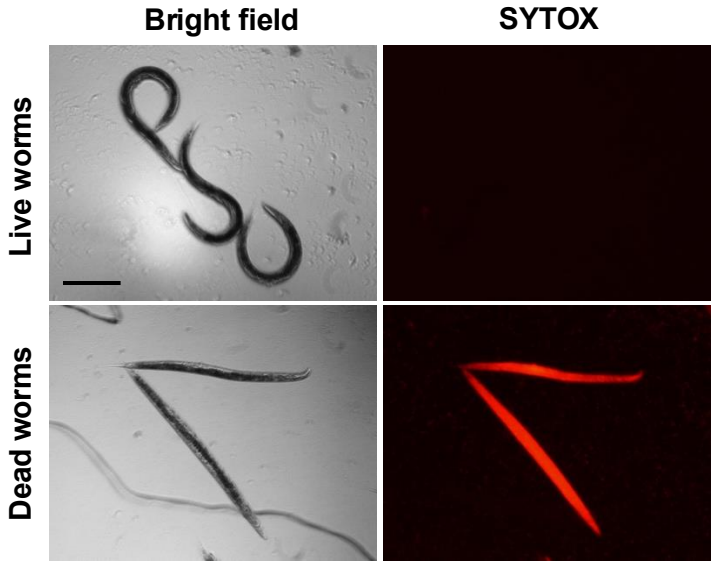


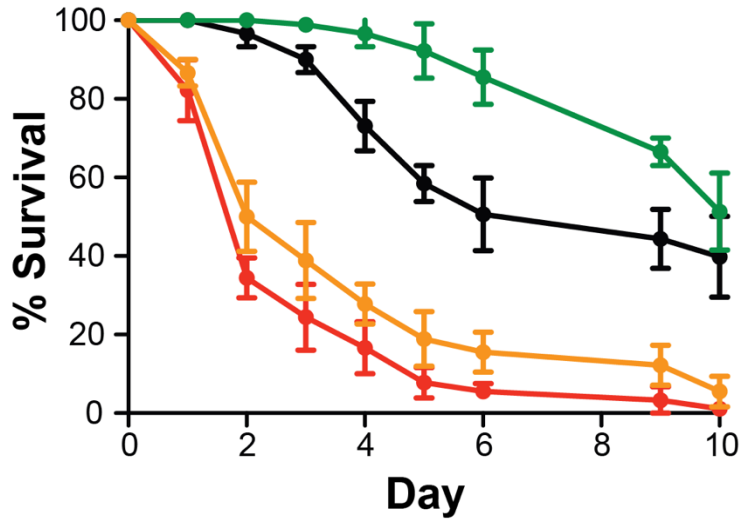
Control bacteria vs.
S. Typhimurium (Stm)

t = 0



Score survival





E. faecium-Stm

E. coli OP50

B. subtilis-Stm

S. Typhimurium

Rangan K et al Science 2016

The Yin and Yang of *Enterococcus*

***Enterococcus* species**

Gram-positive bacteria (Firmicutes), discovered in early 1900s

***"entero" and "cocci"* – intestinal origin and morphology**

Identified in many animals and environment

>70 species, *E. faecalis and E. faecium* - most prominent

~1% of the adult human microbiota (16S rRNA sequencing)

Tolerant to broad pH range, temperature and osmotic conditions

Antibiotic-resistant and pathogenic *Enterococcus* are major cause of healthcare associated infections.

However, non-pathogenic *Enterococcus* can be beneficial and associated with cancer immunotherapy efficacy.

Mechanism of *Enterococcus* modulation of host immunity was unknown.

Worms provided an excellent model system to discover microbiota factors

C. elegans (roundworm)
"model of mammalian gut"

E. faecium (protective)

versus

E. faecalis (not active)

Activity → Assays

Genomics → Proteomics

Biochemistry → Structure

E. faecium

Secreted antigen A (SagA)

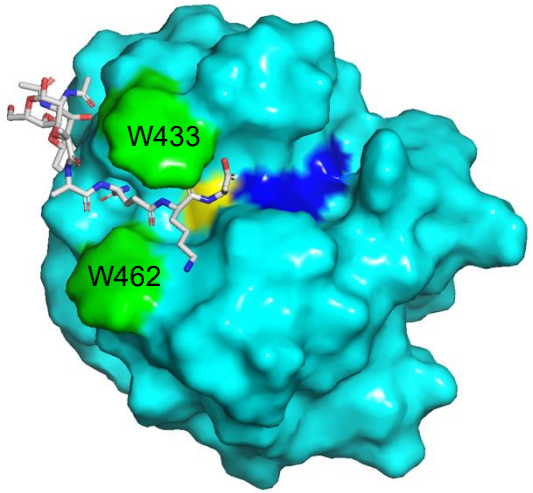
Immuno-reactive metabolites

Comparative analysis of "good" vs "bad" microbiota species in worms revealed protective activity of *E. faecium* SagA.

Rangan K et al Science 2016

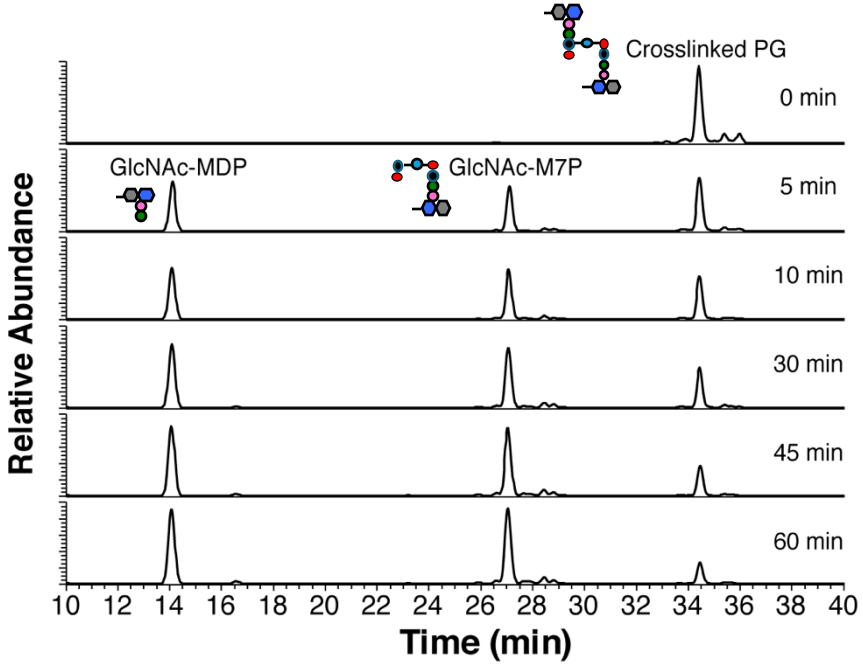
Structure and biochemical activity of SagA-NlpC/p60 peptidase domain

X-ray structure of SagA-NlpC/p60 @ 2.4 Å

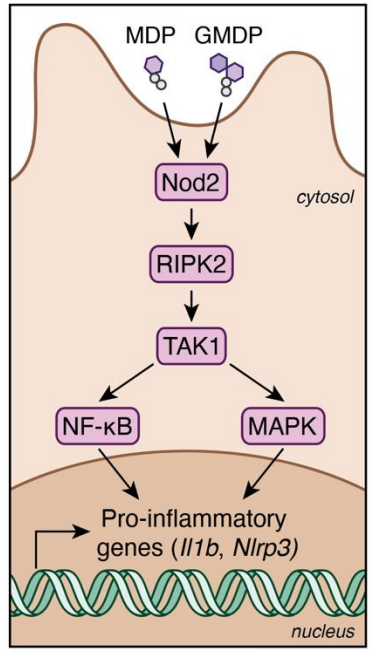


Cys443, H494, W433 and W462 mutants are inactive.

Peptidoglycan cleavage activity of SagA-NlpC/p60



Muropeptides promotes immunity



SagA-NlpC/p60 encodes Cys-endopeptidase activity and selective for cross-linked Lys-type peptidoglycan substrates.

SagA generates immuno-reactive (muropeptides) metabolites that activate and enhance host immunity via NOD2.

SagA x-ray structure and biochemical activity: Kim B et al eLife 2019
Additional mutagenesis and imaging of SagA: Espinosa J et al Biochemistry 2020

E. faecium SagA also prevents *C. difficile* pathogenesis in mice

Antibiotic-resistant Gram-positive bacterium that damages intestinal barrier.

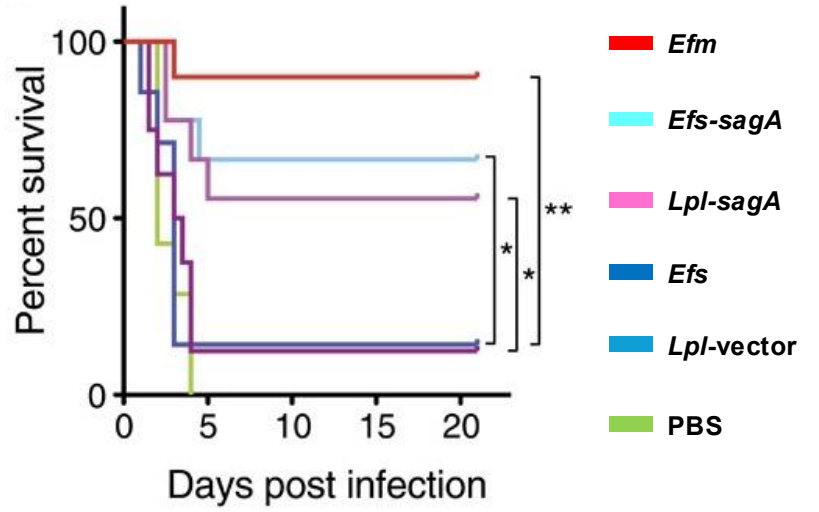
Major cause of antibiotic-induced infection and inflammation.

***C. difficile* ~20% relapse/recurrence, ~80% of mortality in elderly patients.**

Fecal microbiota transplantation is effective, but problematic.

Abt MC, McKenney PT, Pamer EG Nat Rev Microbiol 2016
Kociolek LK, Gerding DN. Nat Rev Gastroenterol Hepatol 2016

Protection against *C. difficile* pathogenesis in mice



SagA-bacteria pre-colonization prevents *S.* Typhimurium and *C. difficile* pathogenesis *in vivo*

Pedicord V et al Science Immunology 2016

SagA activity (Cys443A) was required for protective activity.

Kim B et al eLife 2019

Enterococcus peptidoglycan remodeling promote host immunity and therapy

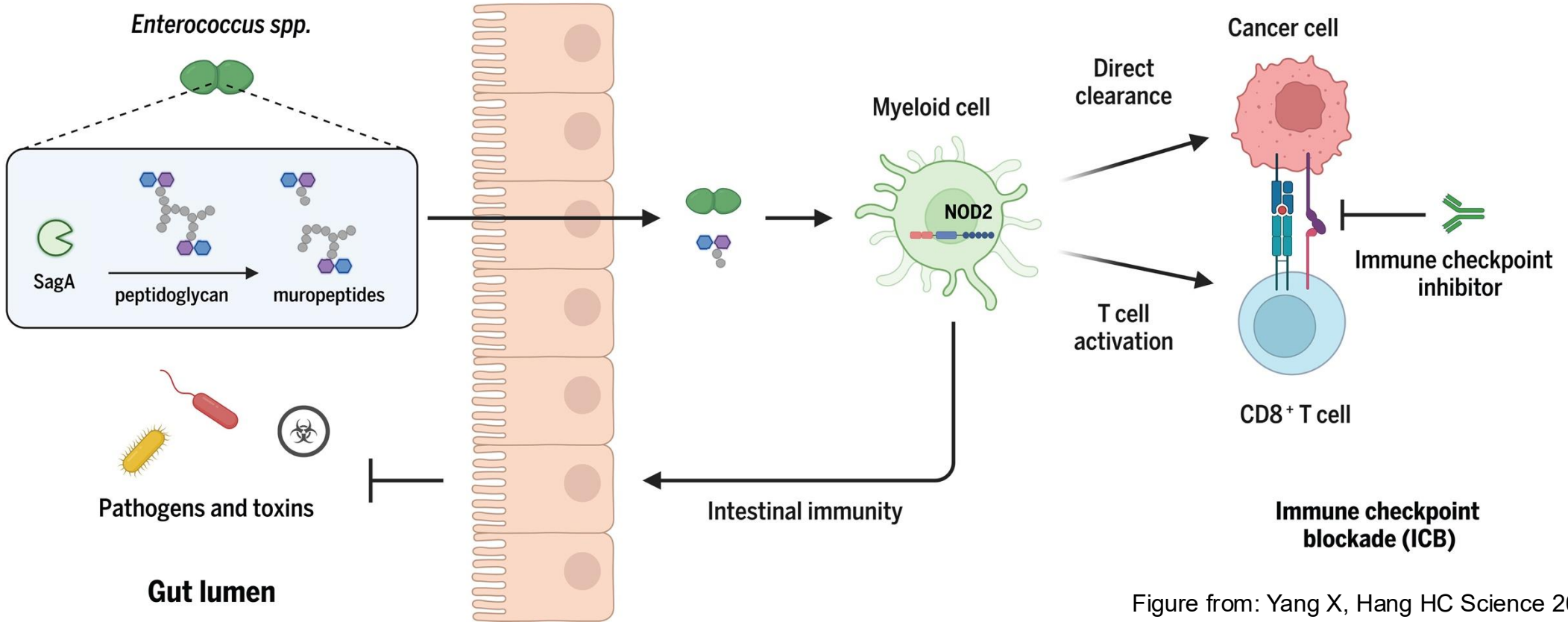


Figure from: Yang X, Hang HC Science 2024

Enterococcus SagA is _sufficient_ and _required_ for promoting host immunity via NOD2 _ex vivo_ and _in vivo_.

Discovery of *E. faecium* SagA activity: Rangan K et al Science 2016
Protection against infection: Pedicord V et al Science Immunology 2016
Protection against colitis: Jang KK et al Cell Host Microbe 2023

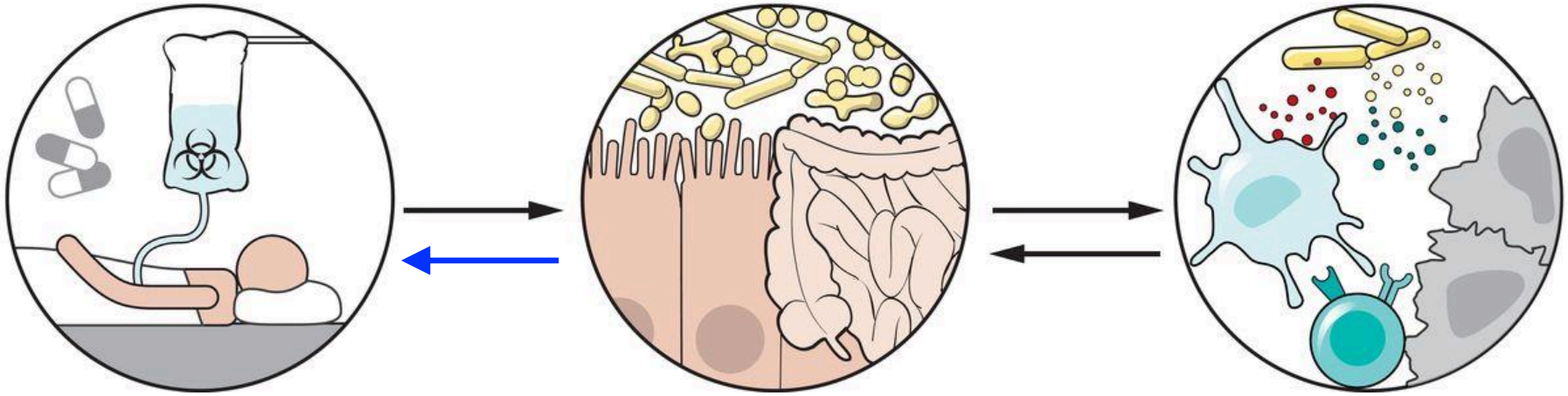
SagA x-ray structure and biochemical mechanism: Kim B et al eLife 2019
Impact on cancer immunotherapy: Griffin M et al Science 2021
Required for cell separation and immune priming: Klupt S et al eLife 2024

Microbiota affects intestinal barrier, immune responses and therapy

Cancer therapies
Anticancer treatment modalities and co-medications (such as antibiotics) affect the integrity of the epithelial barrier.

Microbiome
Gut-resident commensals interacting with epithelial, stromal, endocrine, neural, immune intestinal cells to regulate barrier functions and whole-body metabolism.

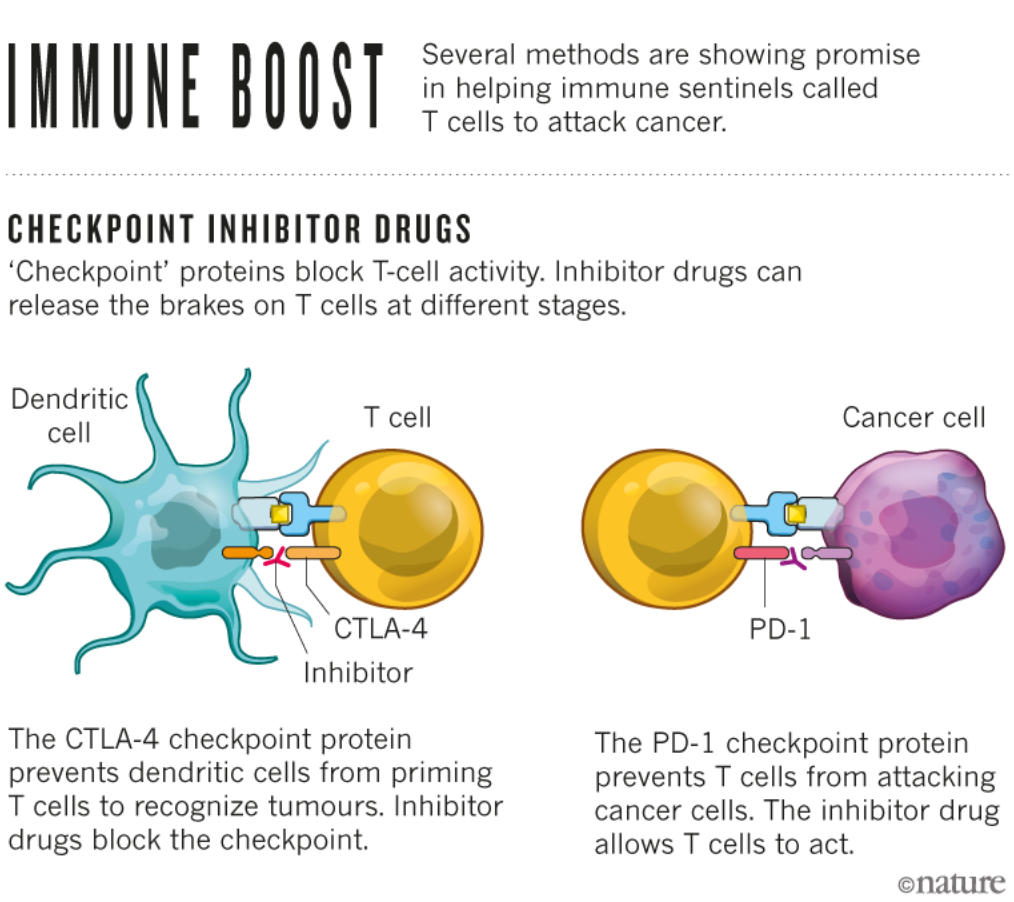
Immune responses
The gut microbiota has systemic effects throughout the meta-organism via secretion of anti-inflammatory cytokine/chemokines, metabolites, antimicrobial and neuropeptides.



Microbiota modulation of metabolism and immunity may also modulate efficacy of chemo- and immuno-therapies.

Reviewed in: Zitvogel L, Ma Y, Raoult D, Kroemer G, Gajewski TF Science 2018

Cancer immunotherapy is transformative, but not effective in all patients



2018 Nobel Prize in Medicine for James Allison and Tasuku Honjo

Phase III melanoma trial: Wolchok JD et al N Engl J Med. 2017

Efficacy of cancer immunotherapy is correlated with microbiota composition

CANCER IMMUNOTHERAPY

Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients

V. Gopalakrishnan,[1,2*] C. N. Spencer,[2,3*] L. Nezi,[3*] A. Reuben,[1] M. C. Andrews,[1] T. V. Karpinets,[3] P. A. Prieto,[1†] D. Vicente,[1] K. Hoffman,[4] S. C. Wei,[5] A. P. Cogdill,[1,5] L. Zhao,[3] C. W. Hudgens,[6] D. S. Hutchinson,[7] T. Manzo,[3] M. Petaccia de Macedo,[6‡] T. Cotechini,[8] T. Kumar,[3] W. S. Chen,[9] S. M. Reddy,[10] R. Szczepaniak Sloane,[1] J. Galloway-Pena,[11] H. Jiang,[1] P. L. Chen,[9§] E. J. Shpall,[12] K. Rezvani,[12] A. M. Alousi,[12] R. F. Chemaly,[11] S. Shelburne,[3,11] L. M. Vence,[5] P. C. Okhuysen,[11] V. B. Jensen,[13] A. G. Swennes,[7] F. McAllister,[14] E. Marcelo Riquelme Sanchez,[14] Y. Zhang,[14] E. Le Chatelier,[15] L. Zitvogel,[16] N. Pons,[15] J. L. Austin-Breneman,[1‖] L. E. Haydu,[1] E. M. Burton,[1] J. M. Gardner,[1] E. Sirmans,[17] J. Hu,[18] A. J. Lazar,[6,9] T. Tsujikawa,[8] A. Diab,[17] H. Tawbi,[17] I. C. Glitza,[17] W. J. Hwu,[17] S. P. Patel,[17] S. E. Woodman,[17] R. N. Amaria,[17] M. A. Davies,[17] J. E. Gershenwald,[1] P. Hwu,[17] J. E. Lee,[1] J. Zhang,[3] L. M. Coussens,[8] Z. A. Cooper,[1,3¶] P. A. Futreal,[3] C. R. Daniel,[4,2] N. J. Ajami,[7] J. F. Petrosino,[7] M. T. Tetzlaff,[6,9] P. Sharma,[5,19] J. P. Allison,[5] R. R. Jenq,[3#] J. A. Wargo[1,3#**]

Gopalakrishnan V et al (Jenq & Wargo labs) Science 2018

CANCER IMMUNOTHERAPY

Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors

Bertrand Routy,[1,2,3] Emmanuelle Le Chatelier,[4] Lisa Derosa,[1,2,3] Connie P. M. Duong,[1,2,5] Maryam Tidjani Alou,[1,2,3] Romain Daillère,[1,2,3] Aurélie Fluckiger,[1,2,5] Meriem Messaoudene,[1,2] Conrad Rauber,[1,2,3] Maria P. Roberti,[1,2,5] Marine Fidelle,[1,3,5] Caroline Flament,[1,2,5] Vichnou Poirier-Colame,[1,2,5] Paule Opolon,[6] Christophe Klein,[7] Kristina Iribarren,[8,9,10,11,12] Laura Mondragón,[8,9,10,11,12] Nicolas Jacquelot,[1,2,3] Bo Qu,[1,2,3] Gladys Ferrere,[1,2,3] Céline Clémenson,[1,13] Laura Mezquita,[1,14] Jordi Remon Masip,[1,14] Charles Naltet,[15] Solenn Brosseau,[15] Coureche Kaderbhai,[16] Corentin Richard,[16] Hira Rizvi,[17] Florence Levenez,[4] Nathalie Galleron,[4] Benoit Quinquis,[4] Nicolas Pons,[4] Bernhard Ryffel,[18] Véronique Minard-Colin,[1,19] Patrick Gonin,[1,20] Jean-Charles Soria,[1,14] Eric Deutsch,[1,13] Yohann Loriot,[1,3,14] François Ghiringhelli,[16] Gérard Zalcman,[15] François Goldwasser,[9,21,22] Bernard Escudier,[1,14,23] Matthew D. Hellmann,[24,25] Alexander Eggermont,[1,2,14] Didier Raoult,[26] Laurence Albiges,[1,3,14] Guido Kroemer,[8,9,10,11,12,27,28*] Laurence Zitvogel[1,2,3,5*]

Routy B et al (Zitvogel lab) Science 2018

CANCER IMMUNOTHERAPY

The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients

Vyara Matson,[1*] Jessica Fessler,[1*] Riyue Bao,[2,3*] Tara Chongsuwat,[4] Yuanyuan Zha,[4] Maria-Luisa Alegre,[4] Jason J. Luke,[4] Thomas F. Gajewski[1,4†]

Anti–PD-1–based immunotherapy has had a major impact on cancer treatment but has only benefited a subset of patients. Among the variables that could contribute to interpatient heterogeneity is differential composition of the patients' microbiome, which has been shown to affect antitumor immunity and immunotherapy efficacy in preclinical mouse models. We analyzed baseline stool samples from metastatic melanoma patients before immunotherapy treatment, through an integration of 16S ribosomal RNA gene sequencing, metagenomic shotgun sequencing, and quantitative polymerase chain reaction for selected bacteria. A significant association was observed between commensal microbial composition and clinical response. Bacterial species more abundant in responders included *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*. Reconstitution of germ-free mice with fecal material from responding patients could lead to improved tumor control, augmented T cell responses, and greater efficacy of anti–PD-L1 therapy. Our results suggest that the commensal microbiome may have a mechanistic impact on antitumor immunity in human cancer patients.

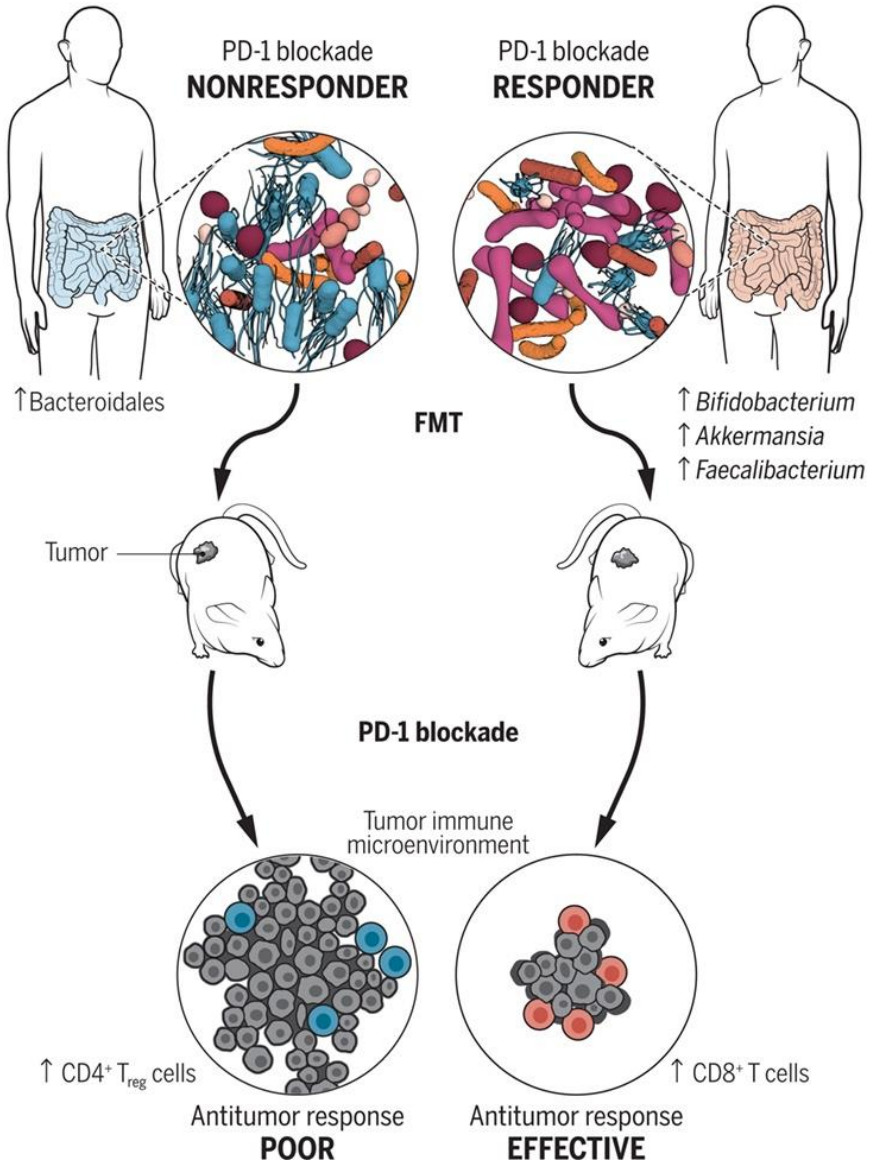
Matson V et al (Gajewski lab) Science 2018

Immunotherapy-responsive cancer patients have unique microbiota.

Antibiotics impair immunotherapy efficacy in animal models.

Specific microbiota species may be important determinant(s) of immunotherapy efficacy in cancer patients.

Fecal microbiota transplantation correlates with immunotherapy response



Fecal microbiota transplantation (FMT) has been FDA approved for preventing recurrence *C. difficile* infections.

FMT of cancer patient microbiota into germ-free mice correlated with immunotherapy response in cancer patients.

Routy B et al (Zitvogel lab) Science 2018
Matson V et al (Gajewski lab) Science 2018
Gopalakrishnan V et al (Jenq & Wargo labs) Science 2018

Microbiota transplantation has unpredictable effects on cancer immunotherapy

CLINICAL TRIALS

Fecal microbiota transplant overcomes resistance to anti–PD-1 therapy in melanoma patients

Diwakar Davar[1]*, Amiran K. Dzutsev[2]*, John A. McCulloch[2], Richard R. Rodrigues[2,3], Joe-Marc Chauvin[1], Robert M. Morrison[1], Richelle N. Deblasio[1], Carmine Menna[1], Quanquan Ding[1], Ornella Pagliano[1], Bochra Zidi[1], Shuowen Zhang[1]†, Jonathan H. Badger[2], Marie Vetizou[2], Alicia M. Cole[2], Miriam R. Fernandes[2], Stephanie Prescott[2], Raquel G. F. Costa[2], Ascharya K. Balaji[2], Andrey Morgun[4], Ivan Vujkovic-Cvijin[5], Hong Wang[6], Amir A. Borhani[7], Marc B. Schwartz[8], Howard M. Dubner[8], Scarlett J. Ernst[1], Amy Rose[1], Yana G. Najjar[1], Yasmine Belkaid[5], John M. Kirkwood[1], Giorgio Trinchieri[2]‡§, Hassane M. Zarour[1,9]‡§

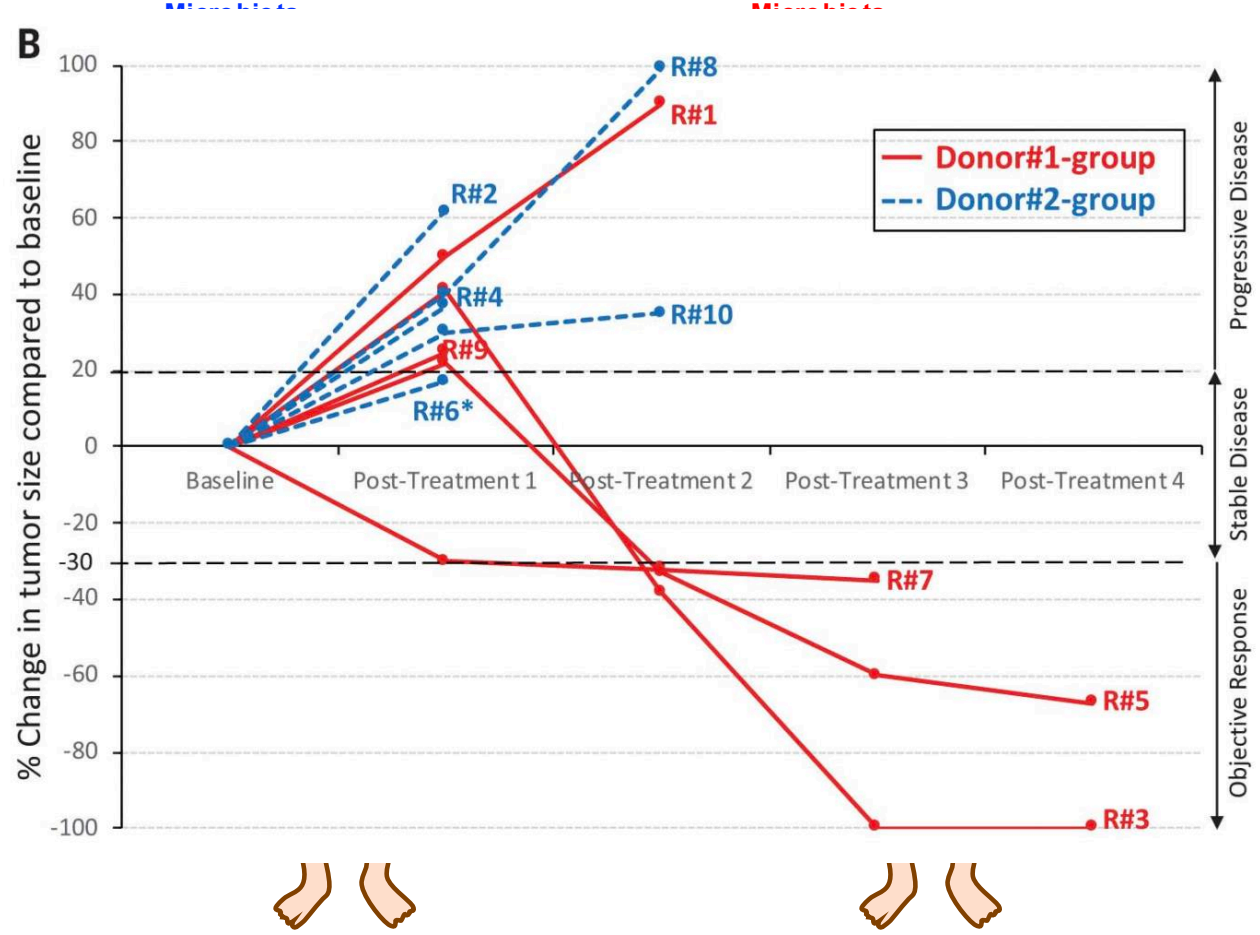
U Pittsburgh Med Center + NIH: Davar D et al (Zarour lab) Science 2021

CLINICAL TRIALS

Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients

Erez N. Baruch[1,2]*†, Ilan Youngster[3,4], Guy Ben-Betzalel[1], Rona Ortenberg[1], Adi Lahat[5], Lior Katz[6], Katerina Adler[7], Daniela Dick-Necula[8], Stephen Raskin[4,9], Naamah Bloch[10], Daniil Rotin[8], Liat Anafi[8], Camila Avivi[8], Jenny Melnichenko[1], Yael Steinberg-Silman[1], Ronac Mamtani[11], Hagit Harati[1], Nethanel Asher[1], Ronnie Shapira-Frommer[1], Tal Brosh-Nissimov[12], Yael Eshet[4,8,13], Shira Ben-Simon[10], Oren Ziv[10], Md Abdul Wadud Khan[14], Moran Amit[15], Nadim J. Ajami[14], Iris Barshack[4,8], Jacob Schachter[1,4], Jennifer A. Wargo[14,16], Omry Koren[10], Gal Markel[1,2,17]*‡, Ben Boursi[4,18,19]‡

Sheba Med Center (Israel): Baruch EN et al (Boursi lab) Science 2021



FMT can be problematic due to microbiota variability between donors and potential pathogens.

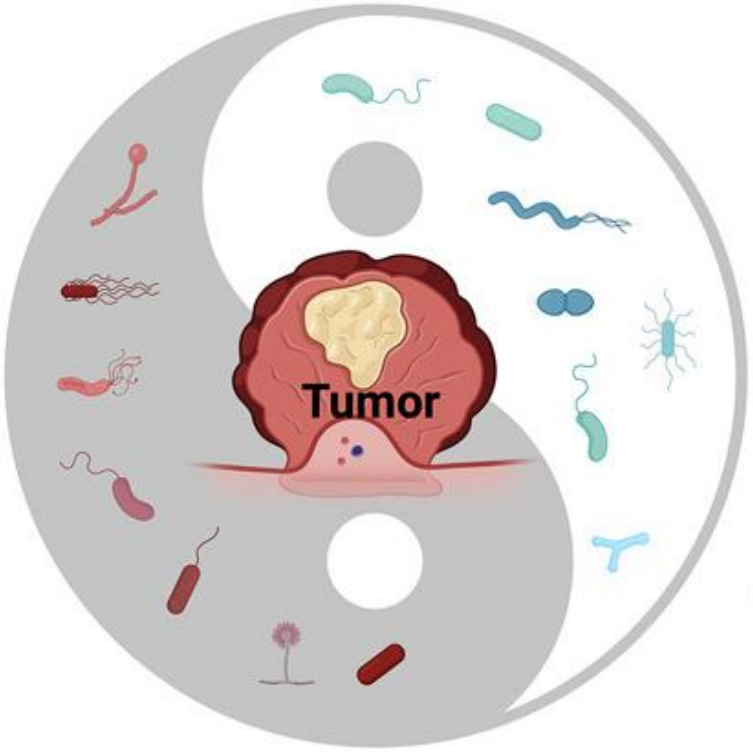
Which microbiota species and mechanisms are important for immunotherapy efficacy in cancer patients?

Jekyll and Hyde of microbiota (bacteria) with cancer and immunotherapy

Cancer promoting bacterial species

Helicobacter pylori - stomach cancer
Nobel Prize 2005 – Marshall & Warren

Fusobacterium nucleatum
colon cancer



Cancer inhibiting bacterial species

Coley's toxins: heat-killed
S. pyogenes and S. marcescens

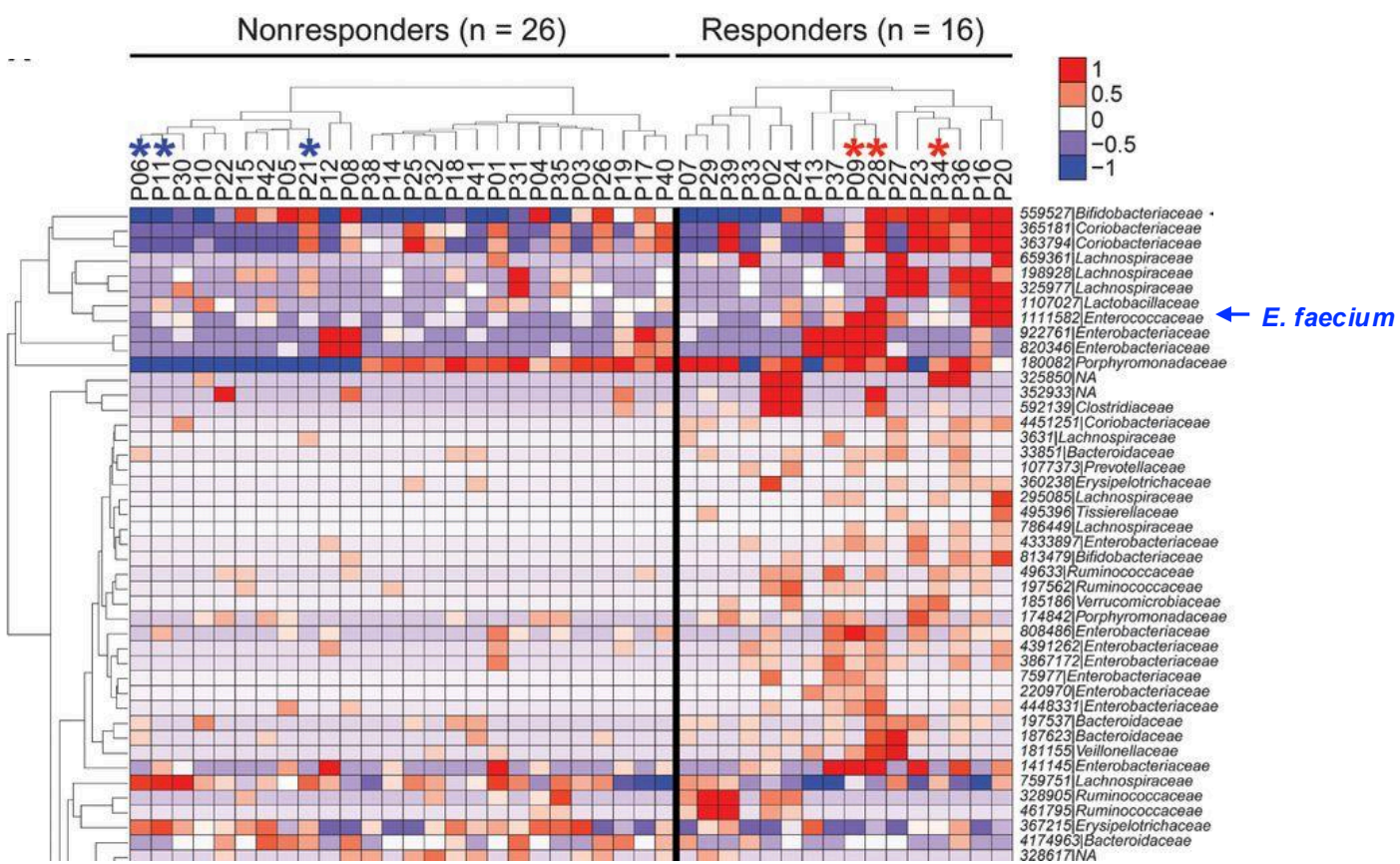
Bacille Calmette Guérin (BCG)
bladder cancer treatment

Different microbiota species have distinct effects on host immunity and cancer progression and therapy.

Reviewed in: Zhang X, Fam KT, Dai T, Hang HC. Cell Chem Biol. 2025

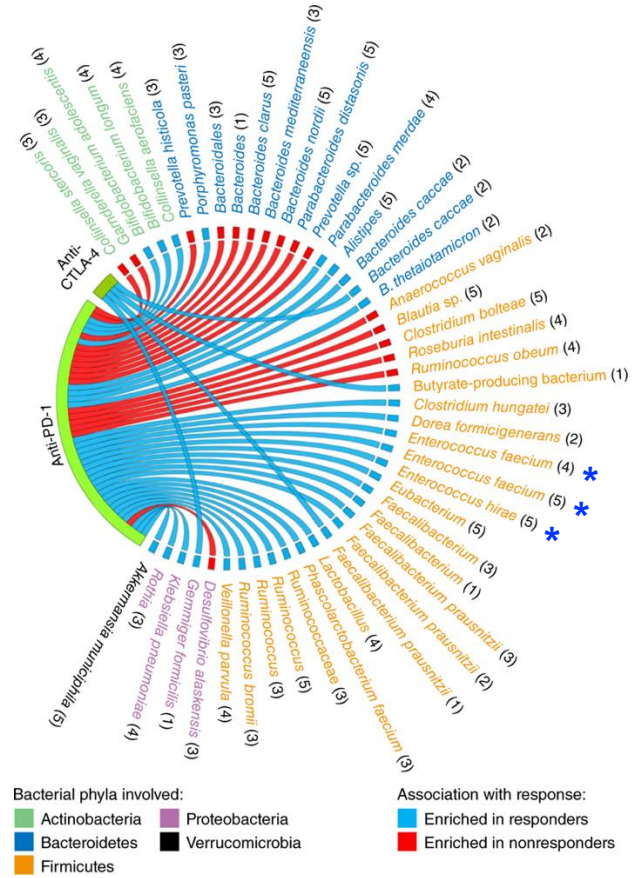
E. faecium was enriched in immunotherapy-responsive cancer patients

Different microbiota species <---> cancer immunotherapy efficacy



Matson V et al (Gajewski lab) Science 2018

Microbiota - immunotherapy correlation



Helmink BA et al Nat Med. 2019

Are *E. faecium* and SagA also sufficient to enhance immunotherapy against cancer?

E. faecium and SagA are sufficient to improve cancer immunotherapy

n = 8/group

-14 days

Abx cocktail in water
(ampicillin, colistin, streptomycin)

Enterococcus strain (3 days)

10^8 CFU/mL commensal
in drinking water

0

10^5 tumor cell line
subQ inj.

20 ug (αPD-L1 or αCTLA4) i.p. injection
9, 11, 13, 15

7 to 17

Immunotherapy treatments

- **Tumor growth**
- **Immune profile**
- **Microbiota analysis**

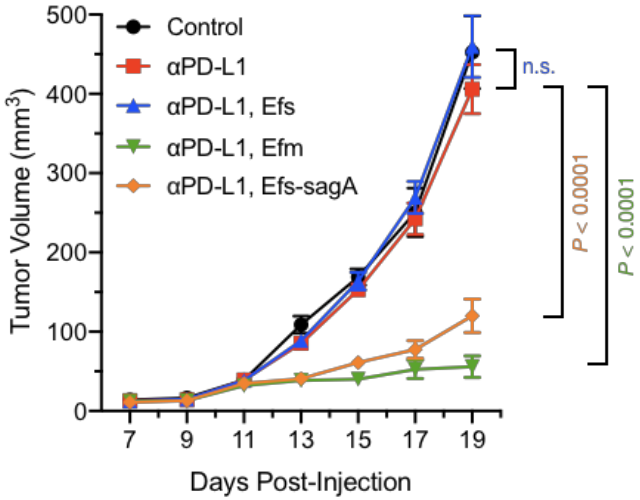
Additional Studies

Matthew Griffin

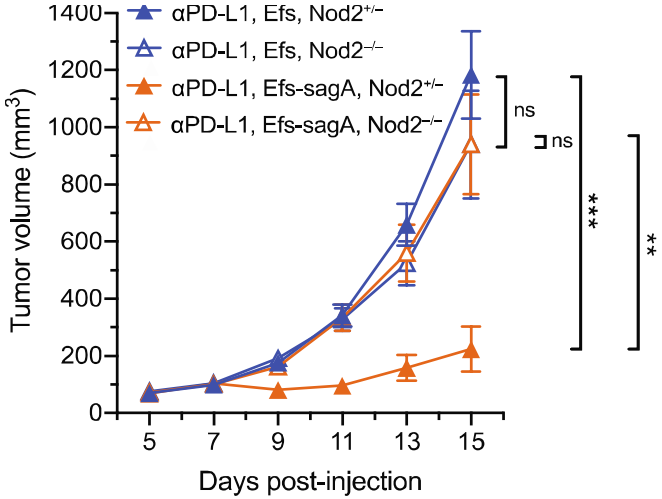
- **Activates myeloid cells**
- **Incre D8 T cells**
- **Activ notherapies and c**
- ***SagA-Enterococcus* species**
  E. faecium, E. mundti, E. hirae, E. durans.

Former Postdoc
Assistant Professor
UC Irvine

Immunotherapy activity on melanoma

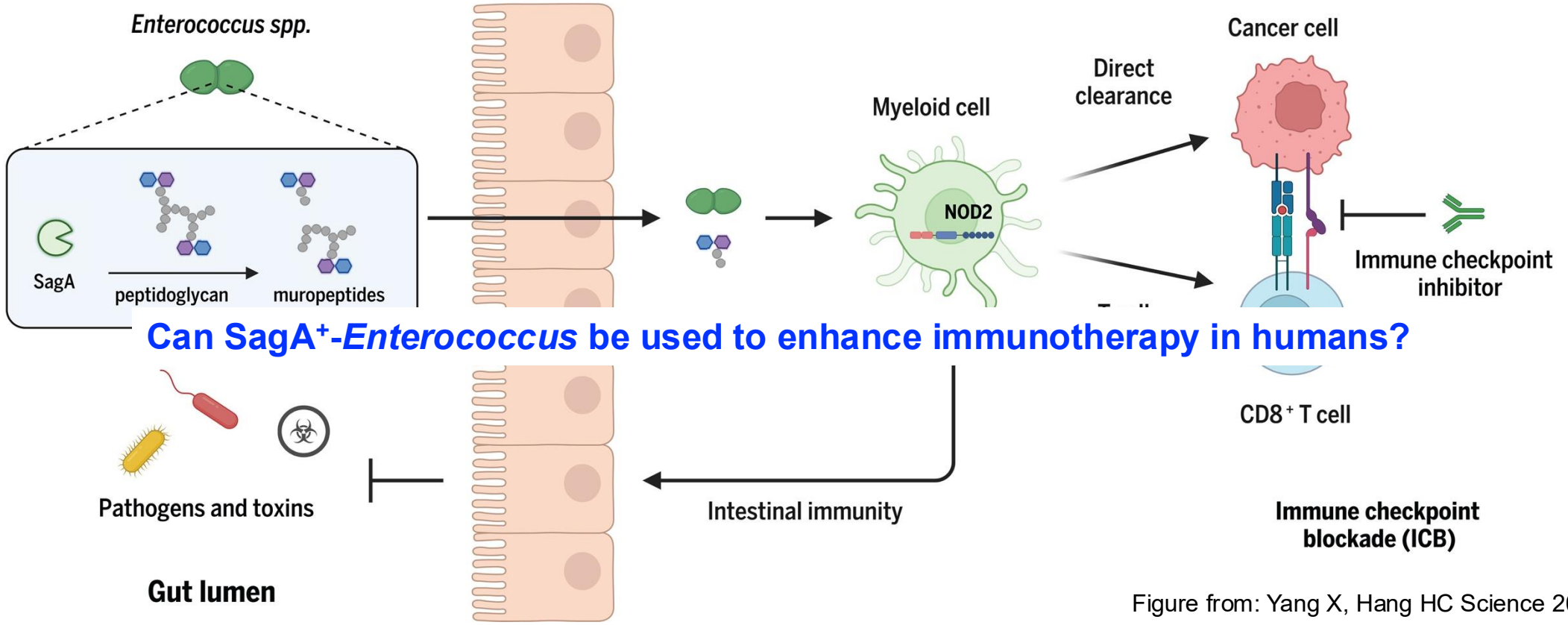


Immune receptor NOD2 is required



Griffin M et al Science 2021

Enterococcus peptidoglycan remodeling promote host immunity and therapy



Can SagA⁺-*Enterococcus* be used to enhance immunotherapy in humans?

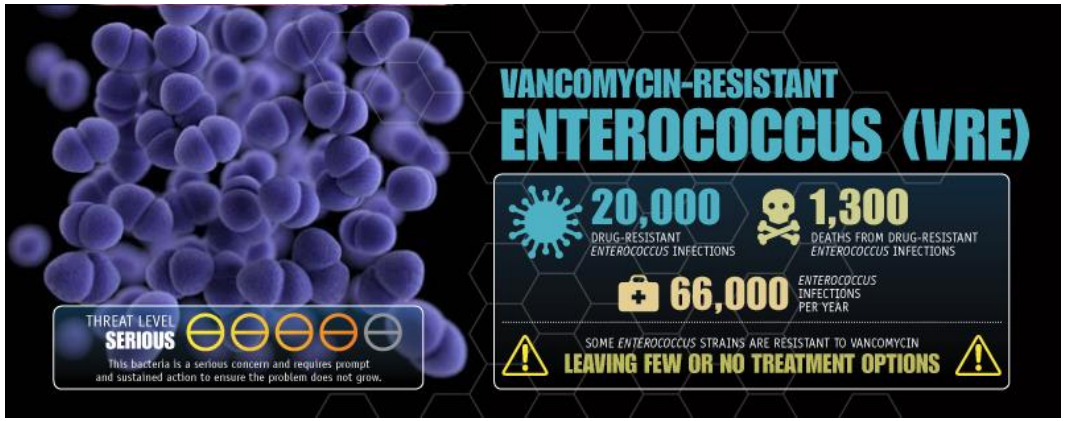
Figure from: Yang X, Hang HC Science 2024

***Enterococcus* SagA is *sufficient* and *required* for promoting host immunity via NOD2 *ex vivo* and *in vivo*.**

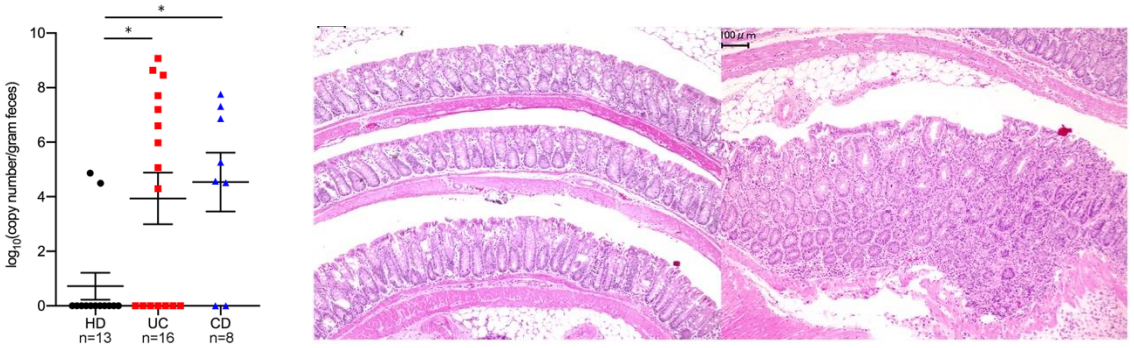
Discovery of *E. faecium* SagA activity: Rangan K et al Science 2016
Protection against infection: Pedicord V et al Science Immunology 2016
Protection against colitis: Jang KK et al Cell Host Microbe 2023

SagA x-ray structure and biochemical mechanism: Kim B et al eLife 2019
Impact on cancer immunotherapy: Griffin M et al Science 2021
Required for cell separation and immune priming: Klupt S et al eLife 2024

Antibiotic-resistant *Enterococcus* are problematic and restricted in humans



E. faecium promotes inflammatory bowel diseases (IBD)

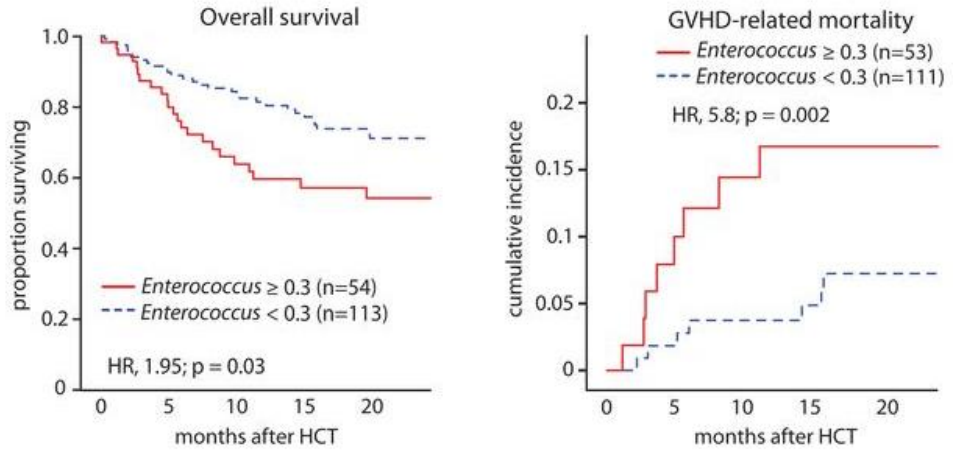


E. faecium from ulcerative colitis patients promotes colitis in IL10-/- mice:
Seishima J et al Genome Biol. 2019, Barnett M et al BMC Immunol. 2019

MICROBIOME

Lactose drives *Enterococcus* expansion to promote graft-versus-host disease

C. K. Stein-Thoeringer[1,2,3], K. B. Nichols[1,2], A. Lazrak[1,2], M. D. Docampo[1,2], A. E. Slingerland[1,2], J. B. Slingerland[1,2], A. G. Clurman[4], G. Armijo[1,2], A. L. C. Gomes[1,2], Y. Shono[1,2], A. Staffas[1,2], M. Burgos da Silva[1,2], S. M. Devlin[5], K. A. Markey[1,2,4], D. Bajic[6], R. Pinedo[7], A. Tsakmaklis[8,9], E. R. Littmann[1,10], A. Pastore[1], Y. Taur[11], S. Monette[12], M. E. Arcila[13], A. J. Pickard[14], M. Maloy[4], R. J. Wright[1], L. A. Amoretti[1], E. Fontana[1], D. Pham[15], M. A. Jamal[15], D. Weber[16], A. D. Sung[17], D. Hashimoto[18], C. Scheid[8], J. B. Xavier[19], J. A. Messina[20], K. Romero[21], M. Lew[17], A. Bush[17], L. Bohannon[17], K. Hayasaka[22], Y. Hasegawa[18], M. J. G. T. Vehreschild[8,9,23], J. R. Cross[14], D. M. Ponce[2,4], M. A. Perales[2,4], S. A. Giralt[2,4], R. R. Jenq[25], T. Teshima[18,22], E. Holler[26], N. J. Chao[27], E. G. Pamer[1,2,10], J. U. Peled[1,2,4,*†], M. R. M. van den Brink[1,2,4,*†]

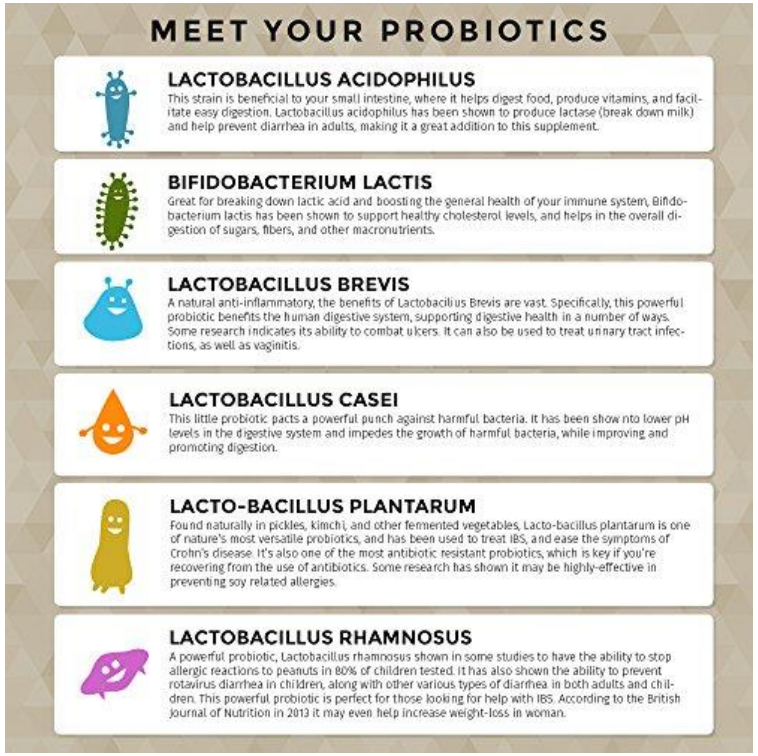


Stein-Thoeringer CK et al (Pamer & van den Brink labs) Science 2019

Vancomycin-resistant _E. faecium_ is a major cause of healthcare-associated infections and very difficult to treat.

Probiotics can be genetically engineered for potential therapeutics

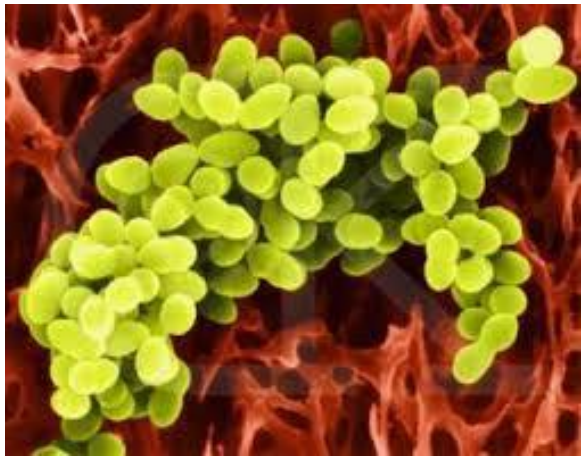
Many probiotics have been explored for human health



Efficacy and actual health benefits are unclear.

Genetic engineering

Lactococcus lactis* + *IL-10



IL-10 (anti-inflammatory cytokine)

Developed for inflammatory bowel diseases

Steidler L et al Nat Biotechnol. 2003
Steidler L et al Science. 2000

Rise Therapeutics: SagA-probiotics?

Probiotics can be engineered to confer novel activity.

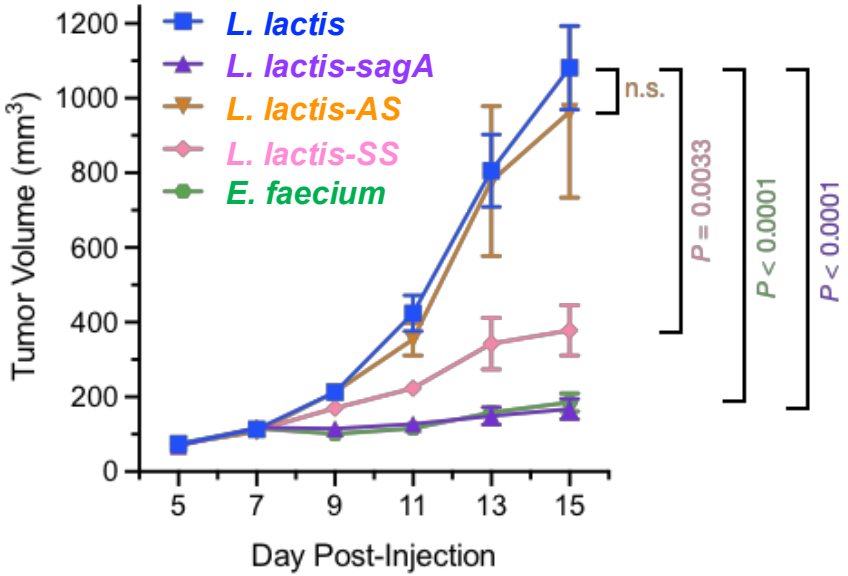
SagA-probiotics can also enhance immune checkpoint inhibitor efficacy

Engineering SagA expression into *L. lactis*

Google Search for "Probiotic Beer"

SagA-Hazy IPA?

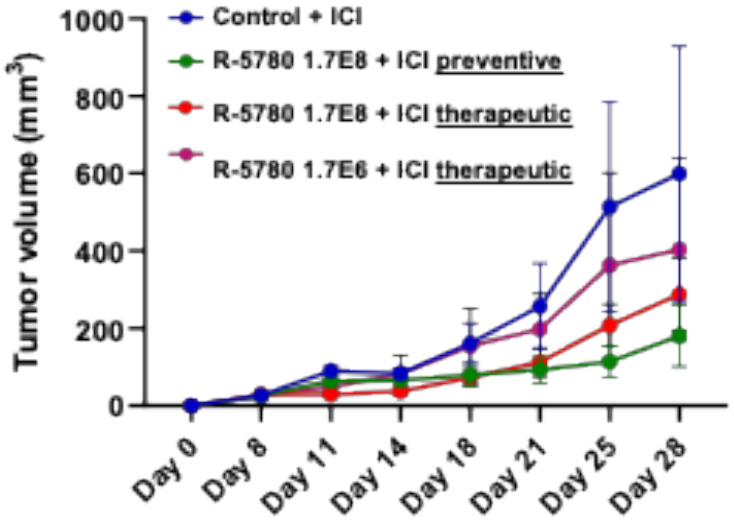
αPD-L1 anti-tumor activity on B16/F10 melanoma



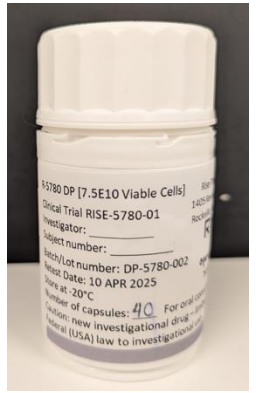
SagA expression confers anti-tumor activity to probiotics and requires hydrolase activity (C443A).

Matthew Griffin (Collaboration with Rise Therapeutics)

Therapeutic Activity and Clinical GMP Manufacturing of R-5780 - *L. lactis-sagA*



L. lactis-sagA **(R-5780) also works as anti-cancer therapeutic with immunotherapy.**



January 2025 - FDA approval for *L. lactis-sagA* (R-5780) as Investigational New Drug (IND).

A Phase 1 Dose-Escalation and Expansion Trial of R-5780 with Immune Checkpoint Inhibitor in Cancer Patients with Solid Tumors (NCT06398418).

RISE THERAPEUTICS

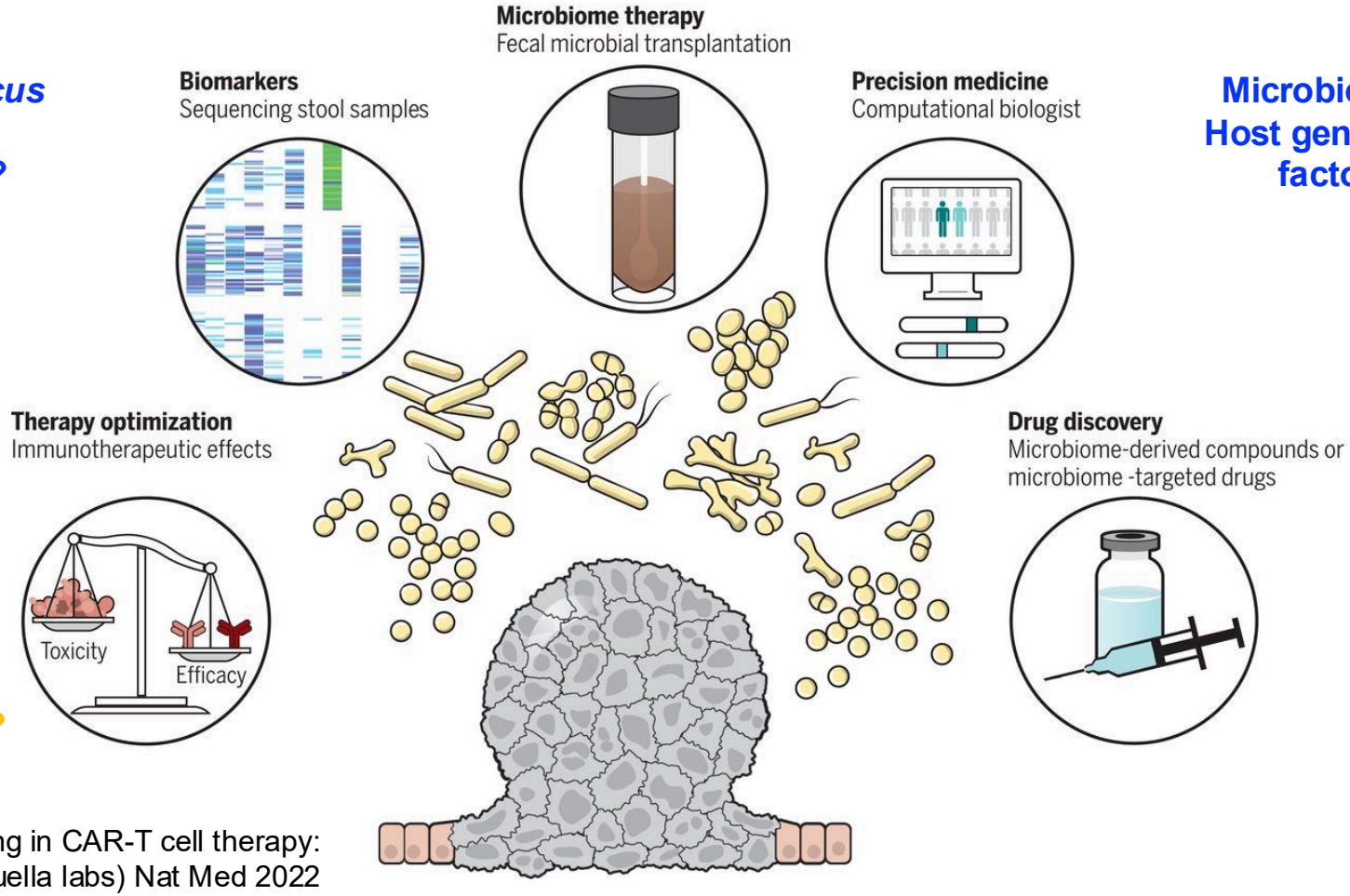
The SagA continues: implications for cancer progression and immunotherapy

SagA-based probiotics (Rise Therapeutics)

Screen for SagA-*Enterococcus*

Other SagA-like enzymes?

Microbiota: SagA-*Enterococcus*
Host genes: NOD2 - major genetic factor in Crohn's disease



SagA-*Enterococcus*

VREfm strains?

Other microbiota species?

New therapeutics?

Muropeptides (MDP)

Griffin M et al Science 2021

Repurpose Mifamurtide

Novel NOD2 agonists

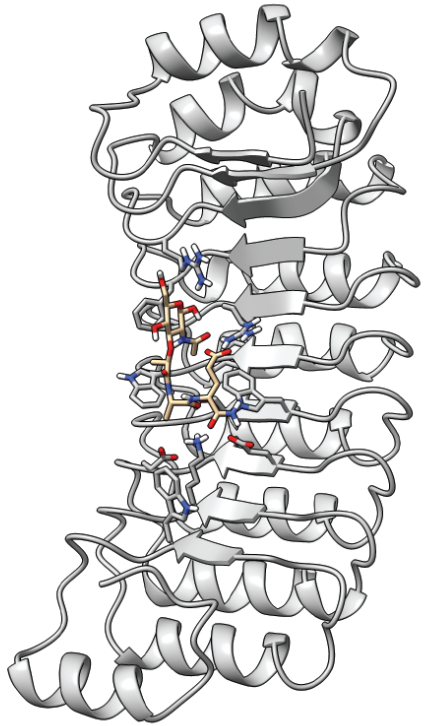
Griffin M, Tsukidate T, Hang HC
ACS Chem Biol 2023

Enterococcus PG remodeling in CAR-T cell therapy: Smith M et al (van den Brink & Ruella labs) Nat Med 2022

SagA^{+}VREfm is enriched in immunotherapy-responsive cancer patients: Gunjur A et al (Lawley lab) Nat Med. 2024

Reviewed in: Zitvogel L, Ma Y, Raoult D, Kroemer G, Gajewski TF Science 2018

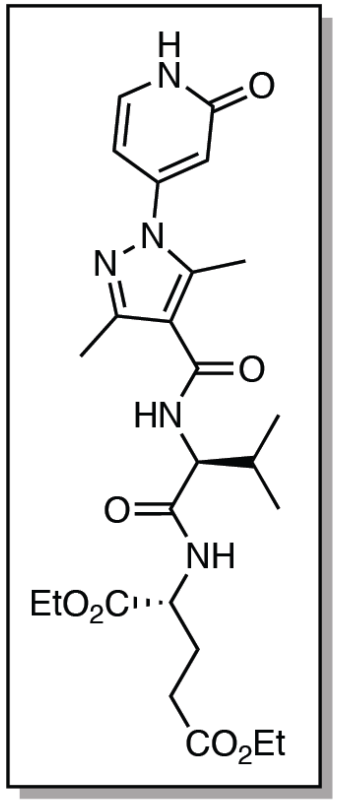
In silico screening and validation reveals novel *N*-arylpyrazole NOD2 agonists



NOD2
virtual docking

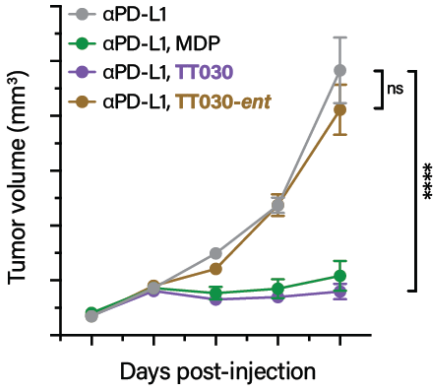
In silico screening

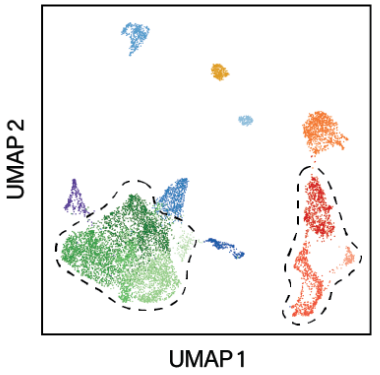
In vitro validation



Lead *N*-arylpyrazole

In vivo activity



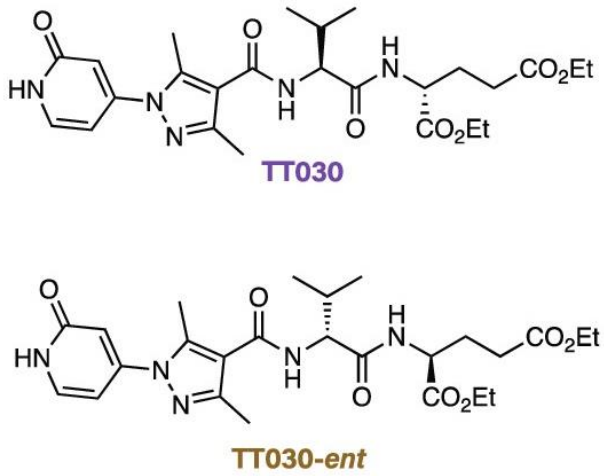


Improved antitumor activity

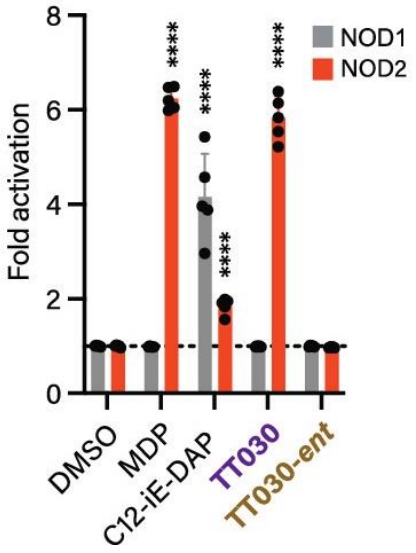
Griffin M, Tsukidate T, Hang HC ACS Chem Biol 2023

Analysis of novel NOD2 agonist specificity *ex vivo* and in mouse models

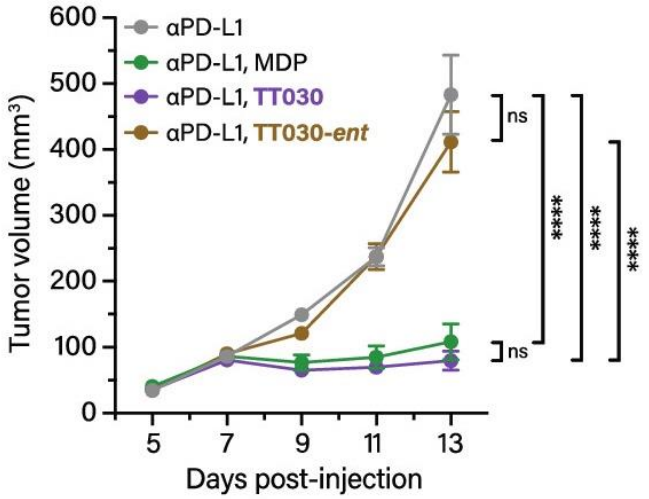
N-arylpyrazole NOD2 agonist / control

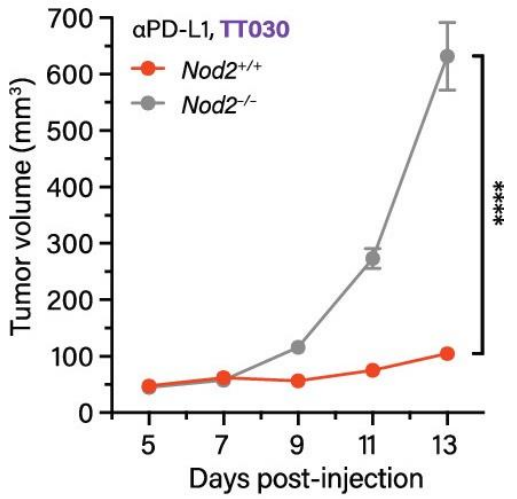


Enantiomer control is inactive



Nod2 is required for activity *in vivo*





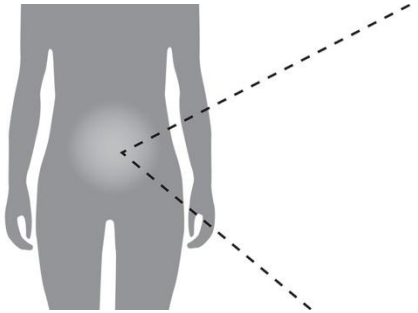
New NOD2 agonist specifically activates NOD2 and enhances checkpoint inhibitor activity in mouse model *in vivo*.

Griffin M, Tsukidate T, Hang HC ACS Chem Biol 2023

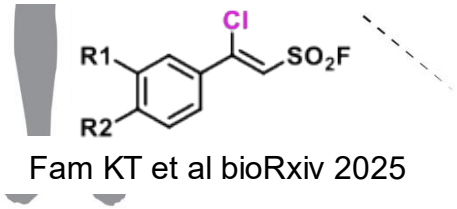
Chemical genetic approaches to dissect microbiota mechanisms

Microbiota species genetics

Chen V et al Appl Env Microbiol 2021



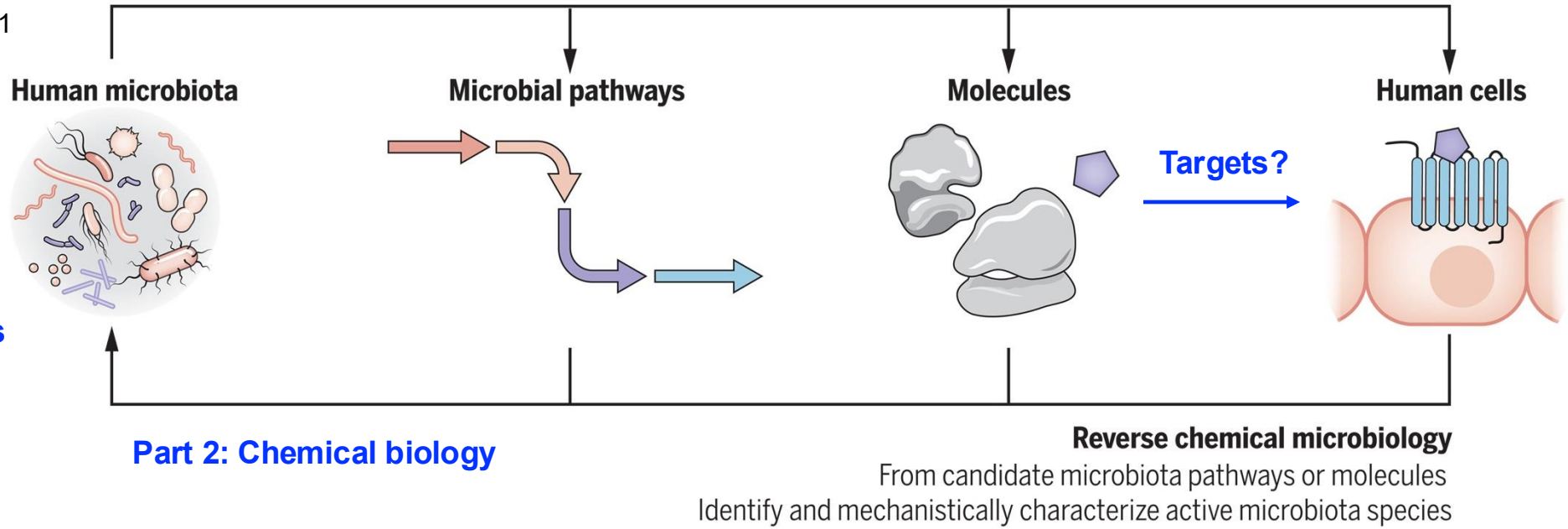
Microbiota enzyme inhibitors



Fam KT et al bioRxiv 2025

Forward chemical microbiology
From active microbiota species
Identify and mechanistically characterize active pathways or molecules

Part 1: model organisms (worms, mice and others)



Part 2: Chemical biology

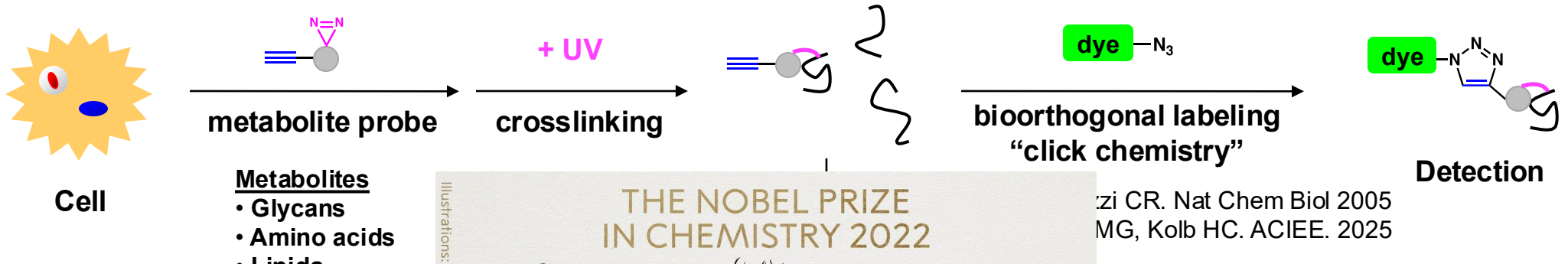
Reverse chemical microbiology
From candidate microbiota pathways or molecules
Identify and mechanistically characterize active microbiota species

Forward and reverse chemical microbiology approaches have revealed specific microbiota mechanisms of action.

Understanding microbiota mechanisms of action provides new opportunities for new diagnostics and therapies.

Reviewed in: Yang X, Hang HC Science 2024

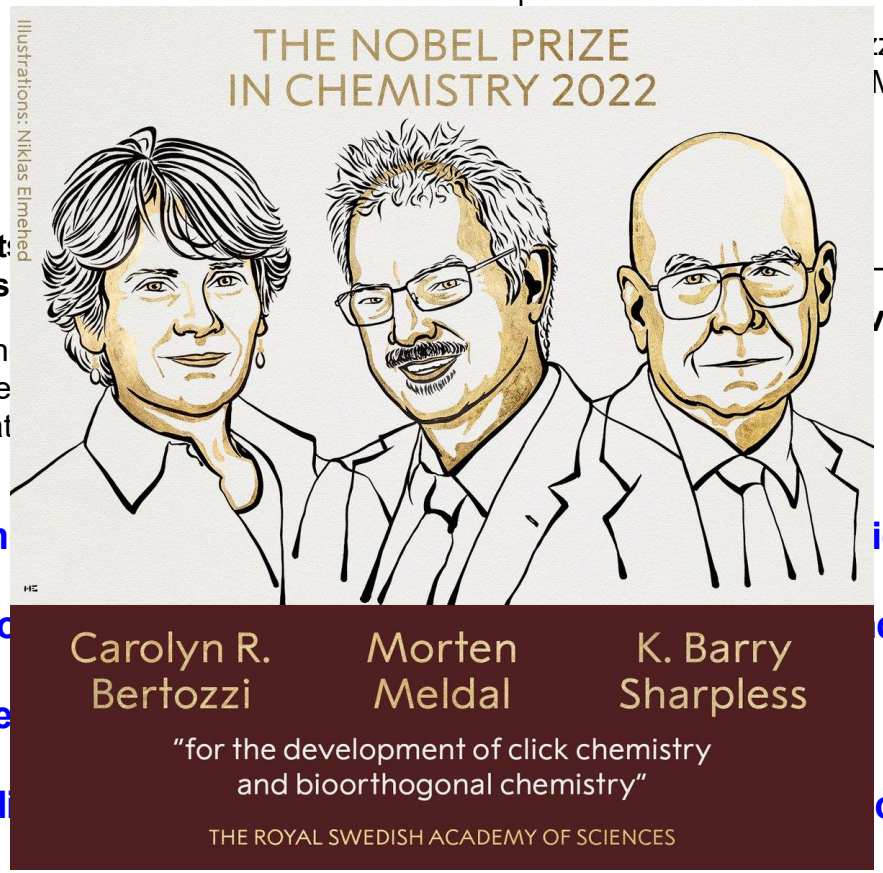
Chemical probes enable direct analysis of metabolite-protein targets



Metabolites
- Glycans
- Amino acids
- Lipids
- Nucleic acids
- Other PTMs
- Natural product
- Synthetic drugs

Reviewed in: Grammel M, Han
Parke
Niphakis MJ, Cravat

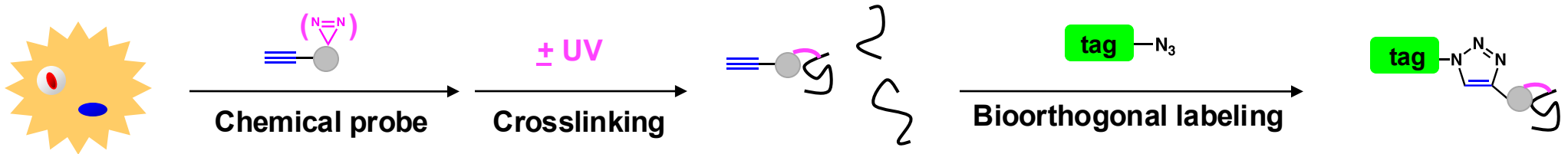
zi CR. Nat Chem Biol 2005
MG, Kolb HC. ACIEE. 2025



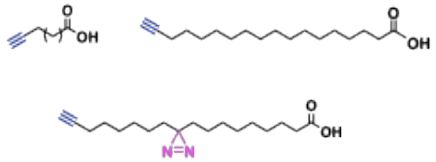
- Discovery of m ions/interactions.
- Large-scale pro d states.
- Analysis of spe
- Applicable to di c drugs.

Chemical probes enable sensitive detection, crosslinking and enrichment of metabolite-targets.

Chemical dissection of microbiota metabolite protein targets

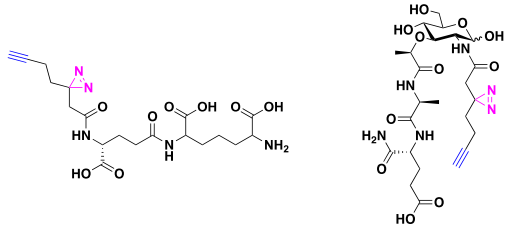


Short and long-chain fatty acid probes



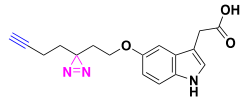
Charron G et al J Am Chem Soc 2009
Yang YY et al J Am Chem Soc 2010
Rangan K et al J Am Chem Soc 2010
Zhang MM, Tsou LK et al PNAS 2010
Zhang MM et al PLoS Biol 2013
Peng T et al J Am Chem Soc 2015
Zhang Z et al Nat Chem Biol 2020

Peptidoglycan metabolite probes



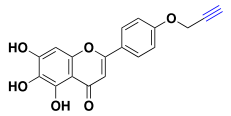
Wang Y-C et al ACS Chem Biol 2019
Hespen CW et al Chem Commun 2022

Tryptophan metabolite probe



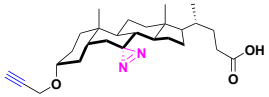
Zhao X et al Nat Chem Biol 2023

Plant metabolite probe



Tsou LK et al J Am Chem Soc 2016

Bile acid probes

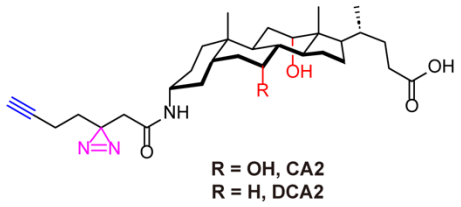


Salmonella: Yang X et al Nat Chem Biol 2023
C. difficile: Foster E et al ACS Chem Biol 2022
Enterococcus: Yang X et al RCS Chem Biol 2022
C. difficile TcdB toxin: Miletic S et al Nat Microbiol 2025
DCA-STING activation: Yang X et al bioRxiv 2025

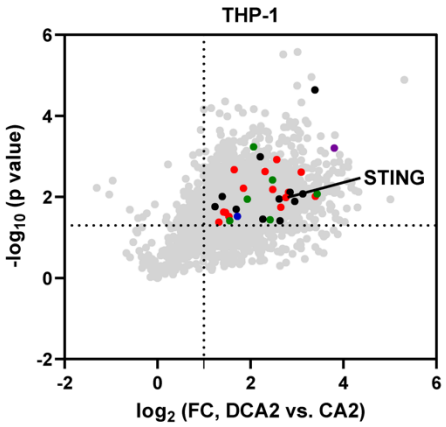
Chemoproteomics has reveal new microbiota metabolite protein targets and mechanisms.

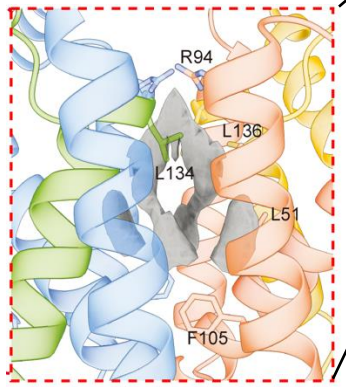
Reviewed in: Microbial metabolite probes: Zhang ZJ, Wang YC, Yang X, Hang HC Chembiochem 2020
Chemical proteomics of microbiota metabolites: Zhao X, Yang X, Hang HC Biochemistry 2022

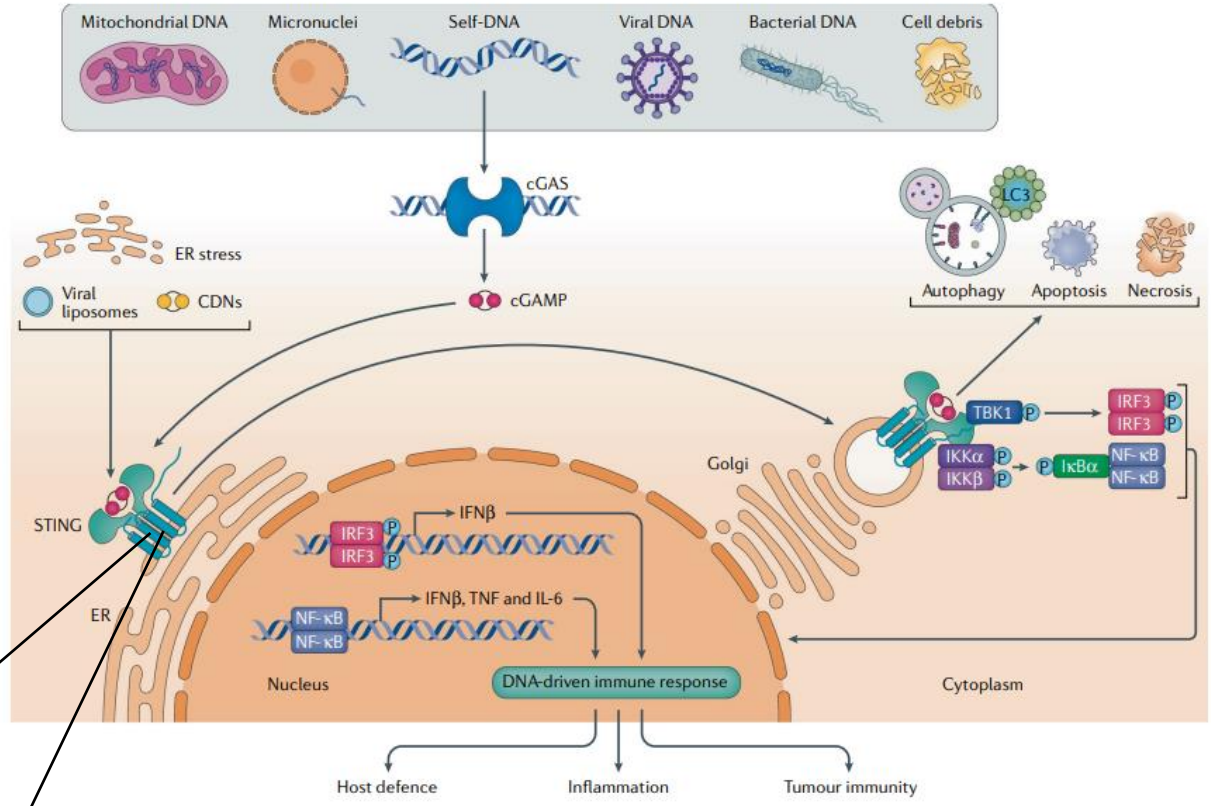
Chemoproteomics revealed bile acid regulation of STING activation



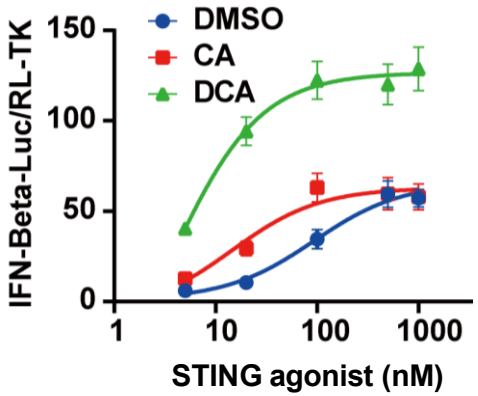
R = OH, CA2
R = H, DCA2

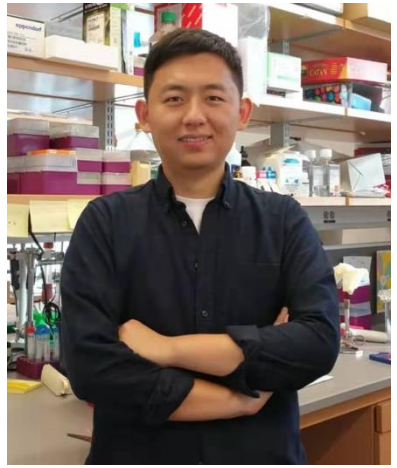






Motwani M et al Nat Rev Genetics 2019





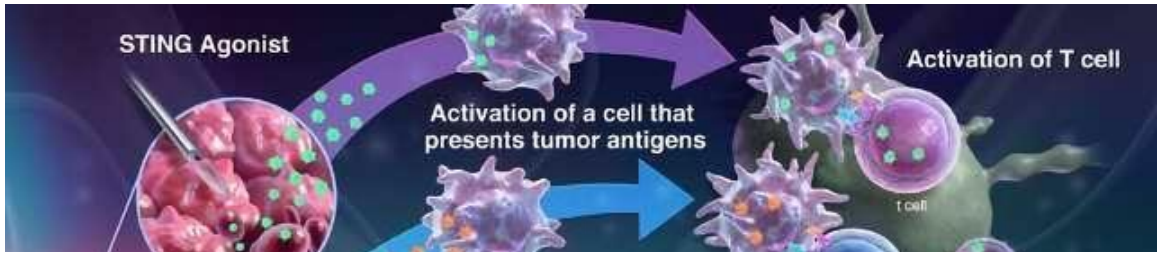
Deoxycholic acid (DCA), but not cholic acid (CA), binds STING-TM and promotes agonist activity *ex vivo*.

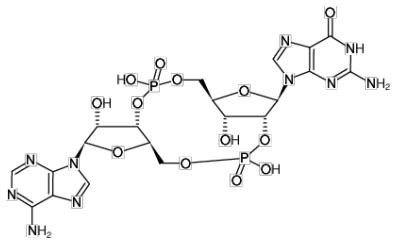
Xinglin Yang, Former Postdoc

Assistant Professor @ Peking University

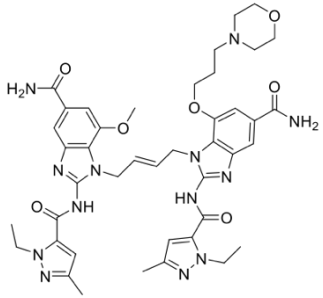
Yang X*, Zhang X* et al (collaboration with Parker, Ward, Lairson and Forli labs at Scripps) bioRxiv 2025

STING senses cyclic dinucleotides and is target for immunotherapy





2',3'-cGAMP



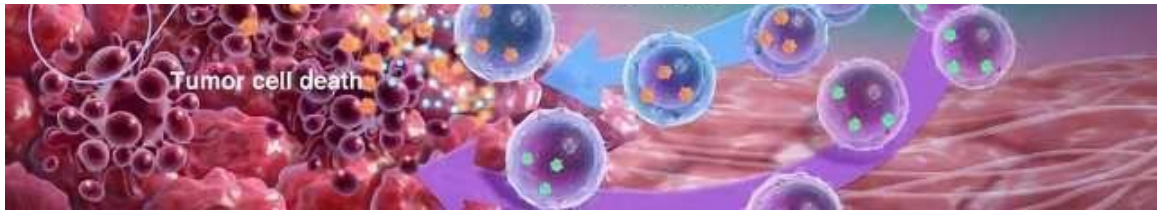
diABZI

Ramanjulu JM et al (GSK) Nature 2018

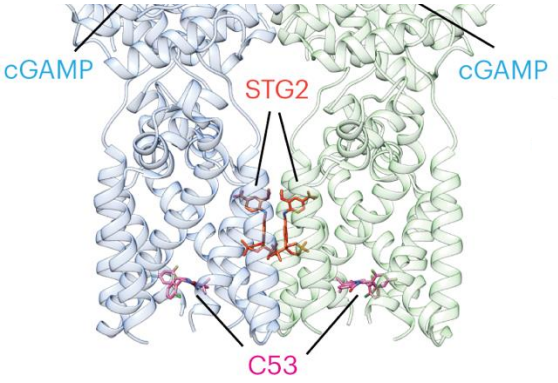
Dimer 1 Dimer 2

DCA binds STING transmembrane dimer-dimer interface similar to STG2.

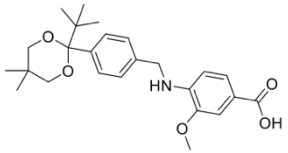
Bile acid (DCA) binding of STING promote cancer immunotherapy?



Reviewed in: Samson N, Ablasser A. Nat Cancer. 2022
Chin EN, Sulpizio A, Lairson LL. Trends Cell Biol. 2023



Chin EN et al (Scripps/Calibr) Science 2020

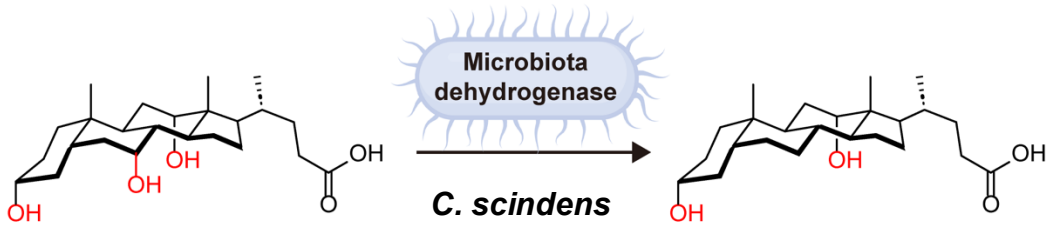


STG2

STG2 agonist binds/glues STING-TM interface:
Li J et al (UT-Southwestern & Novartis) Nat Chem Biol 2024

STING agonists activate antitumor immune responses and promotes clearance of cancer cells.

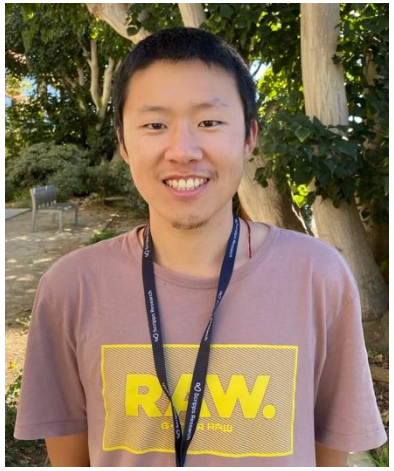
DCA and microbiota-producer promotes STING agonist antitumor activity *in vivo*



CA

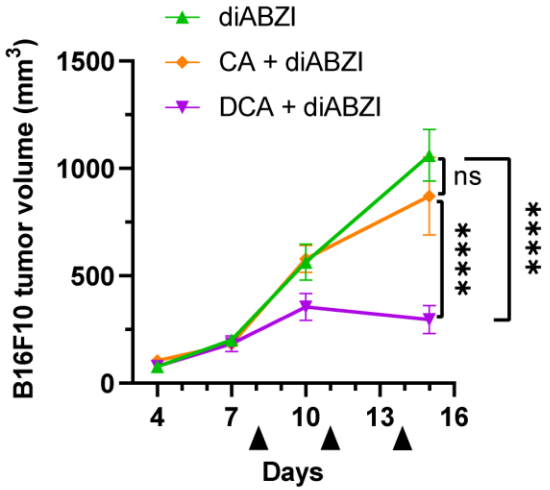
DCA

Funabashi M et al
(Fischbach lab) Nature 2020

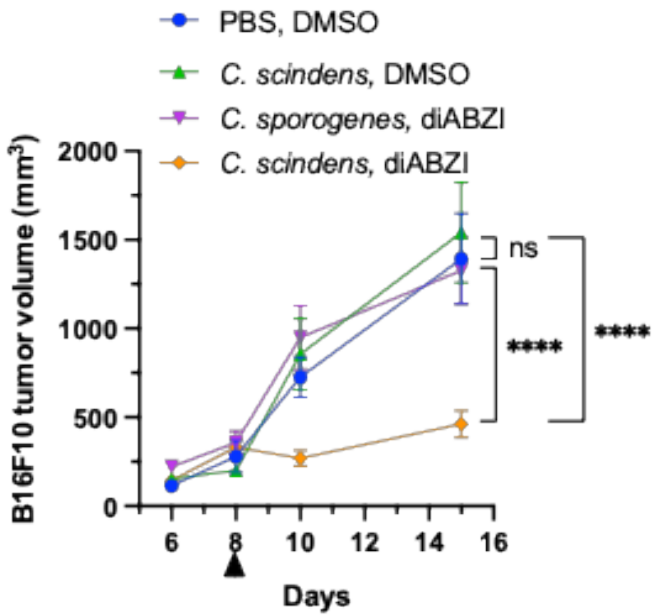


Xing Zhang, Postdoc

Intratumoral injection (BAs and STING agonist)



Intratumoral injection (STING agonist)
Oral gavage of bacteria

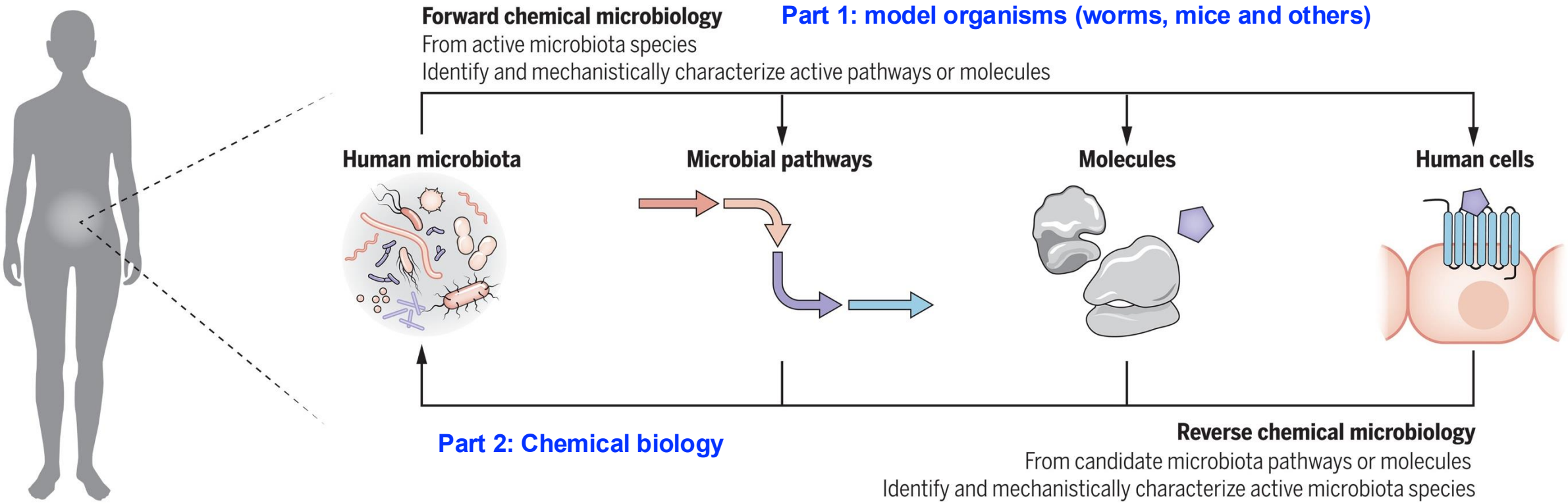


DCA, but not CA, and microbiota-producer (*C. scindens*) promotes STING agonist antitumor activity *in vivo*.

Specific bile acids and microbiota species may modulate STING-targeted immunotherapies.

Yang X*, Zhang X* et al (collaboration with Parker, Ward, Lairson and Forli labs at Scripps) bioRxiv 2025

Chemical genetic approaches to dissect microbiota mechanisms

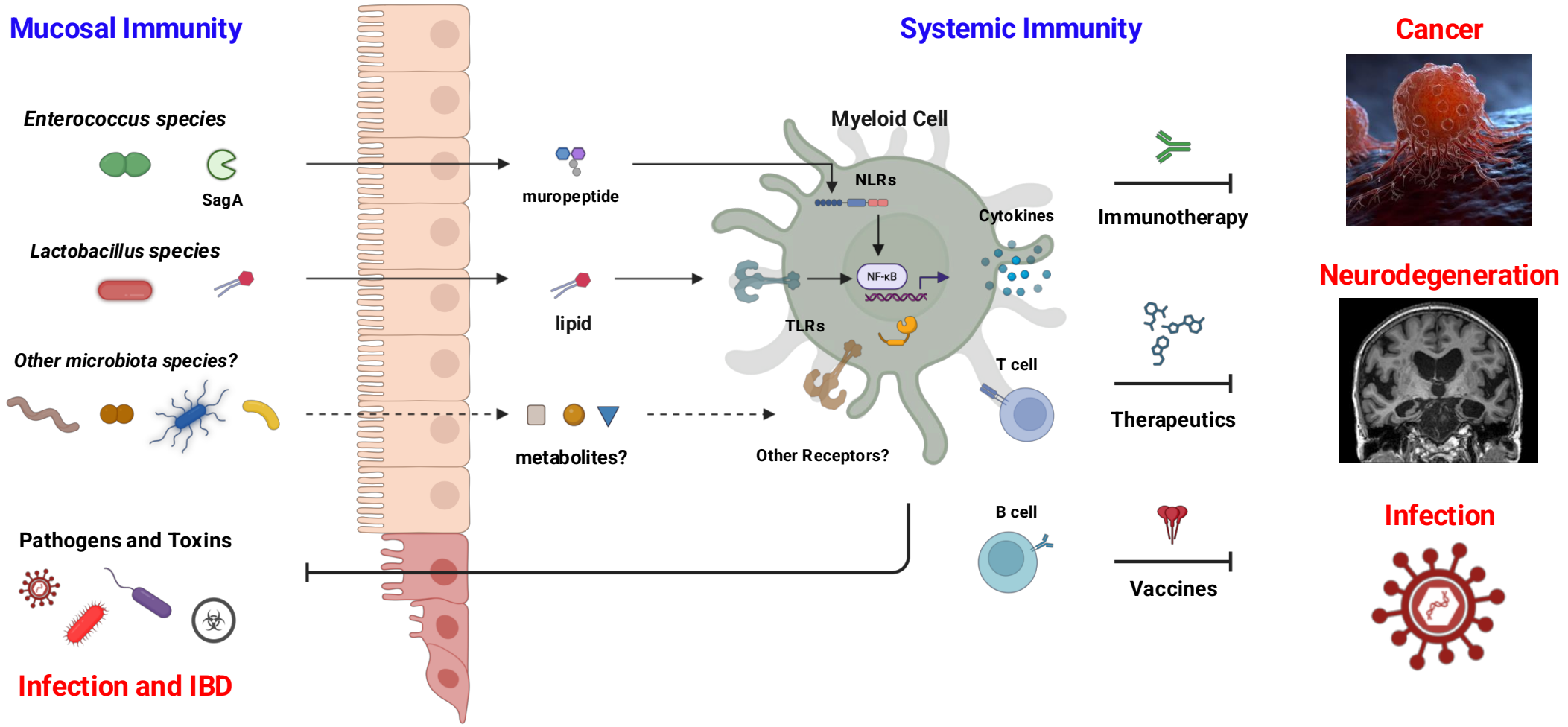


Forward and reverse chemical microbiology approaches have revealed specific microbiota mechanisms of action.

Understanding microbiota mechanisms of action provides new opportunities for new diagnostics and therapies.

Reviewed in: Yang X, Hang HC Science 2024

Hang Laboratory Ongoing and Future Studies



Still many more microbiota species and metabolites to characterize in health and disease!

Hang Lab Collaborations at Scripps Research

Raphael Park
Cryo-electron tomography

Stefano Forli
Metabolite/drug docking

Ben Cravatt
Chemoproteomics

Alejandra Mendoza
Mucosal immunology

Shannon Miller
Mammalian gene editing

Andrew Ward
Cryo-electron microscopy

Ilia Droujinine
Cell-specific proteomics

Chris Parker
Chemoproteomics

John Teijaro
Antiviral immunity

Amy Lightner
Inflammatory bowel disease

Ian Wilson
X-ray crystallography

Stuart Lipton
Neurodegeneration

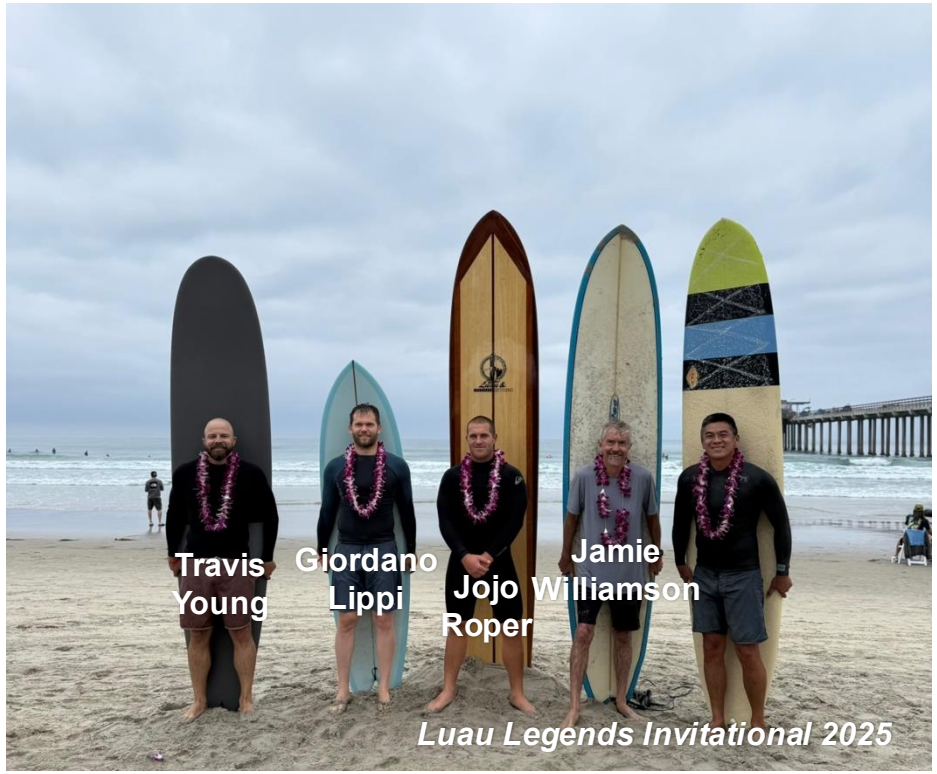
Mia Huang
Glycobiology

Ian Seiple
New antibiotics

Keary Engle
New antibiotics

Need your support and talent to innovative, collaborate and translate!

Board Meetings for a Good Cause - Luau & Legends of Surfing Invitational



Scripps Research – Calibr-Skaggs Surf Teams

Annual Funding Raising Event for Cancer Research

Microbiota has a major impact on health, disease, therapies and environment

Microbes are ubiquitous and important.

Microbes are diverse and have unique features.

Microbiota modulates host health, disease and therapy.

Microbial pathogens are major challenge to global health.

Antibiotic resistance is significant healthcare crisis.

Microbiota also contributes to environmental sustainability.

Understanding microbiota mechanisms is important for human health and environment.

Checkout Hang lab Microbiota Booths at Reception!

Acknowledgements and Support

Current Members
Nadine Berenst (& Miller lab)
Varaang Bhandula
Morgan Birabaharan (T32 Fellow)
Pavan Kumar Chodisetti
Tingting Dai
Stas Dikiy (CRI Fellow)
Natavan Dudkina (Hewitt Fellow)
Kyong Fam
Yan-Zhu Hsieh
Abeera Mehmood (& Mendoza lab)
Anh Nguyen
Bao Anh Nguyen
Stephanie Smelyansky
Siyue Yang (& Droujinine lab)
Adrianna Turner
Chongyang Wu (& Lipton lab)
Xing Zhang
Angela Kong (manager)
Liz LaBlond (coordinator)

SagA/NOD2 Team / Alumni
Daniel Mucida (Rockefeller)
Gary Fanger (Rise Therapeutics)
Ken Cadwell (Univ. Penn)

Victor Chen
Juliel Espinosa
Steven Klupt
Matthew Griffin (UC Irvine)
Charles Hespen
Byungchul Kim
Ti-Yu Lin
Virginia Pedicord (Cambridge)
Kavita Rangan (Johns Hopkins)
Taku Tsukidate
Yen-Chih Wang
Nathan Westcott

Bile acid/STING Team / Alumni
Chris Parker (Scripps)
Weichao Li
Andrew Ward (Scripps)
Anant Gharpure
Stefano Forli (Scripps)
Althea Hansel-Harris
Andreas F. Tillack
Luke L. Lairson (Scripps)
Ariana Sulpizio

Xinglin Yang (Peking Univ.)
Xiaohui Zhao (Zheijang Univ.)

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Collaborative Innovation Fund at Scripps Research
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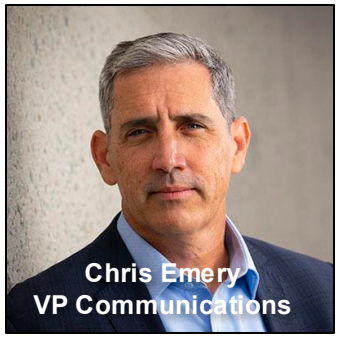
NIH NATIONAL CANCER INSTITUTE
NIH National Institute of Allergy and Infectious Diseases
NIH National Center for Complementary and Integrative Health
THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

Thank you Scripps Research Leadership and Staff!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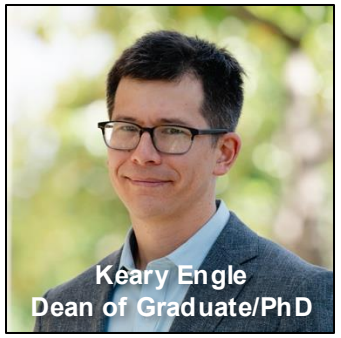
Keary Engle
Dean of Graduate/PhD

Graduate Office


Flow Cytometry and Other Cores

I am immensely privileged to be a scientist. I feel such joy to be on this incredible intellectual journey, to be working with diverse and dedicated colleagues, and to be experiencing amazing opportunities that science has given me, all while unraveling the mysteries of biology…..and chemistry, ***especially at Scripps Research.***

Ardem Patapoutian - Biographical, Nobel Prize in Physiology or Medicine 2021

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IMM Department Staff

Thank you for joining the Front Row Lecture!