Harnessing the power of the microbiota to boost immunity against infection and cancer

Wednesday, August 18, 2021
1:00 PM PT/4:00 PM ET

Howard Hang, PhD
Professor
Departments of Immunology & Microbiology and Chemistry

THE FRONT ROW
at Scripps Research

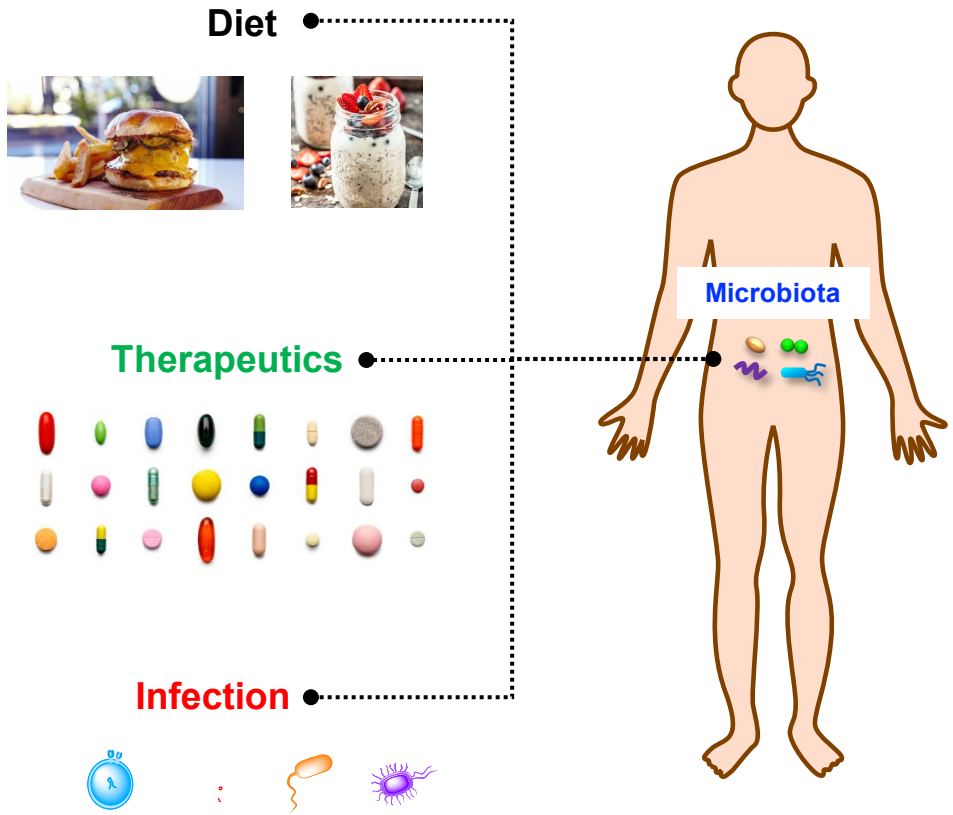
Microbes are ubiquitous and have beneficial affects

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.

Microbiota are important for host physiology, disease and therapy



Microbiota

- 4 x 10^{13} of bacteria/colon ~ 3 x 10^{13} host red blood cells.
- ~10^{2-3} bacterial species per individual.
- microbiome (>500K genes) vs human genome (~20K genes)
- unique genes, proteins and metabolites.
- varies: location, individuals, age, diet and drugs.

Host Physiology & Disease

- Metabolism ---> Malnutrition, Obesity, Others
- Immunity ---> Infection, Inflammatory diseases, Cancer
- Behavior ---> Neurological disorders

Drug Metabolism and Immunotherapy

Microbiota (bacteria, fungi, viruses) encodes unique activity, but are complex, heterogenous and dynamic.

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 on human health!

Microbiota and microbiome studies at Scripps Research

Can Medicines that Alter the Microbiome Prevent Cardiovascular Disease?

Speaker:
Reza Ghadiri, PhD
Professor
Department of Chemistry

THURSDAY, NOVEMBER 12, 2020

Scripps Research

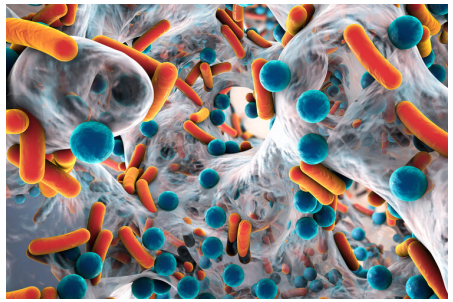
Register at frontrow.scripps.edu

ARTICLES
https://doi.org/10.1038/s41587-020-0549-5
nature biotechnology
Check for updates

Directed remodeling of the mouse gut microbiome inhibits the development of atherosclerosis

Poshen B. Chen[1], Audrey S. Black[1], Adam L. Sobel[1], Yannan Zhao[1], Purba Mukherjee[1], Bhuvan Molparia[2,3], Nina E. Moore[1], German R. Aleman Muench[4], Jiejun Wu[4], Weixuan Chen[4], Antonio F. M. Pinto[5], Bruce E. Maryanoff[1], Alan Saghatelian[5], Pejman Soroosh[4], Ali Torkamani[2,3], Luke J. Leman[1,6] and M. Reza Ghadiri[1,6]

Chen PB et al (Ghadiri lab) Nat Biotechnol 2020



Michael G. Constantinides, Ph.D. Assistant Professor
Department of Immunology & Microbiology @ Scripps

IMMUNOLOGY

MAIT cells are imprinted by the microbiota in early life and promote tissue repair

Michael G. Constantinides, Verena M. Link, Samira Tamoutounour, Andrea C. Wong, P. Juliana Perez-Chaparro, Seong-Ji Han, Y. Erin Chen, Kelin Li, Sepideh Farhat, Antonin Weckel, Siddharth R. Krishnamurthy, Ivan Vujkovic-Cvijin, Jonathan L. Linehan, Nicolas Bouladoux, E. Dean Merrill, Sobhan Roy, Daniel J. Cua, Erin J. Adams, Avinash Bhandoola, Tiffany C. Scharschmidt, Jeffrey Aubé, Michael A. Fischbach, Yasmine Belkaid*

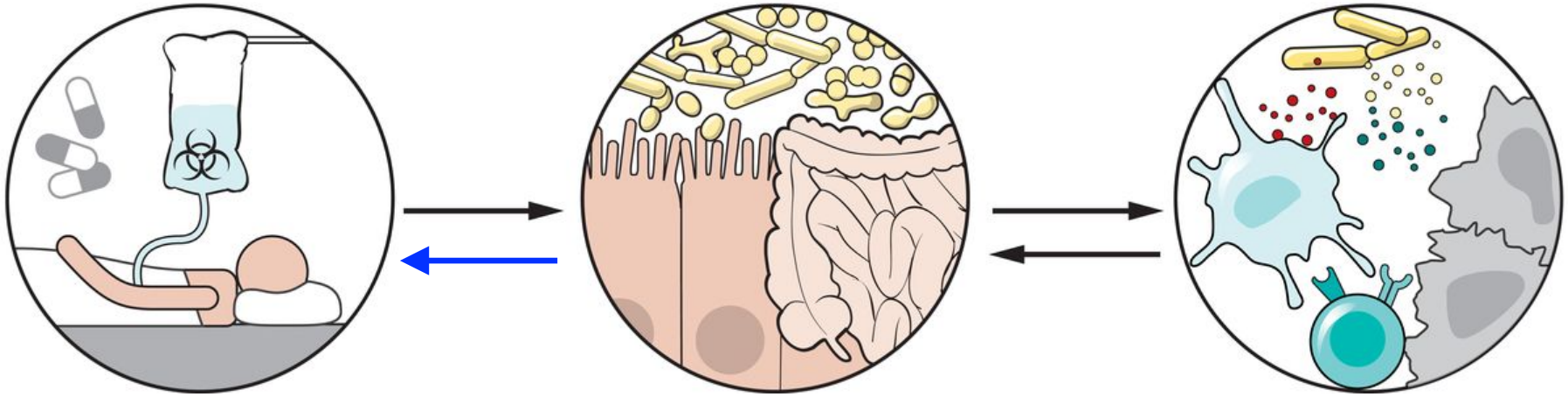
Constantinides MG et al (Belkaid lab) Science 2019

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.



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

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

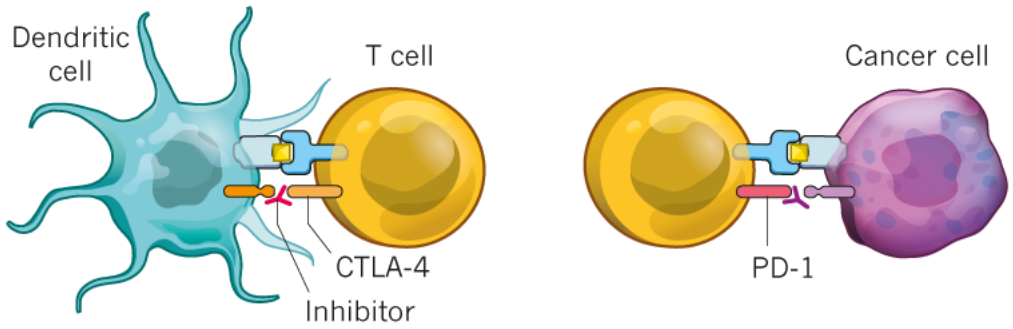
Cancer immunotherapy is transformative, but not effective in all patients

IMMUNE BOOST

Several methods are showing promise in helping immune sentinels called T cells to attack cancer.

CHECKPOINT INHIBITOR DRUGS

'Checkpoint' proteins block T-cell activity. Inhibitor drugs can release the brakes on T cells at different stages.



The CTLA-4 checkpoint protein prevents dendritic cells from priming T cells to recognize tumours. Inhibitor drugs block the checkpoint.

The PD-1 checkpoint protein prevents T cells from attacking cancer cells. The inhibitor drug allows T cells to act.

©nature

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 (Wargo lab) 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.

Microbiota transplantation can improve efficacy of 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]‡§

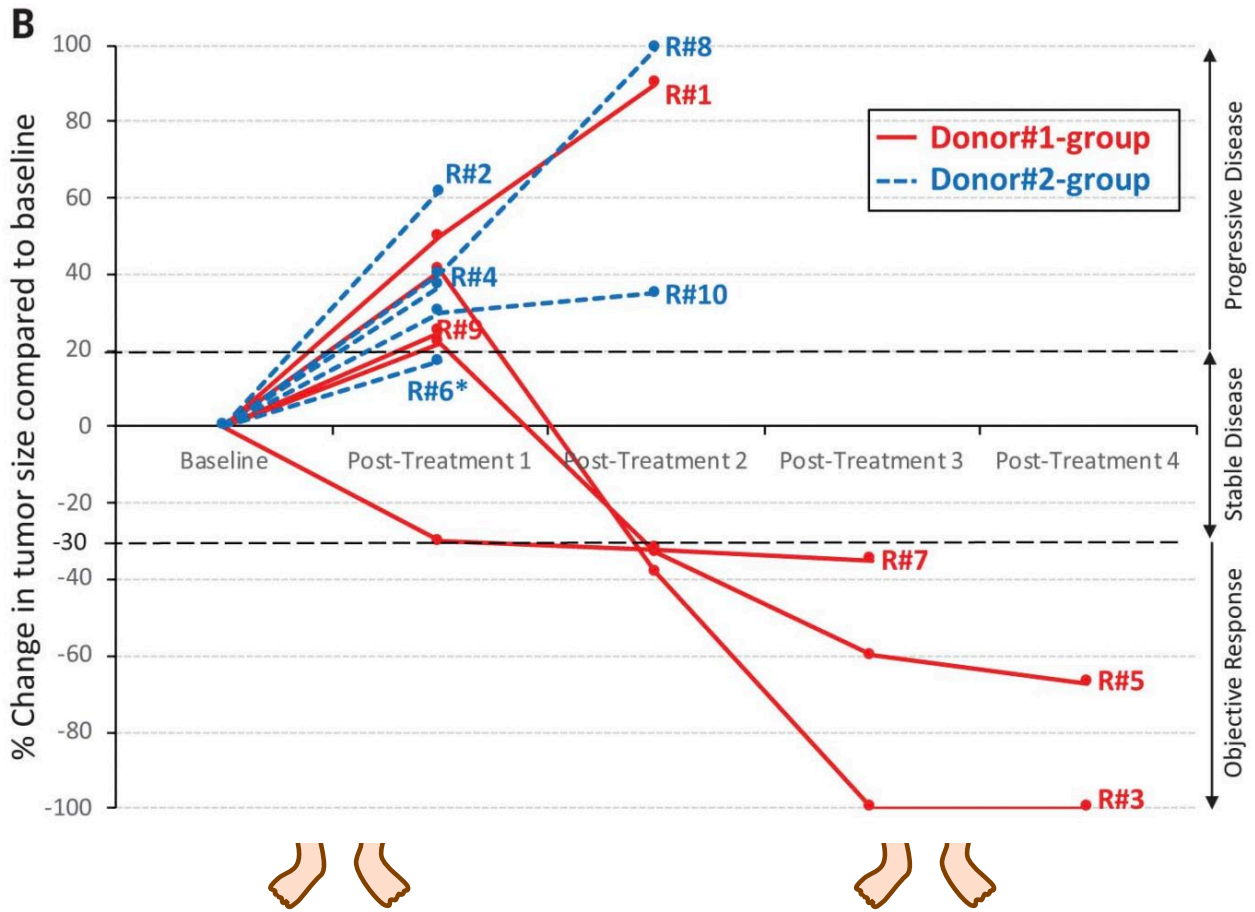
U Pittsburgh Med Center + NIH: Davar D et al (Zarour lab) Science 2021

CLINICAL TRIALS

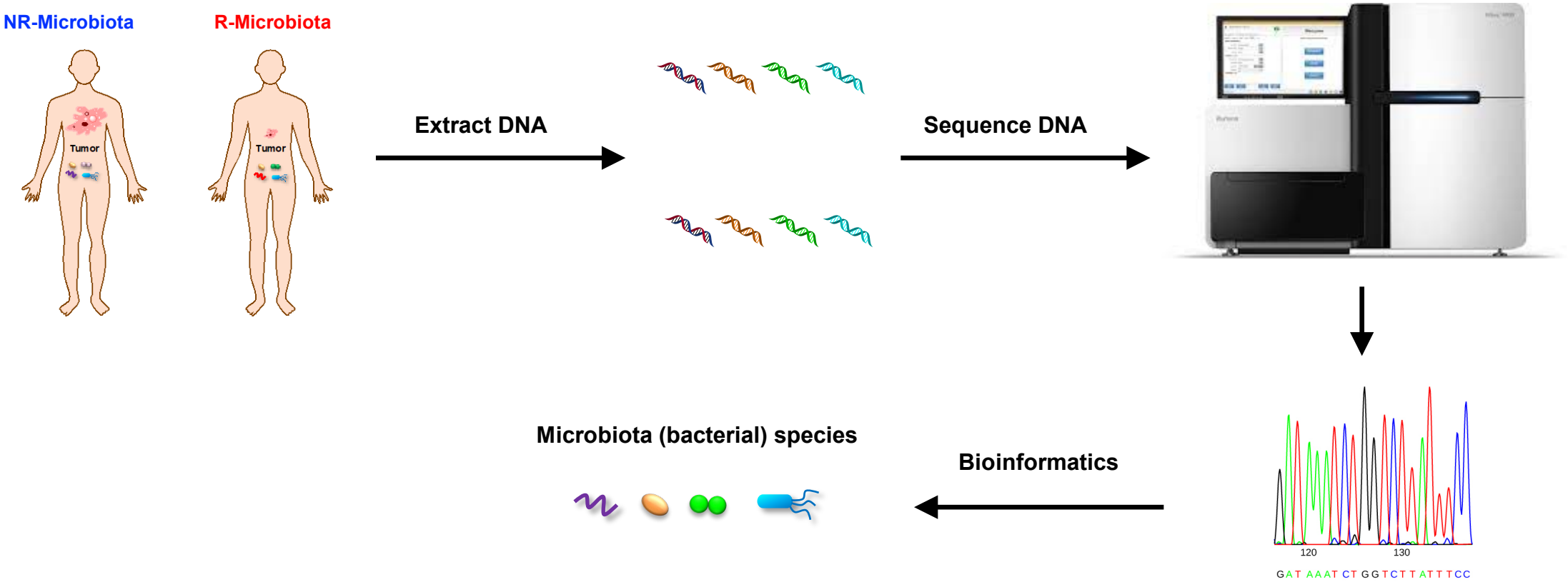
Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients

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



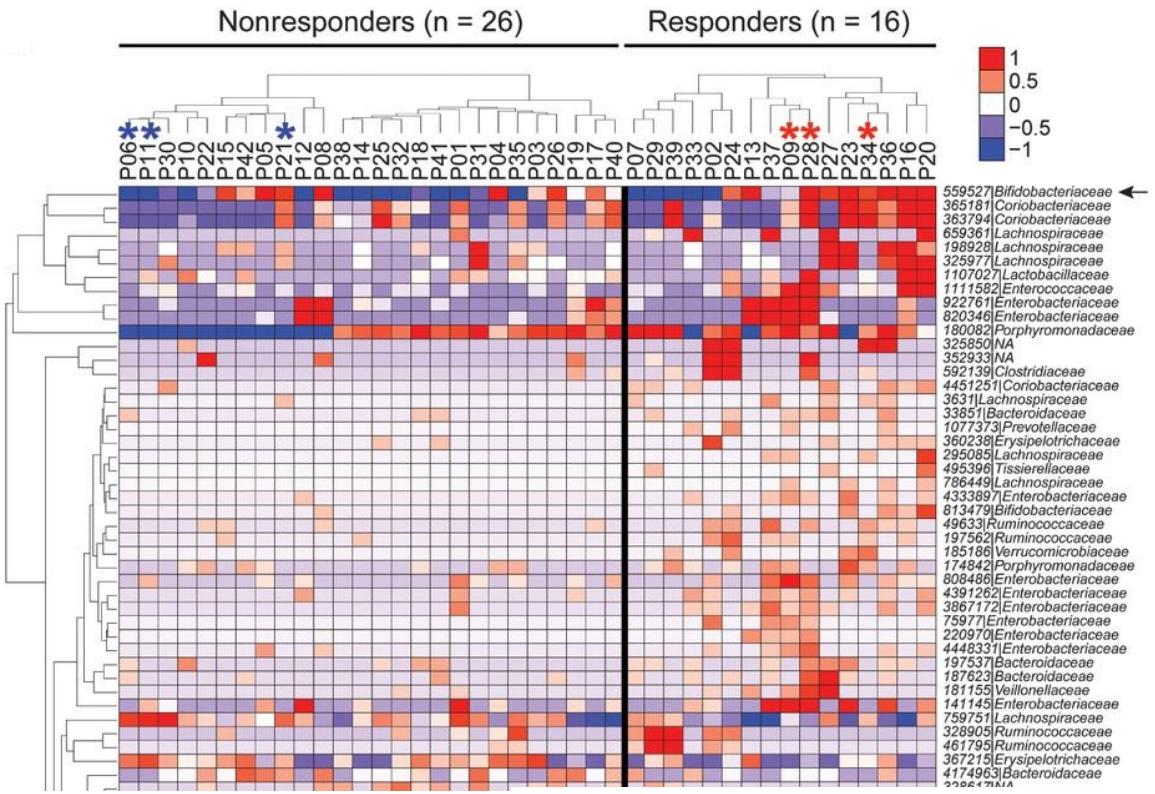
DNA sequencing has also transformed microbiome studies



DNA sequencing allows comparative genomic analysis of microbiota samples.

Microbiota studies are exciting, but still challenging to mechanistically dissect

Different microbiota species <---> cancer immunotherapy efficacy



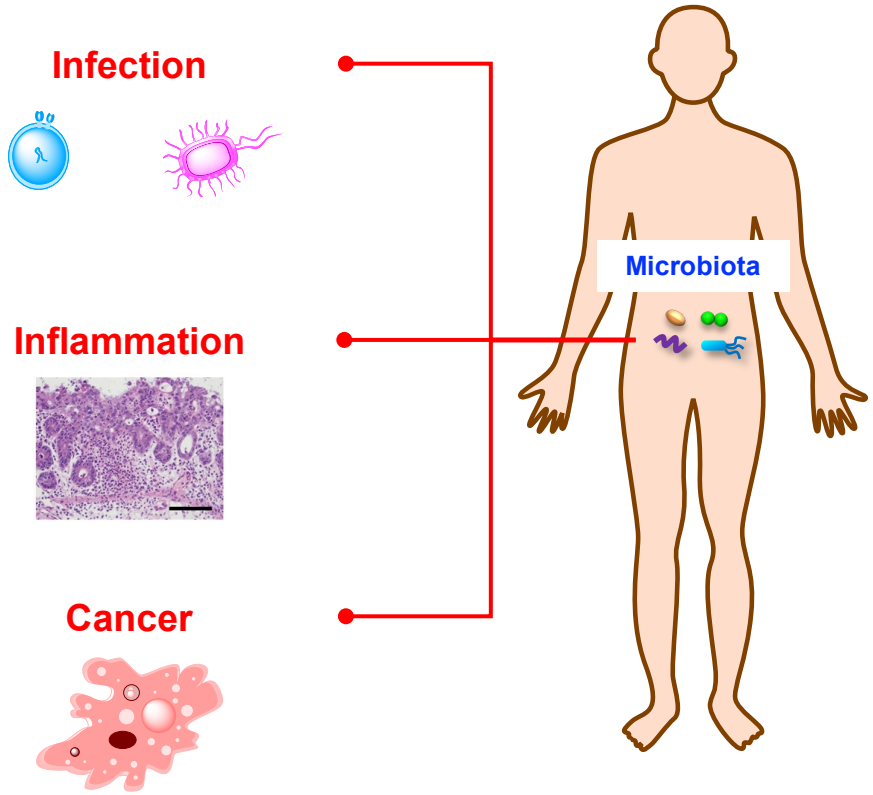
Matson V et al (Gajewski lab) Science 2018

Key challenges in microbiota studies

- **Many microbes are host/disease-specific.**
- **Many microbes cannot be cultured *ex vivo*.**
- **Intra- and inter-microbial interactions.**
- **Activity often from rare and minor species/strain.**
- **Limited genetics in many species.**
- **Limited chemical tools in many species.**

Complex and highly variable microbiota composition between individuals is a major challenge.

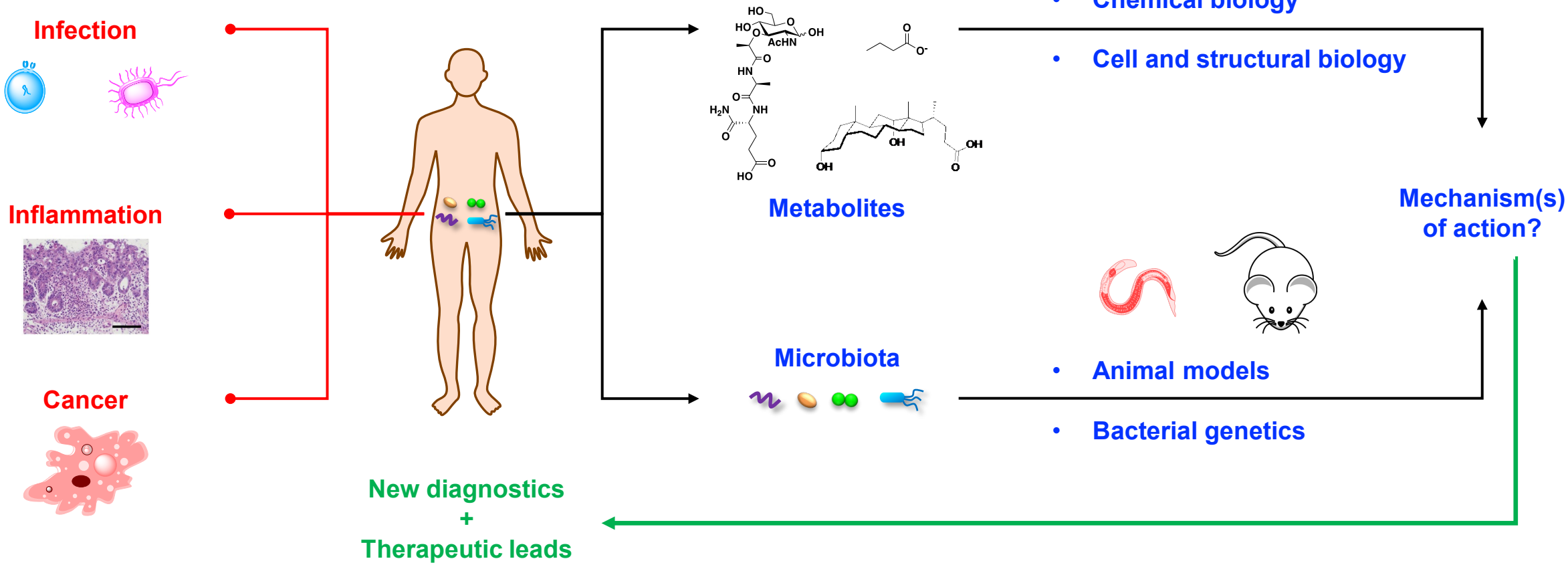
Innovative approaches are need to dissect specific microbiota functions



Microbiota (diverse bacteria, fungi, viruses)

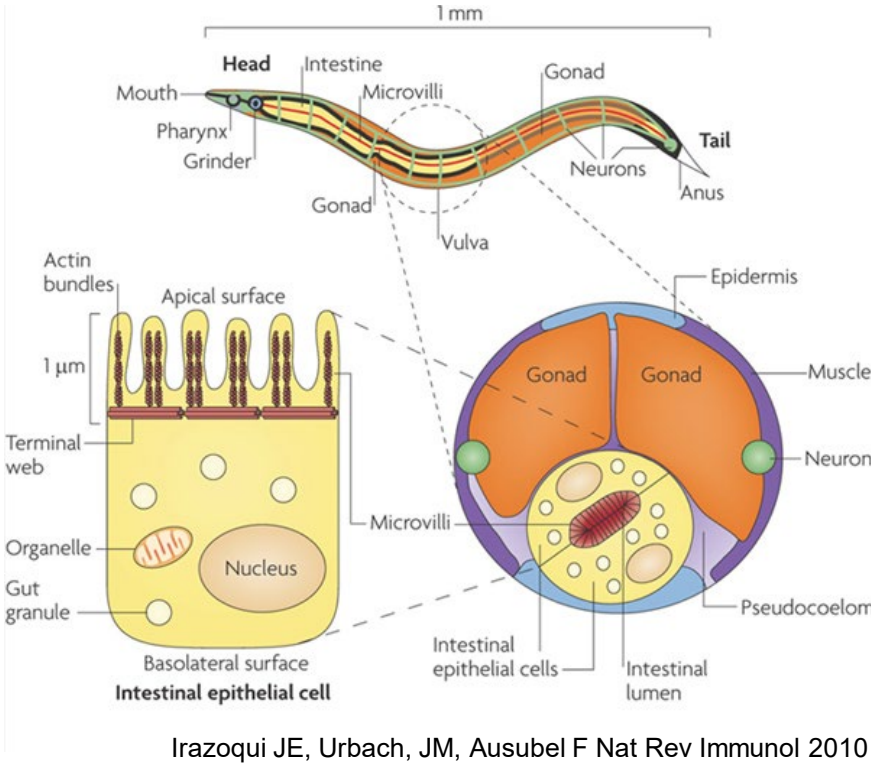
- **Correlated with health and disease.**
- **May functions as endogenous “adjuvants” to modulate host immunity.**
- **Need new approaches to dissect the functions of specific microbiota species and factors.**

Dissection and translation of microbiota mechanisms @ Scripps Research

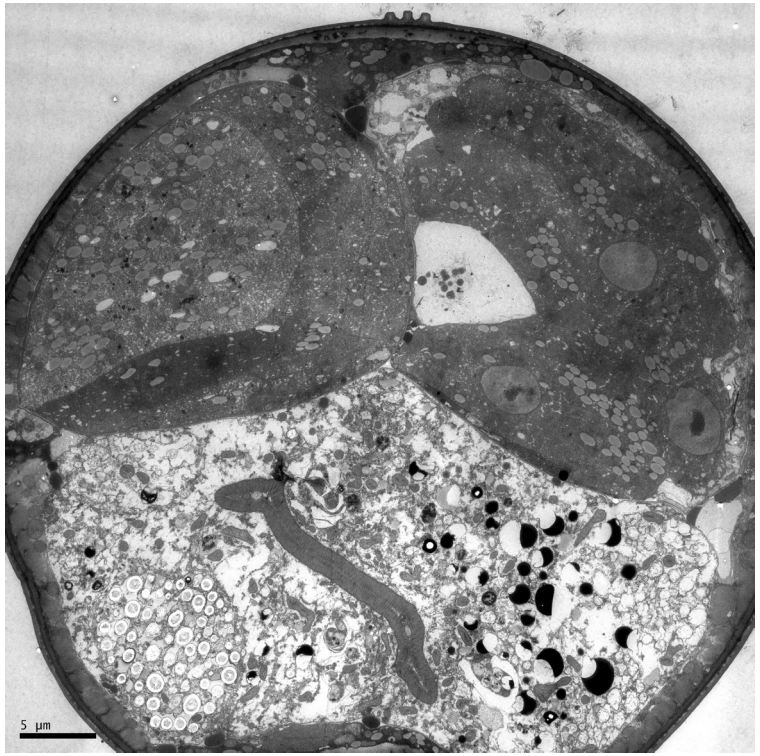


Elucidation of specific metabolite and microbiota mechanism(s) of action may reveal new targets, therapeutic leads and biomarkers.

Small animal models are useful for exploring specific microbiota species



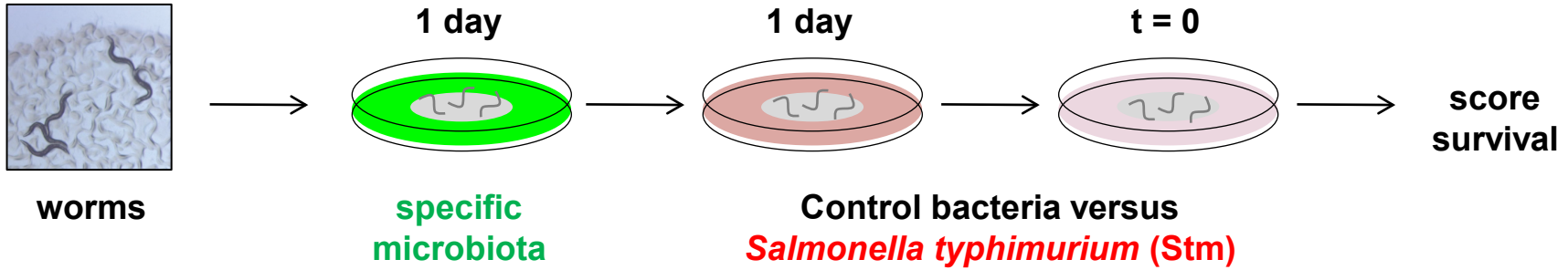
Irazoqui JE, Urbach, JM, Ausubel F Nat Rev Immunol 2010



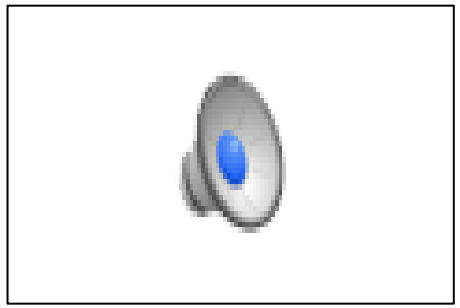
- **Worms (*C. elegans*) interact with diverse microbes in their natural habitat.**
- **No adaptive immune system, but have innate immunity and share similar features to mammalian intestine.**
- ***C. elegans* can be colonized by different microbiota species and infected by diverse pathogens.**

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

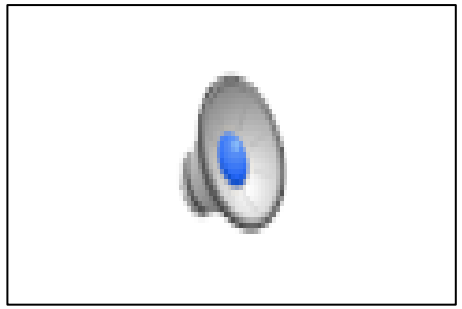
Pulsed infection assay:



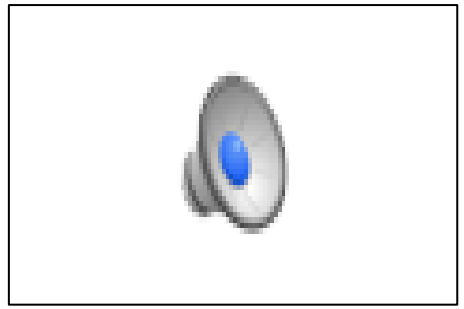
Control bacteria; day 3



Control – Stm; day 3

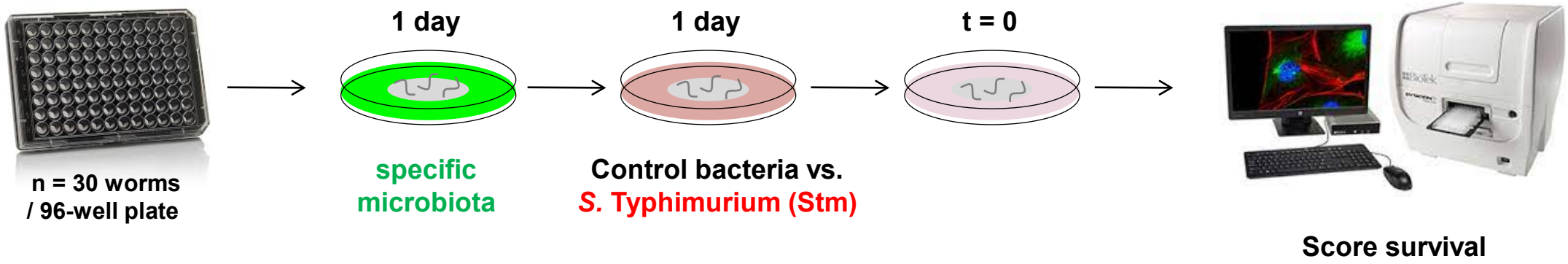


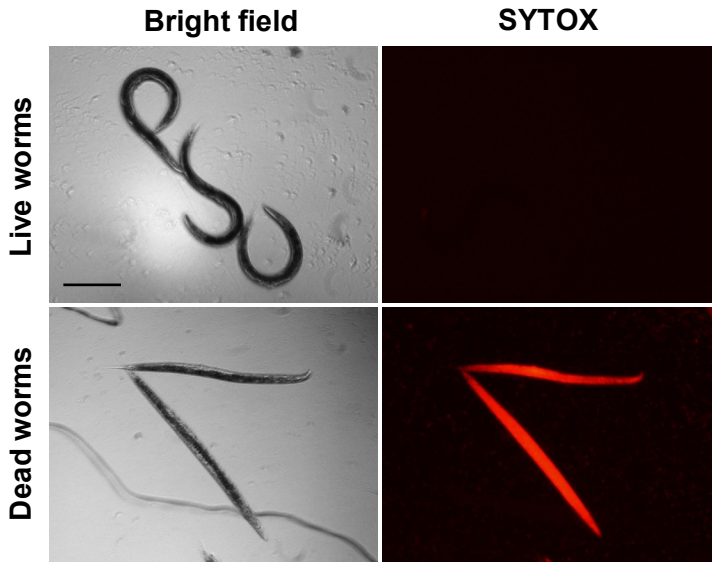
***E. faecium* – Stm; day 3**

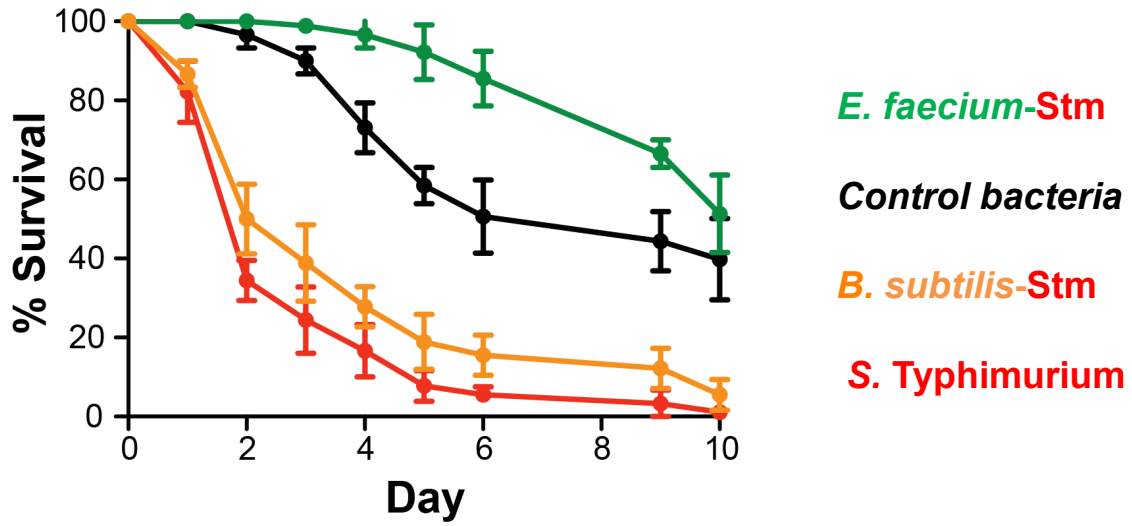


Roundworms (*C. elegans*) provides an inexpensive animal model for exploring complex biology – microbiota mechanisms.

High-throughput analysis of beneficial microbiota species (infection)







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

>60 species, *E. faecalis and E. faecium* - most prominent

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

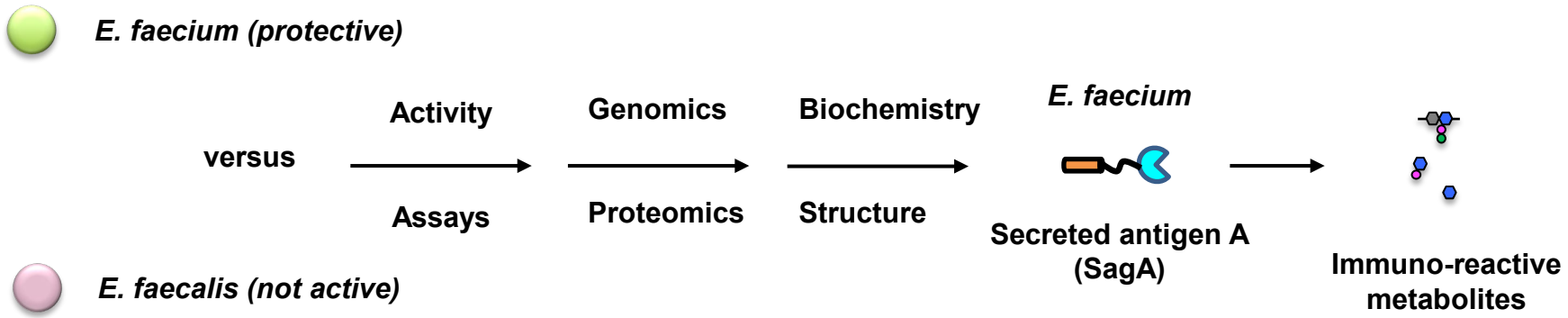
Tolerant to broad pH range, temperature and osmotic conditions

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.

Worms provided an excellent model system to discover microbiota factors

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



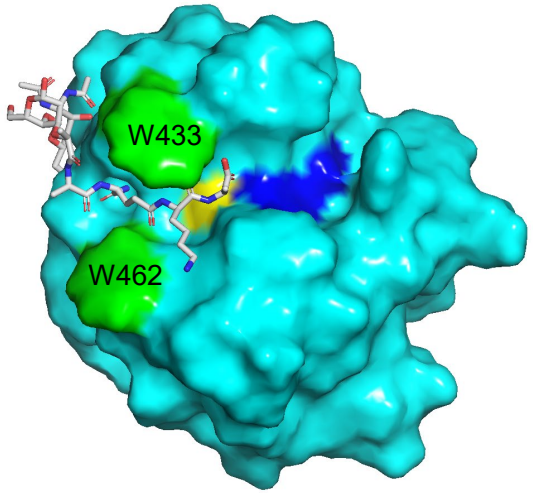
Comparative analysis of "good" vs "bad" microbiota species in worms revealed protective activity of *E. faecium* SagA.

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

Rangan K et al Science 2016

Structure and activity of *E. faecium* SagA and muropeptides

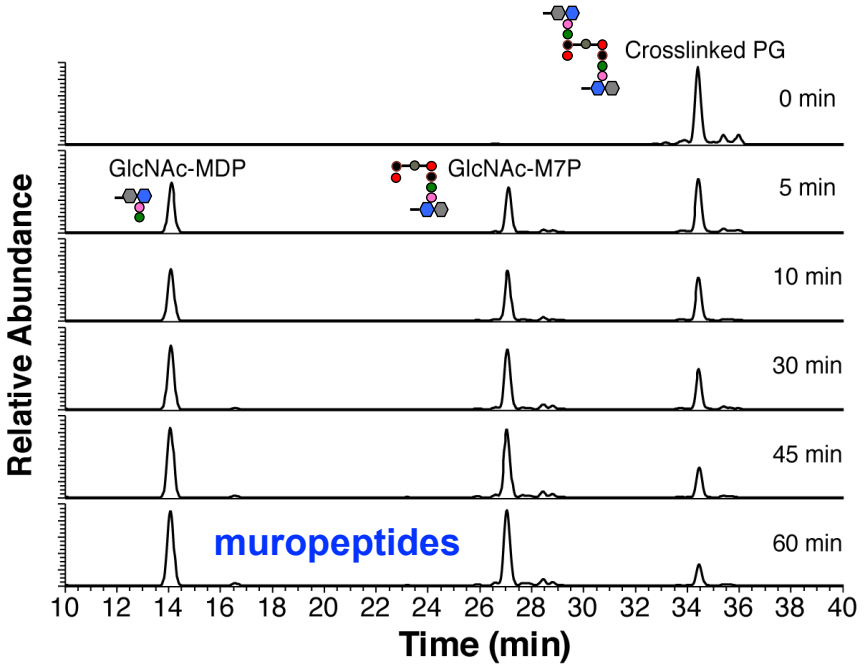
X-ray structure of SagA-NlpC/p60



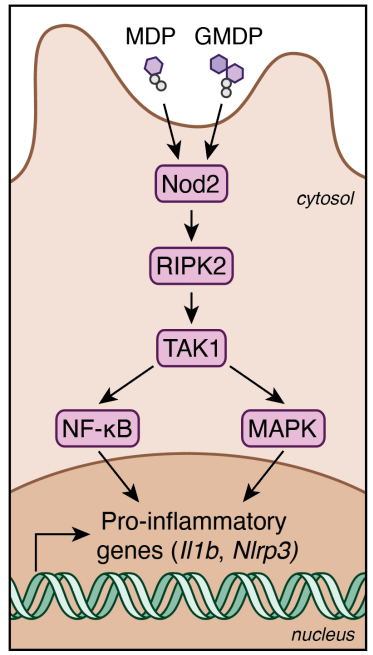
Solved by molecular replacement with *S. aureus* CwlT-NlpC/p60 (35% identity)

CwlT structure: Xu Q et al (Wilson lab @ Scripps) J Mol Biol 2014

Biochemical activity of SagA-NlpC/p60



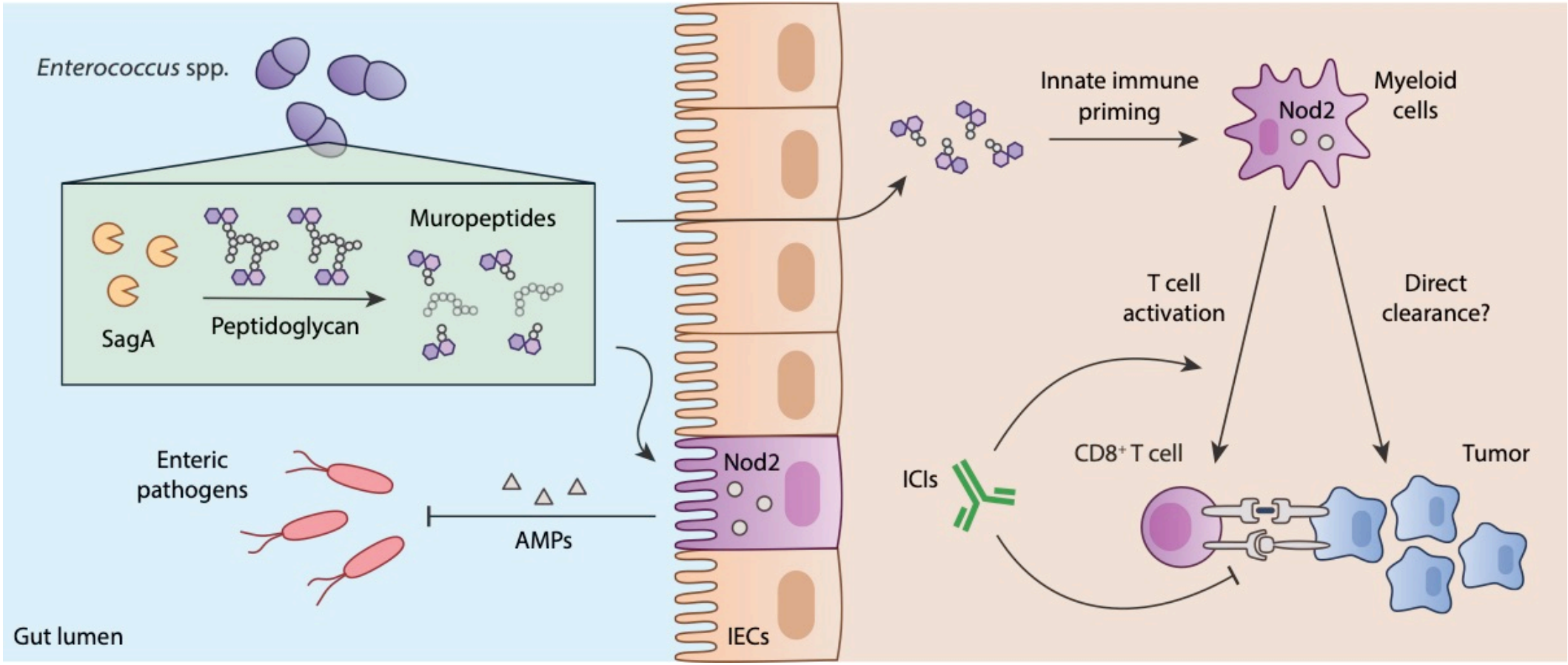
Muropeptides promotes immunity



Kim B et al eLlife 2019
Griffin M et al Science 2021

SagA-NlpC/p60 cleaves x-linked peptidoglycan substrates and generates immune-active muropeptides.

Specific *Enterococcus* species and factors enhance host immunity



Discovery of *E. faecium* SagA activity: Rangan K et al Science 2016
Protection against infection: Pedicord V et al Science Immunology 2016

SagA x-ray structure and biochemical mechanism: Kim B et al eLife 2019
Impact on cancer immunotherapy: Griffin M et al Science 2021

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

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

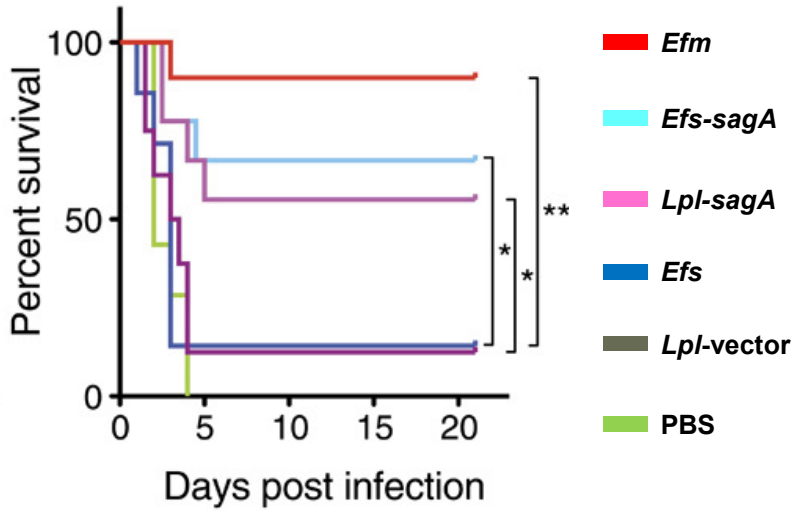
Major cause of antibiotic-induced infection and inflammation.

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

Fecal microbiota transplantation is effective, but problematic.

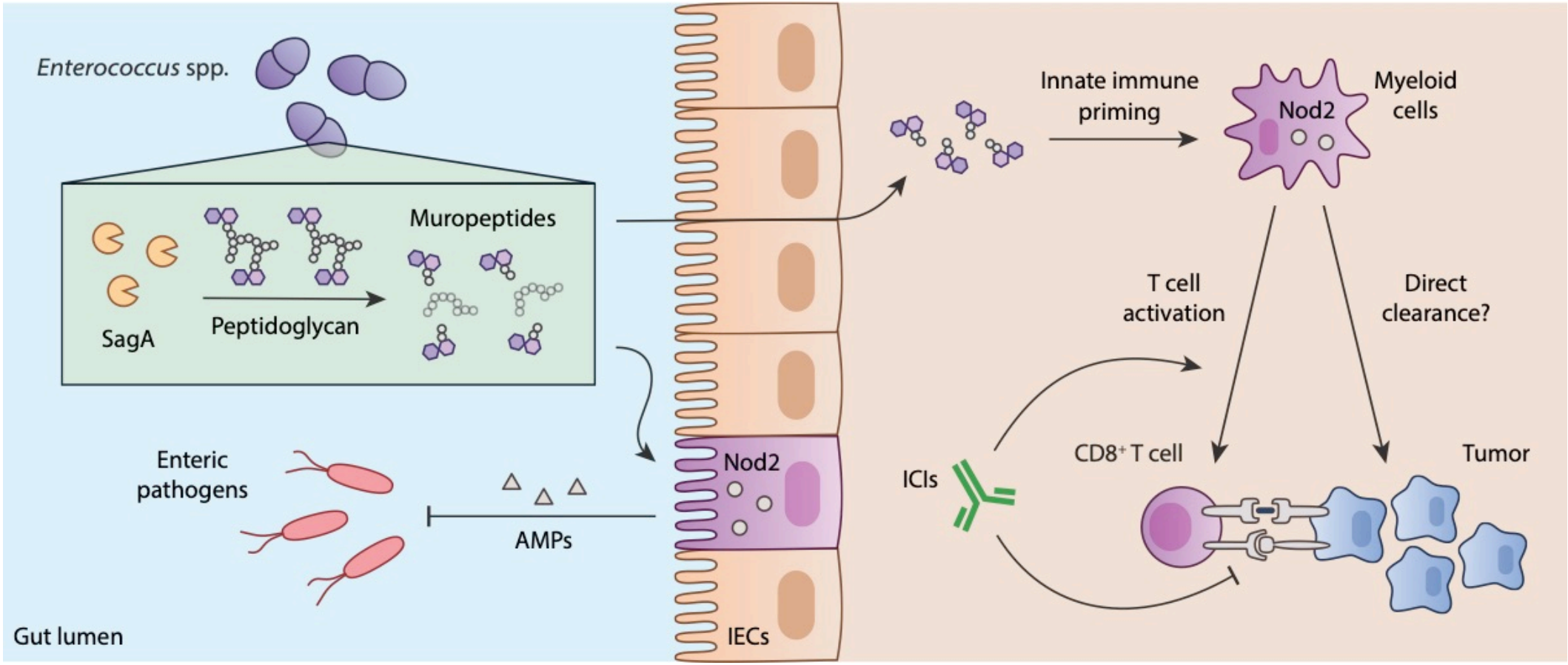
Abt MC, McKenney PT, Pamer EG Nat Rev Microbiol 2016
Kociolek LK, Gerding DN. Nat Rev Gastroenterol Hepatol 2016

SagA-bacteria pre-colonization prevents *C. difficile* pathogenesis in mice



Pedicord V et al Science Immunology 2016

Specific *Enterococcus* species and factors enhance host immunity



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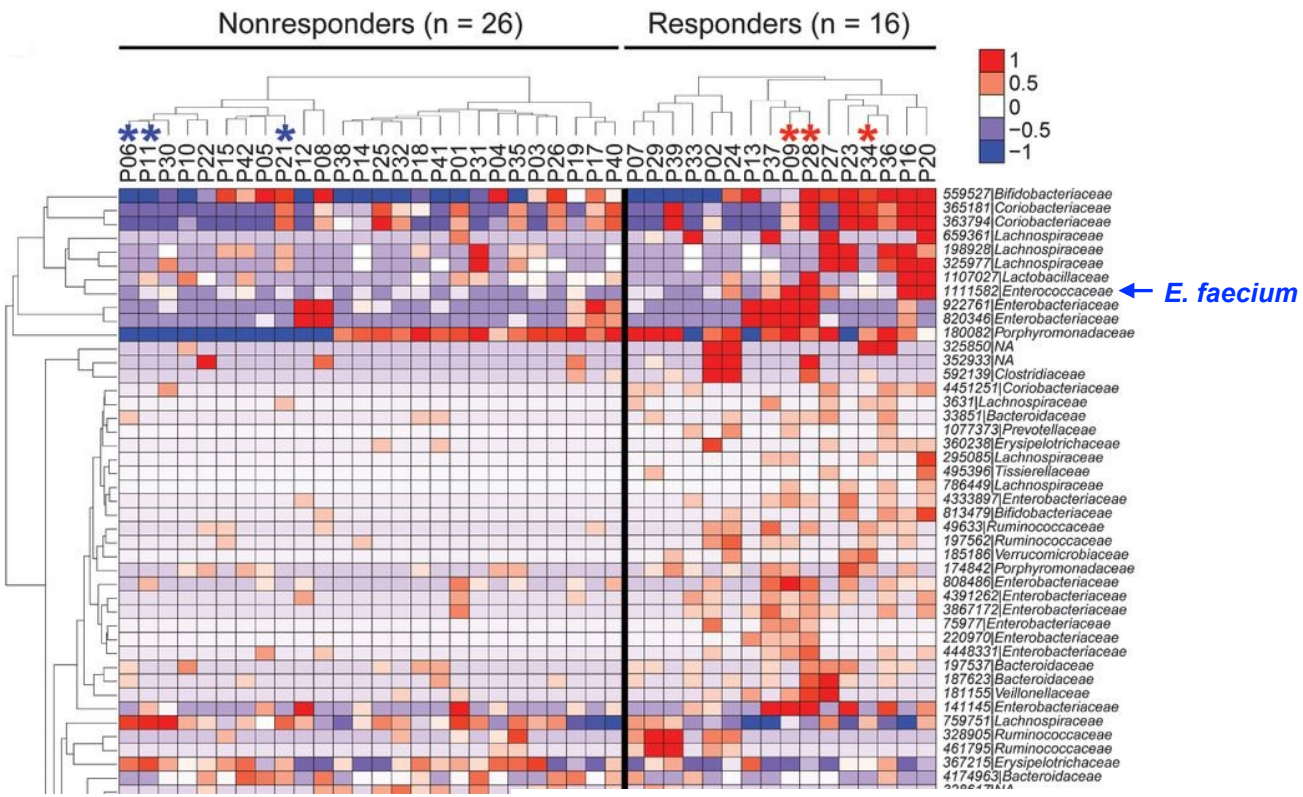
Protection against infection: Pedicord V et al Science Immunology 2016

SagA x-ray structure and biochemical mechanism: Kim B et al eLife 2019
Impact on cancer immunotherapy: Griffin M et al Science 2021

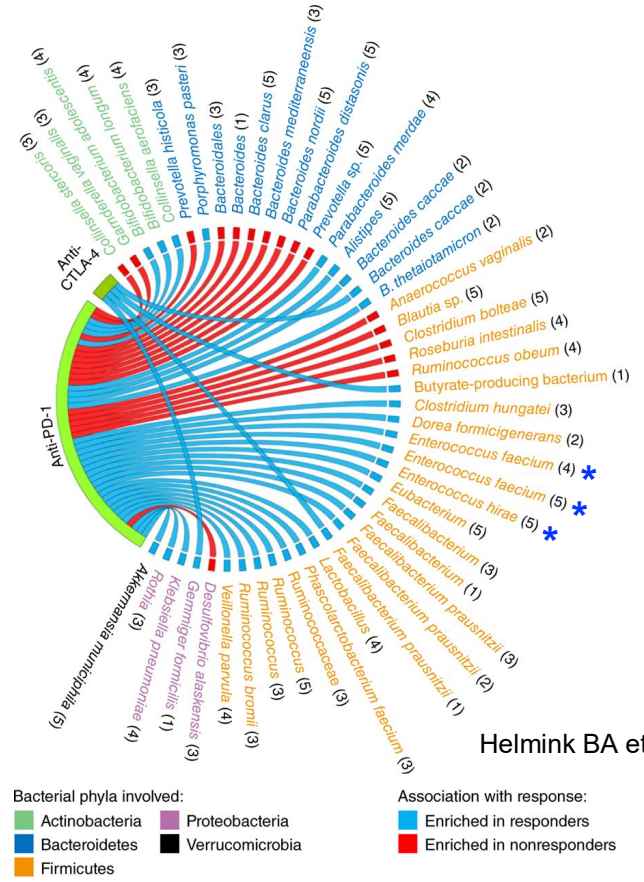
E. faecium was enriched in immunotherapy responsive cancer patients

Different microbiota species <---> cancer immunotherapy efficacy



Matson V et al (Gajewski lab) Science 2018

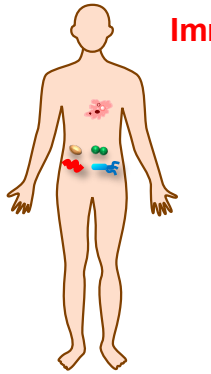
Microbiota - immunotherapy correlation



Helmink BA et al Nat Med. 2019

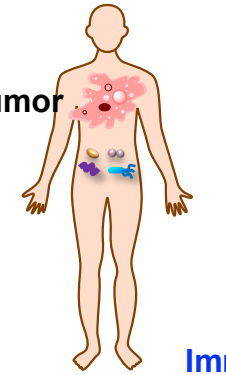
Are *E. faecium* and SagA also sufficient to enhance immunotherapy against cancer?

Reprogramming of non-responsive microbiota for immunotherapy?



Immunotherapy-responsive (R) cancer patient

immunotherapy



tumor

SagA-probiotic or metabolite

or

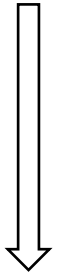
Long-term goal: treatment of non-responsive patients

Immunotherapy non-responsive (NR) cancer patient

Tumor mouse models

NR-microbiota

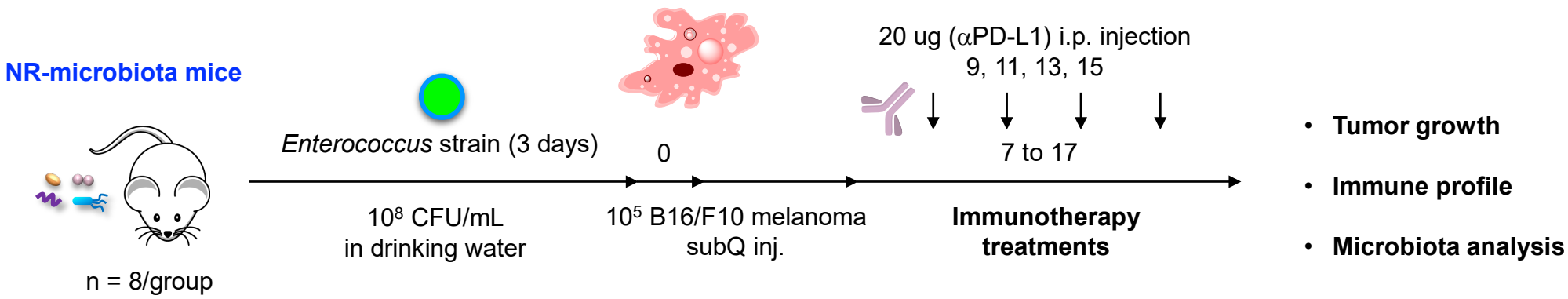
SagA-probiotic or metabolite



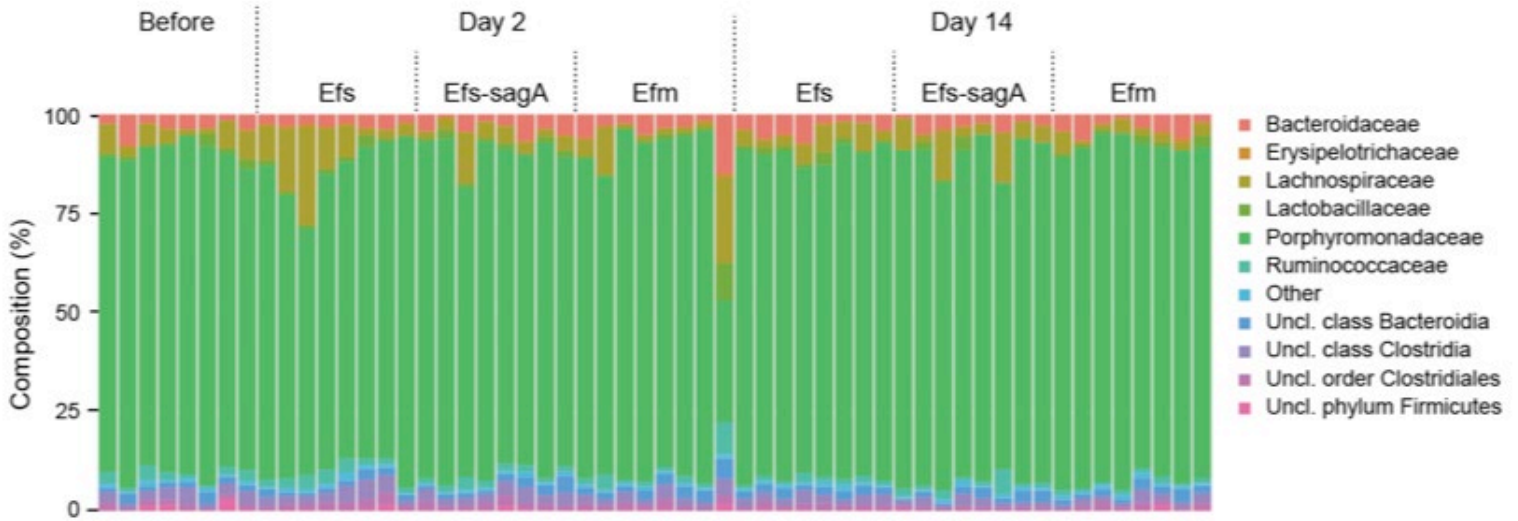
Immunotherapy

Evaluate tumor progress and immune responses

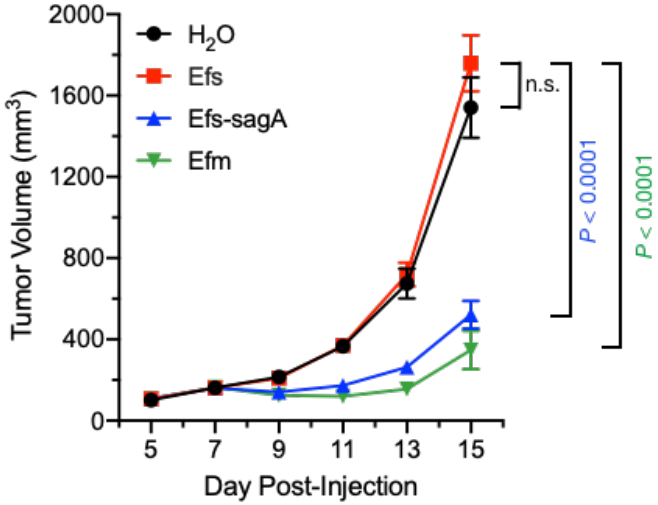
SagA-bacteria can reprogram immunotherapy non-responsive microbiota



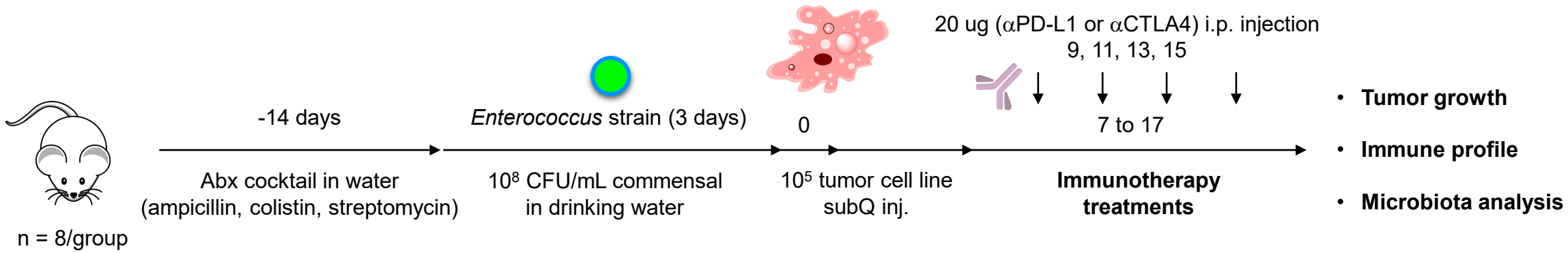
16S rRNA analysis of microbiota composition



Immunotherapy activity



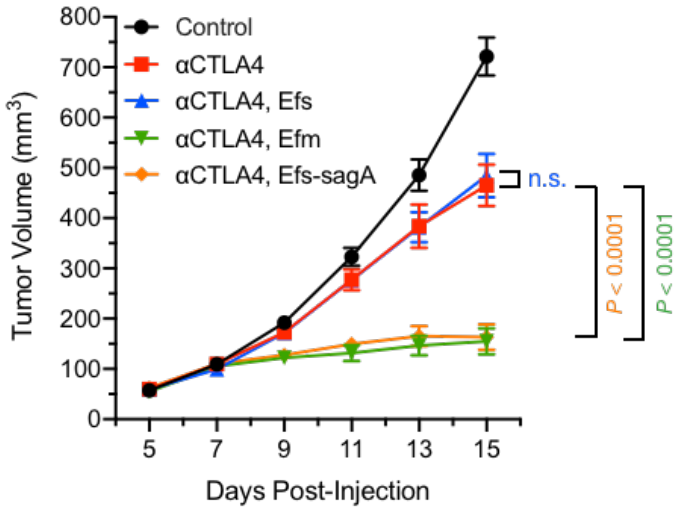
SagA is sufficient to improve efficacy of immune checkpoint inhibitors



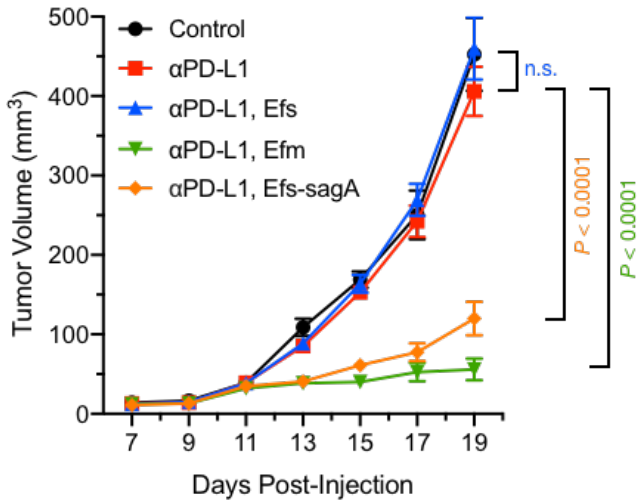
Other mechanistic studies

- **Activates myeloid cells**
- **Increases tumor-specific CD8 T cells**
- **Requires immune receptor NOD2**

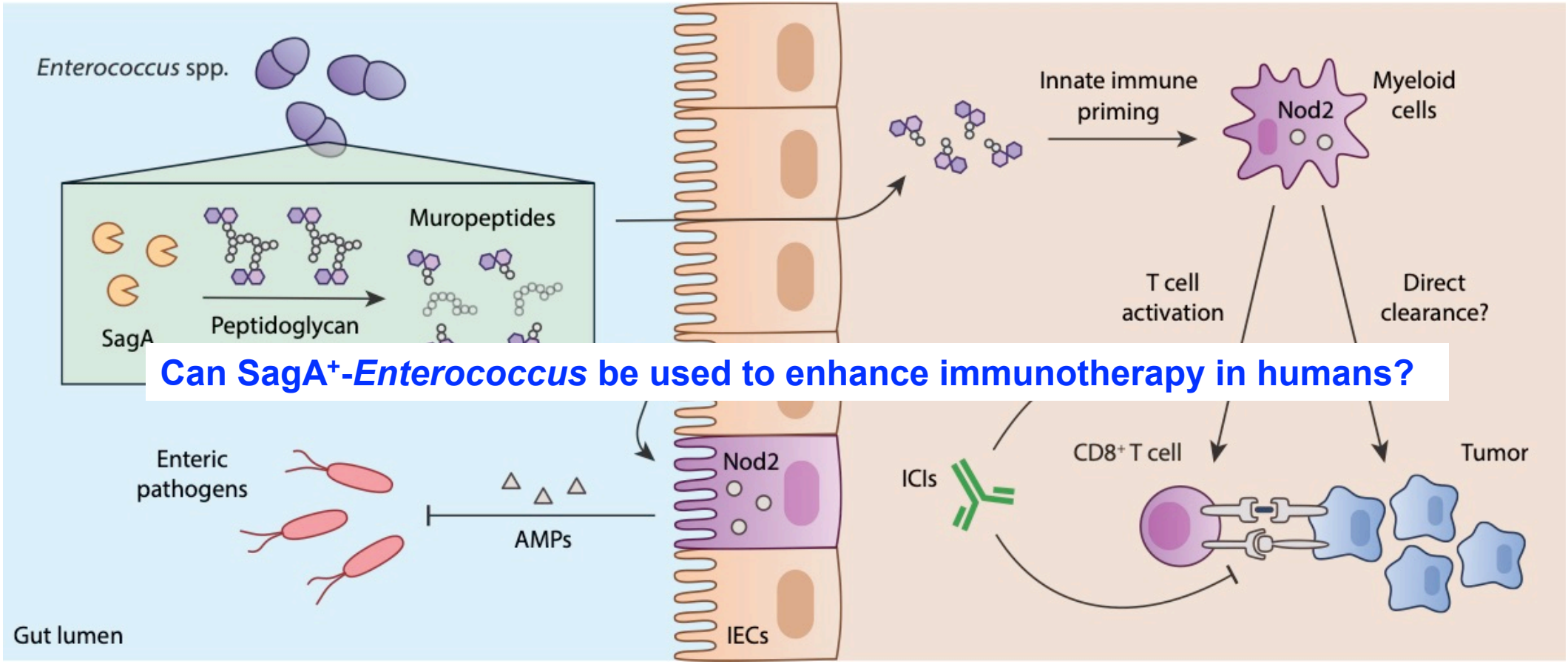
αCTLA4 activity on colon adenocarcinoma



αPD-L1 activity on melanoma



Specific *Enterococcus* species and factors enhance host immunity



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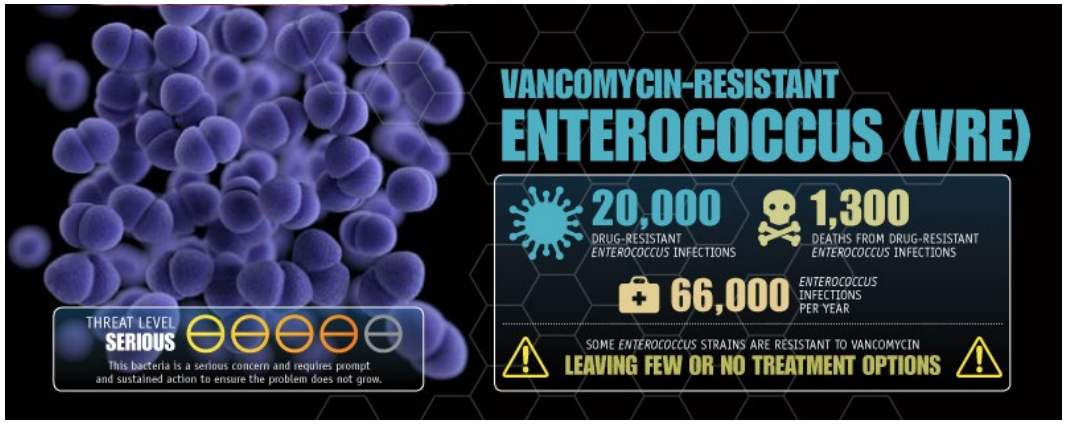
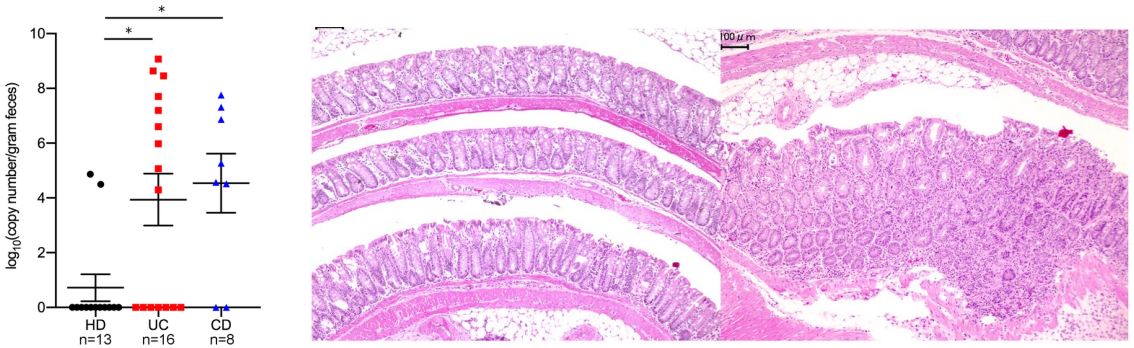
Impact on cancer immunotherapy: Griffin M et al Science 2021

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

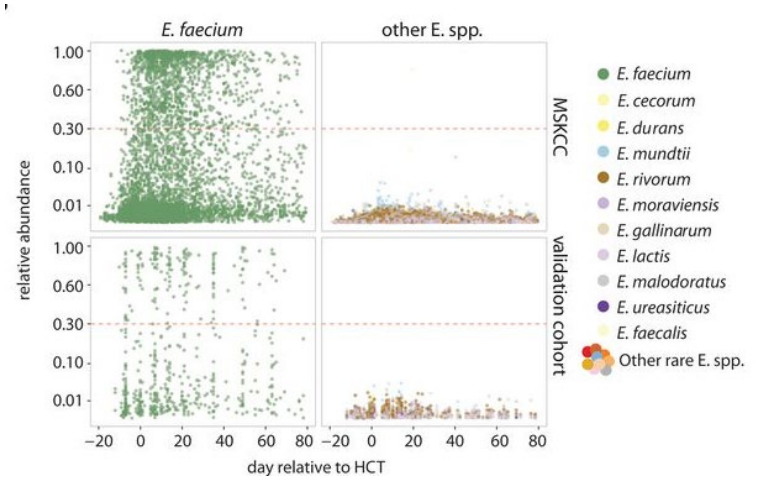


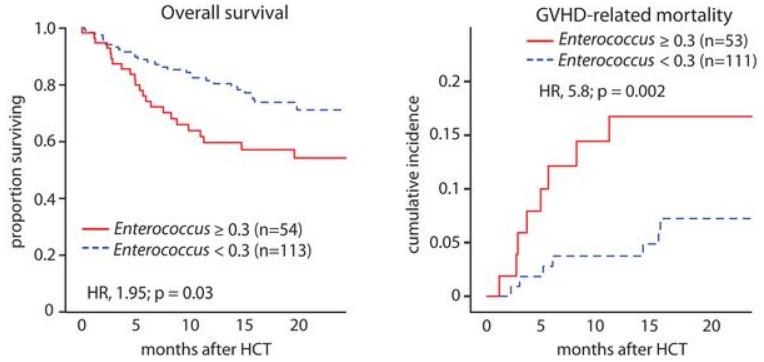
E. faecium promotes inflammatory bowel diseases (IBD)



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

VRE exacerbates graft vs host disease (GVHD) in transplantation

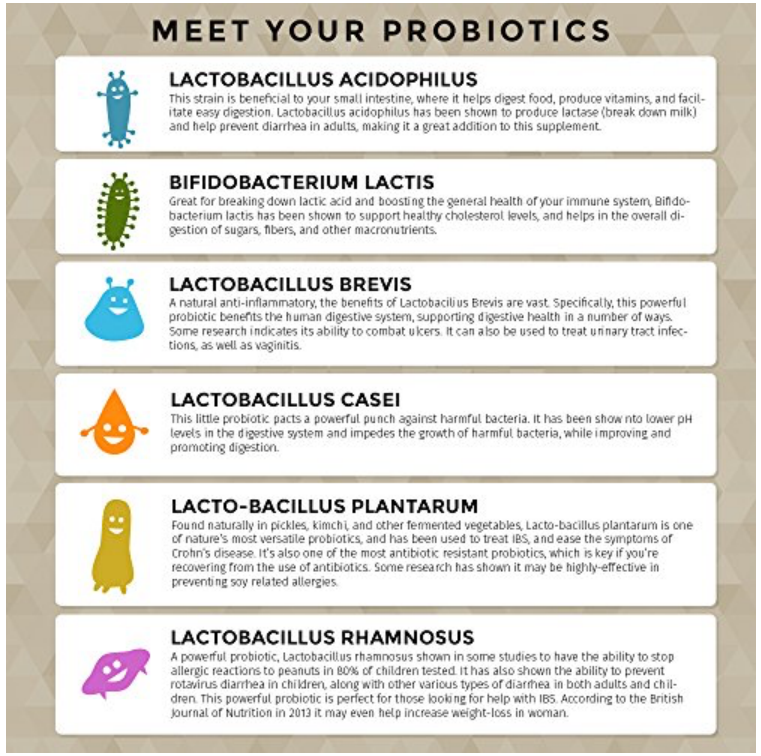




Stein-Thoeringer CK et al (Pamer & van den Brink labs) Science 2019

Probiotics can be genetically engineered for potential therapeutics

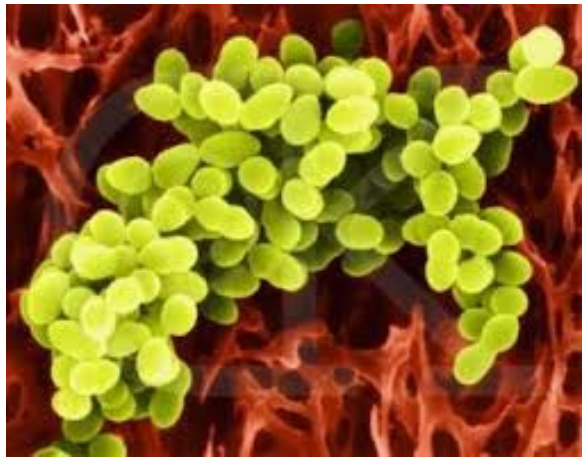
Many probiotics have been explored for human health



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

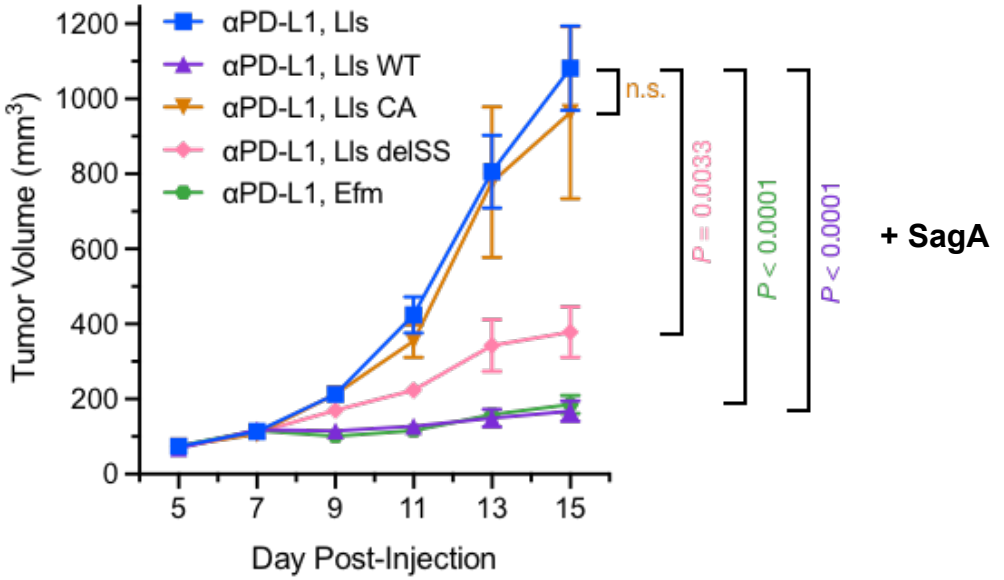
Engineering SagA expression into *L. lactis*

Google Search for "Probiotic Beer"

SagA-engineered L. lactis

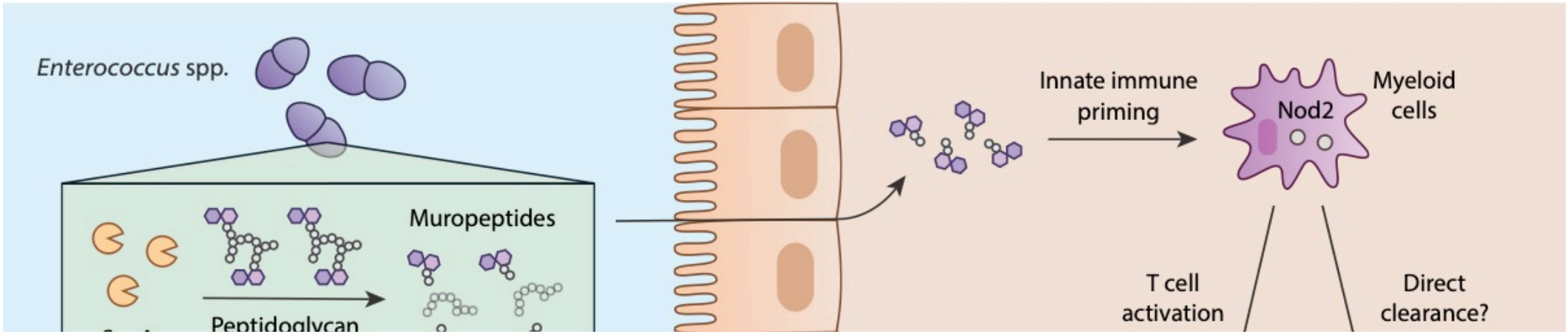
SagA-IPA?

αPD-L1 activity on B16/F10 melanoma

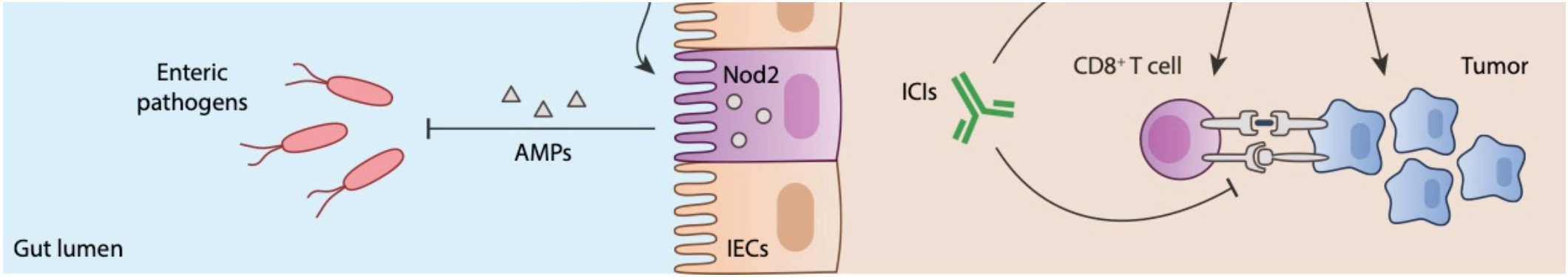


SagA expression confers anti-tumor activity to probiotics and requires hydrolase activity (C443A).

Specific *Enterococcus* species and factors enhance host immunity



Are there other bacteria that may function like SagA⁺-*Enterococcus* in human microbiomes?



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SagA x-ray structure and biochemical mechanism: Kim B et al eLife 2019
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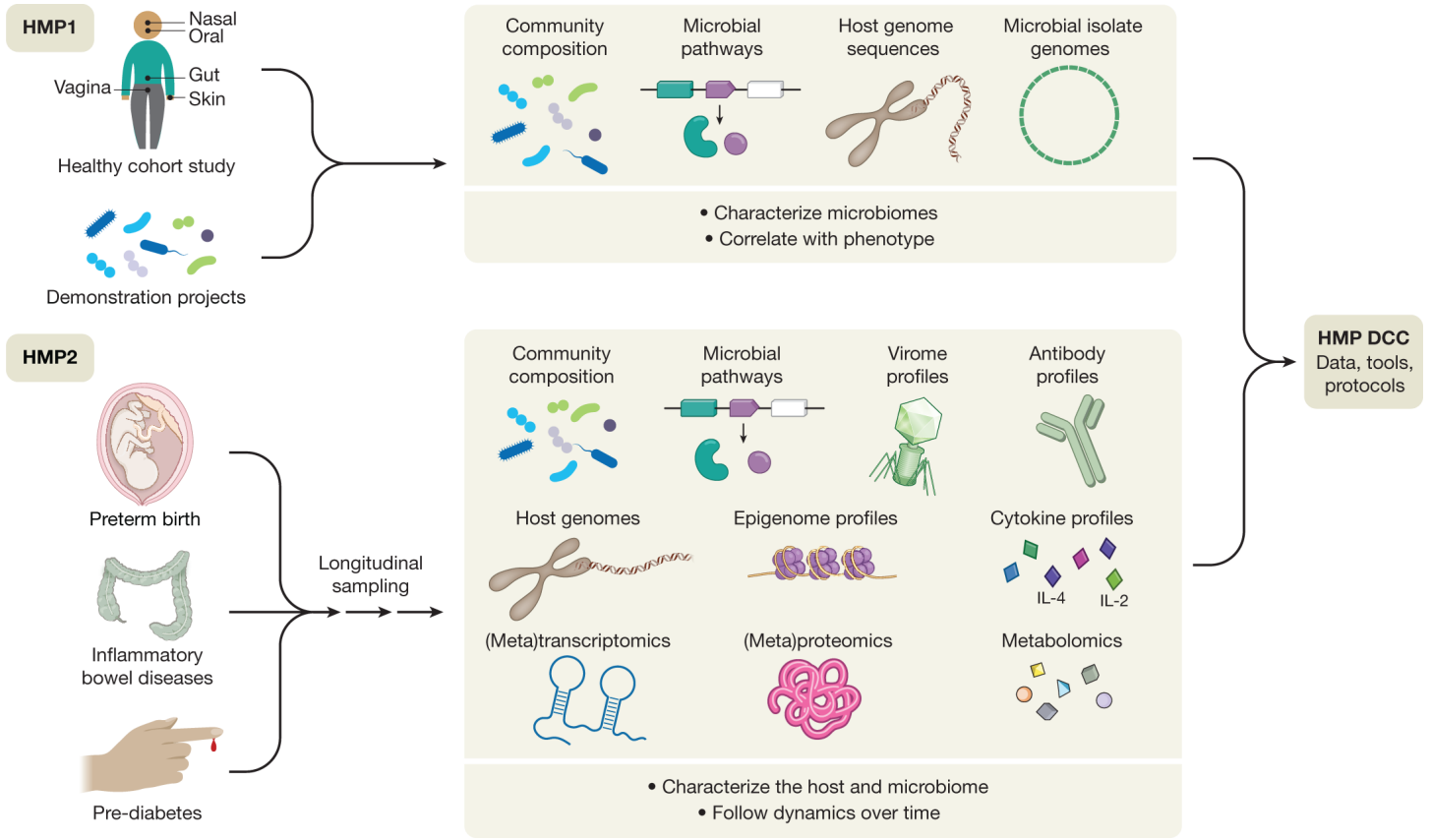
Human microbiome project (HMP) has profiled many individuals and diseases



HMP has analyzed >30,000 samples from different individuals, tissues and diseases (IBD, pre-T2D and others).

The Integrative Human Microbiome Project, Nature 2019

Human microbiome contains other SagA-like peptidoglycan hydrolases

Secreted antigen A (SagA) – NlpC/p60 hydrolase

Human Microbiome Genome Assemblies from HMP (2,216)

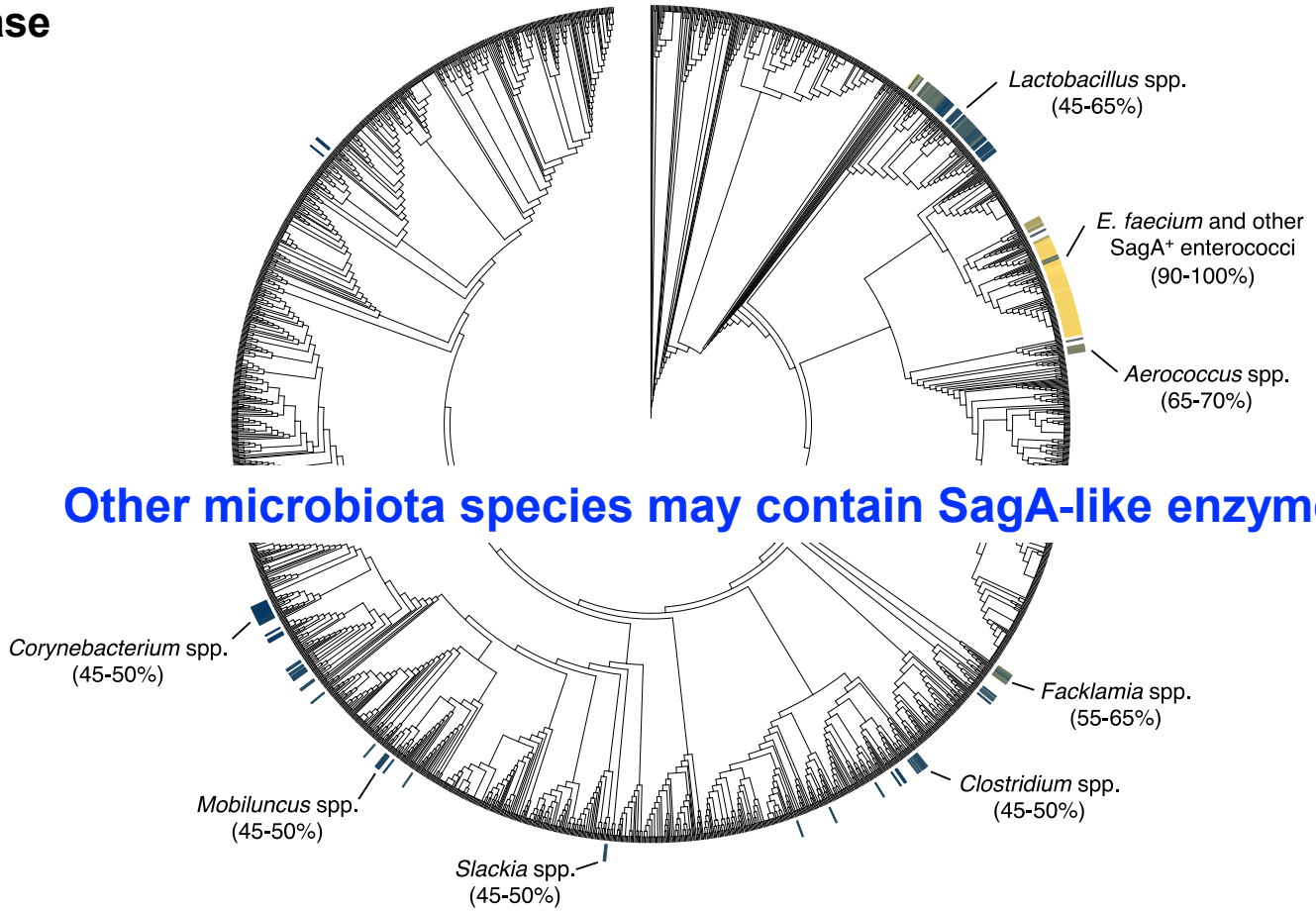
↓

Queried for orthologs of *E. faecium* COM15 SagA NlpC/p60

↓

Mapped onto 16S rRNA Phylogenetic Tree

Sequence Identity (%)



Other microbiota species may contain SagA-like enzymes!

Enterococcus peptidoglycan remodeling promotes cancer immunotherapy

RESEARCH

REPORT

MICROBIOME

Enterococcus peptidoglycan remodeling promotes checkpoint inhibitor cancer immunotherapy

Matthew E. Griffin[1,2], Juliel Espinosa[1], Jessica L. Becker[1], Ji-Dung Luo[3], Thomas S. Carroll[3], Jyoti K. Jha[4], Gary R. Fanger[4], Howard C. Hang[1,2]*

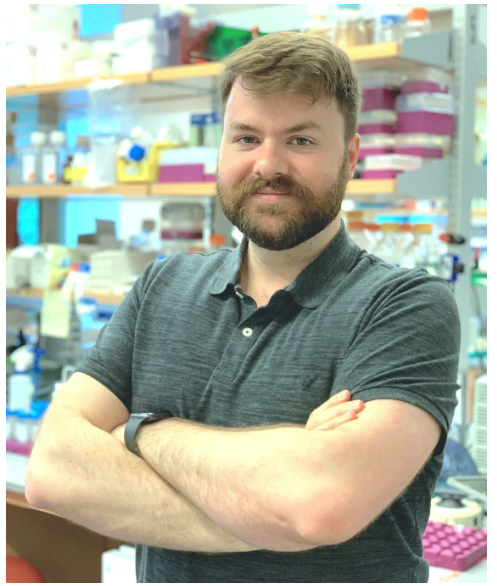
Science 2021 in press

Available online August 26th, 2021

Funded by

NIH - National Cancer Institute

Melanoma Research Foundation



Matthew Griffin – Hope Funds for Cancer Postdoctoral Fellow
@ Scripps Research

The SagA continues: implications for infection, inflammation and immunotherapy

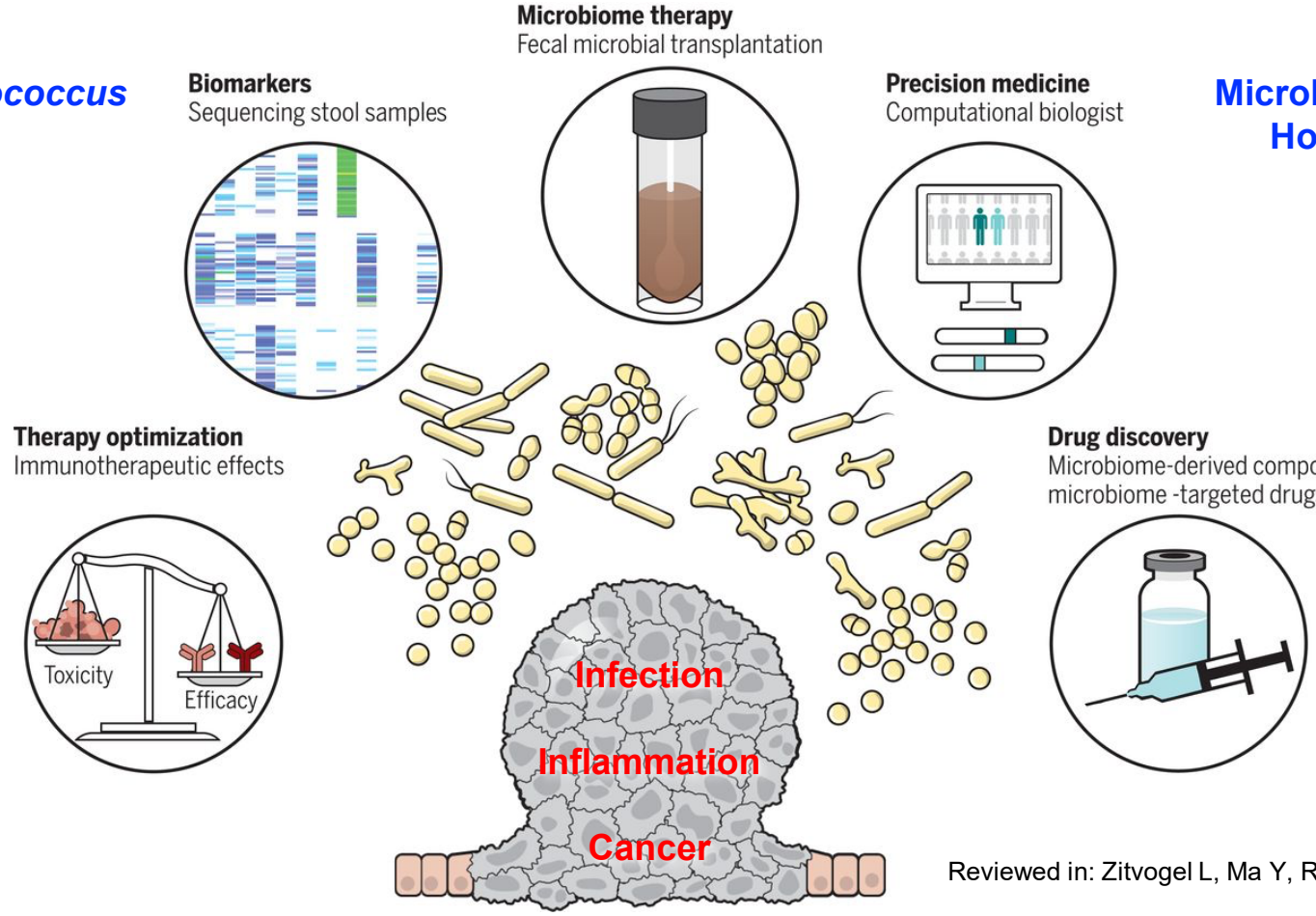
SagA-based probiotics (Rise Therapeutics)

Screen for SagA-*Enterococcus*

SagA-*Enterococcus*

+

Other microbiota species?



Microbiota: SagA-*Enterococcus*
Host genes: NOD2 alleles

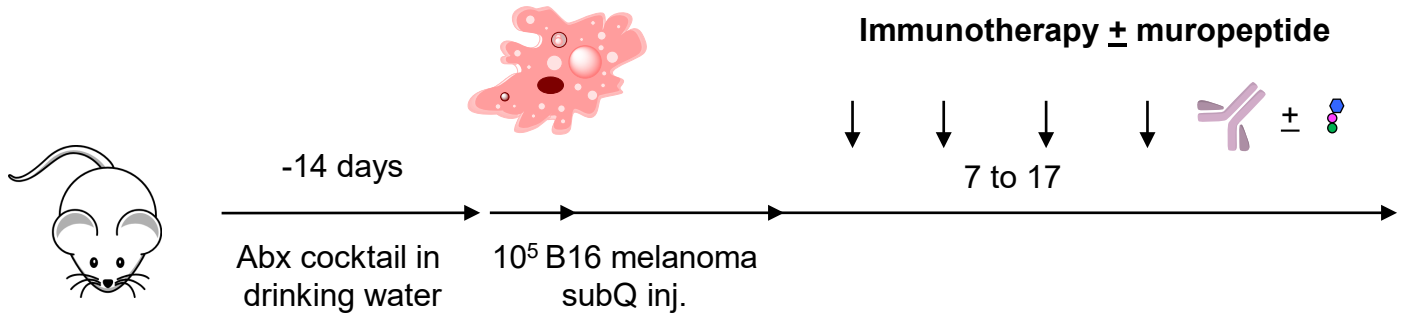
New therapeutics?
Muropeptides?

Repurpose existing drugs?

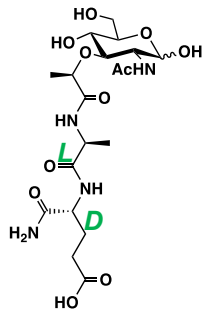
Novel NOD2 agonists @ Scripps Research?

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

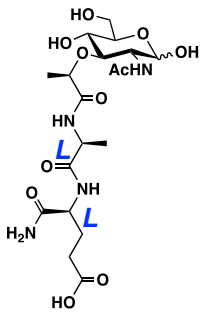
MDP co-injection restores immune checkpoint inhibitor efficacy *in vivo*



MDP-*LD*
NOD2-active

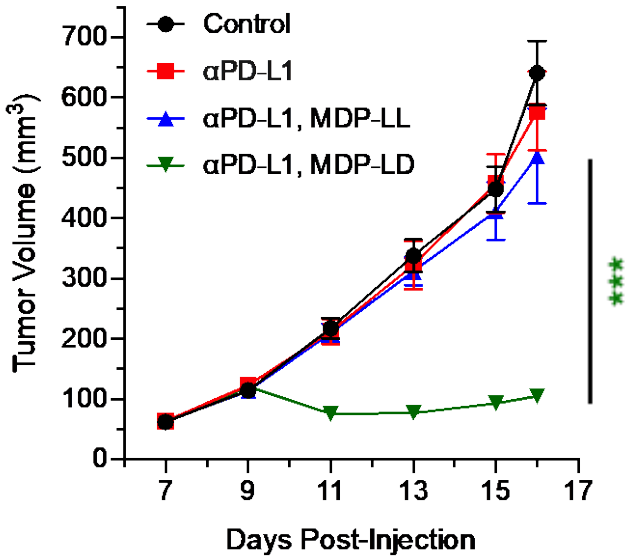


MDP-*LL*
NOD2-inactive

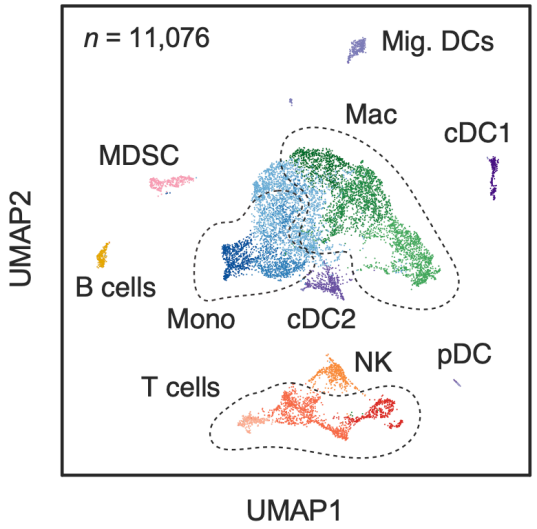


In cells: Girardin SE et al J Biol Chem 2003
In in vitro: Grimes CL et al J Am Chem Soc 2012

MDP-*LD* inhibits tumor growth



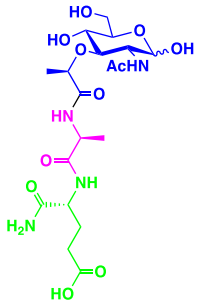
MDP-*LD* activates myeloid cells



Griffin M et al Science 2021 in press

Discovery and therapeutic development of muramyl-dipeptide (MDP)

Freund's adjuvant

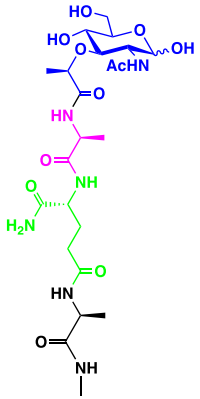


muramyl-dipeptide (MDP)
Adam A, Ciorbaru R, Ellouz F, Petit JF, Lederer E.
Biochem Biophys Res Commun 1974

NOD1 sensing of DAP
Girardin SE et al Science 2003
Chamaillard M et al Nat Immunol 2003

NOD2 sensing of MDP
Girardin SE et al J Biol Chem 2003
Inohara N et al J Biol Chem 2003

Mifamurtide (MTP-PE)



PE-phospholipid

Better delivery into cells

Better half-life in mouse models

Active against different cancer types

Prevent tumor growth, but not effective for treatment as single agent

Fidler IJ et al J Immunol 1987

Mepact (Takeda)

FDA orphan drug in 2001

Approved in Europe 2009 to prevent recurrence of osteosarcoma

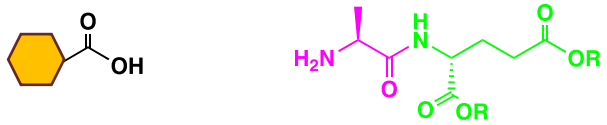
Meyers PA, Chou AJ. Adv Exp Med Biol 2014

Repurpose Mepact?

Next-generation NOD2 agonists?

MDP-based drugs / NOD2 agonists are effective adjuvants, safe in humans and should be employed for immunotherapy.

In silico screen for novel MDP-analogs / NOD2 agonists



Carboxylic acids
(catalogue, ~ 30 k)

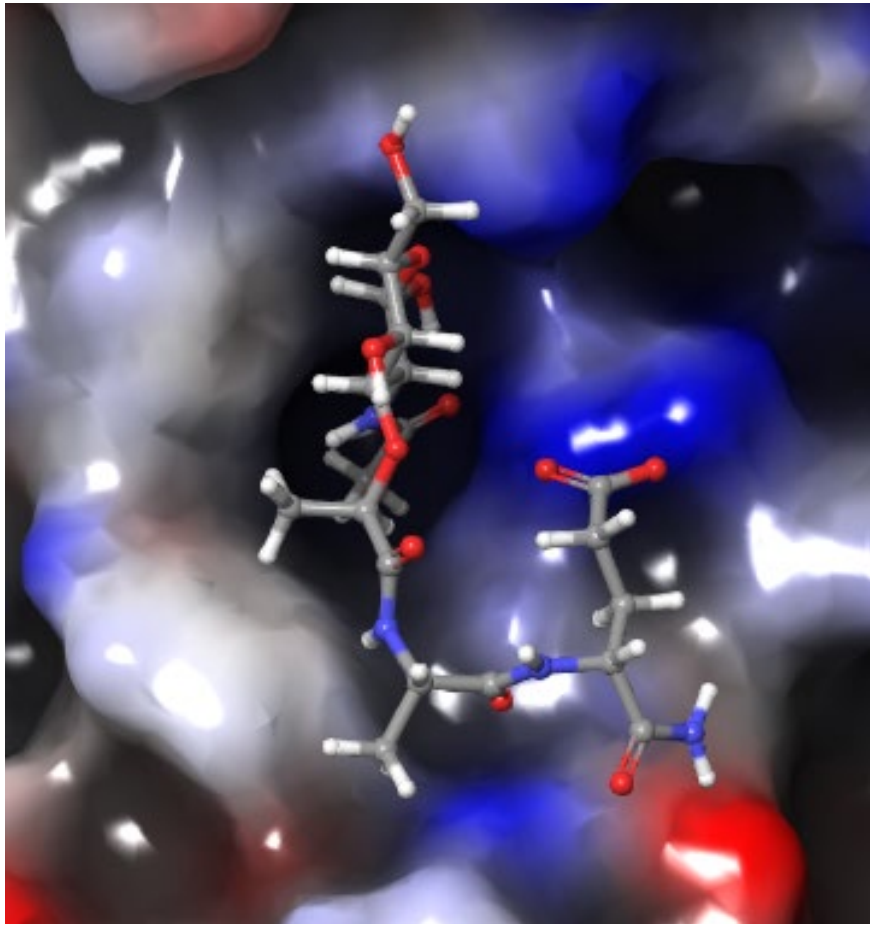
Dipeptide

RDKit

Desmuramyl-dipeptides (dMDPs)

Schrödinger

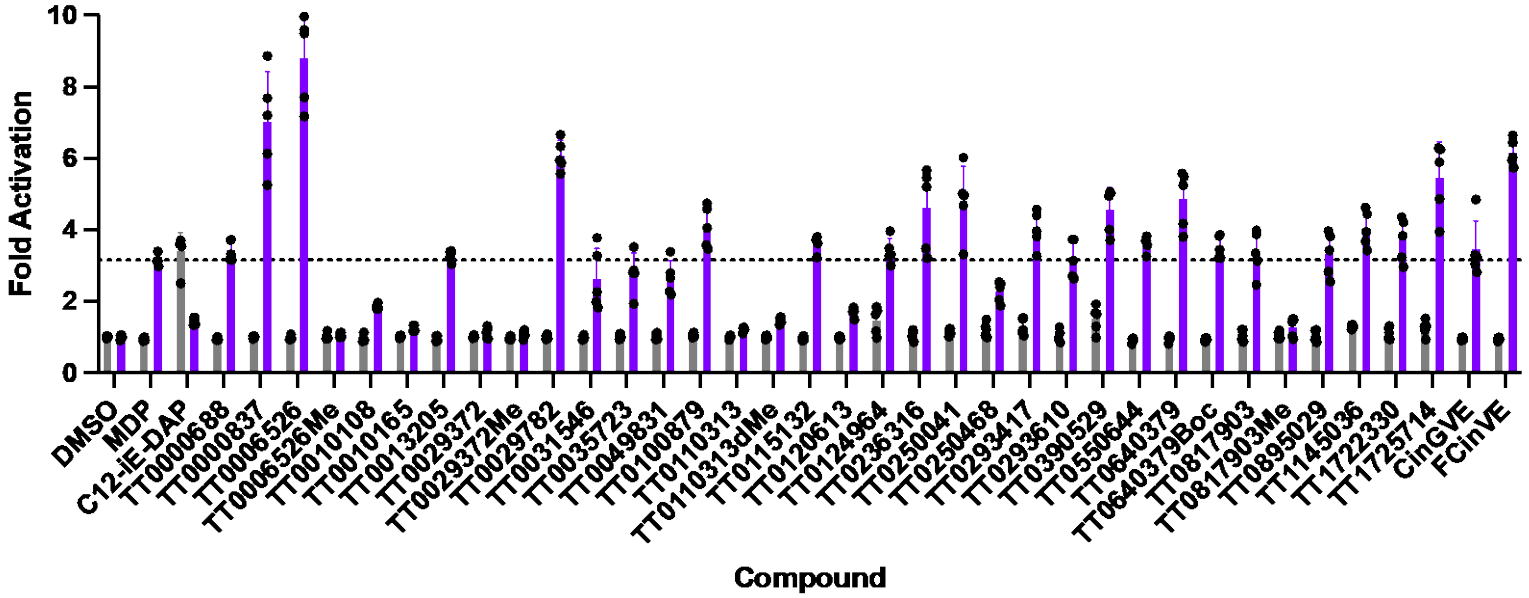
Property filter	Total: ~ 30,000 compounds
Docking	Hits: 1683 compounds
Clustering	Top 10% unique: ~100 compounds
Synthesis	Representatives: ~30 compounds
Biological testing	Actives: ~15 compounds



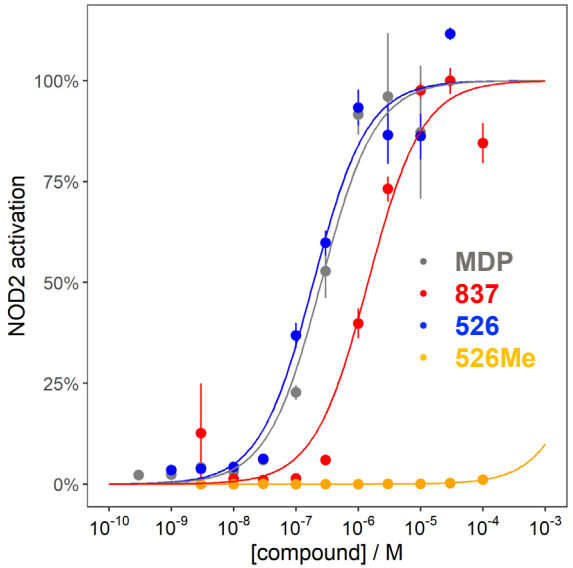
Based on NOD2 X-ray structure and mutagenesis: Maekawa et al Nat Commun 2016

Validation and optimization of novel NOD2 agonists

Selective activation of NOD2 versus NOD1



Dose-response activity



Several novel dMDP / NOD2 agonists have comparable or better activity than original microbiota metabolite - MDP.

Calibr - Scripps Research provides new opportunities for drug discovery

Article

Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing

https://doi.org/10.1038/s41586-020-2577-1

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Accepted: 17 July 2020

Published online: 24 July 2020

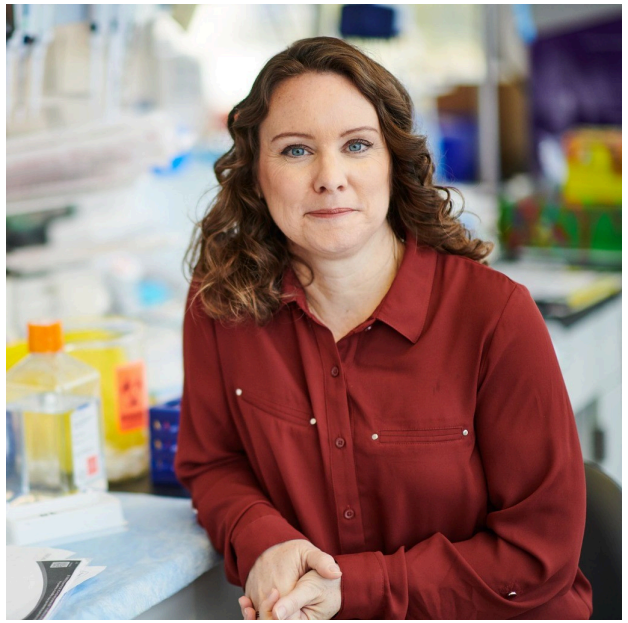
Check for updates

Laura Riva[1,23], Shuofeng Yuan[2,3,4,23], Xin Yin[1], Laura Martin-Sancho[1], Naoko Matsunaga[1], Lars Pache[1], Sebastian Burgstaller-Muehlbacher[5], Paul D. De Jesus[1], Peter Teriete[1], Mitchell V. Hull[6], Max W. Chang[7], Jasper Fuk-Woo Chan[2,3,4], Jianli Cao[2,3,4], Vincent Kwok-Man Poon[2,3,4], Kristina M. Herbert[1], Kuoyuan Cheng[8,9], Tu-Trinh H. Nguyen[6], Andrey Rubanov[1], Yuan Pu[1], Courtney Nguyen[1], Angela Choi[10,11,12], Raveen Rathnasinghe[10,11,12], Michael Schotsaert[10,11], Lisa Miorin[10,11], Marion Dejosez[13], Thomas P. Zwaka[13], Ko-Yung Sit[14], Luis Martinez-Sobrido[15], Wen-Chun Liu[10,11], Kris M. White[10,11], Mackenzie E. Chapman[16], Emma K. Lendy[17], Richard J. Glynne[18], Randy Albrecht[10,11], Eytan Ruppin[6], Andrew D. Mesecar[16,17], Jeffrey R. Johnson[10], Christopher Benner[7], Ren Sun[19], Peter G. Schultz[6], Andrew I. Su[20], Adolfo García-Sastre[10,11,21,22], Arnab K. Chatterjee[6]✉, Kwok-Yung Yuen[2,3,4]✉ & Sumit K. Chanda[1]✉

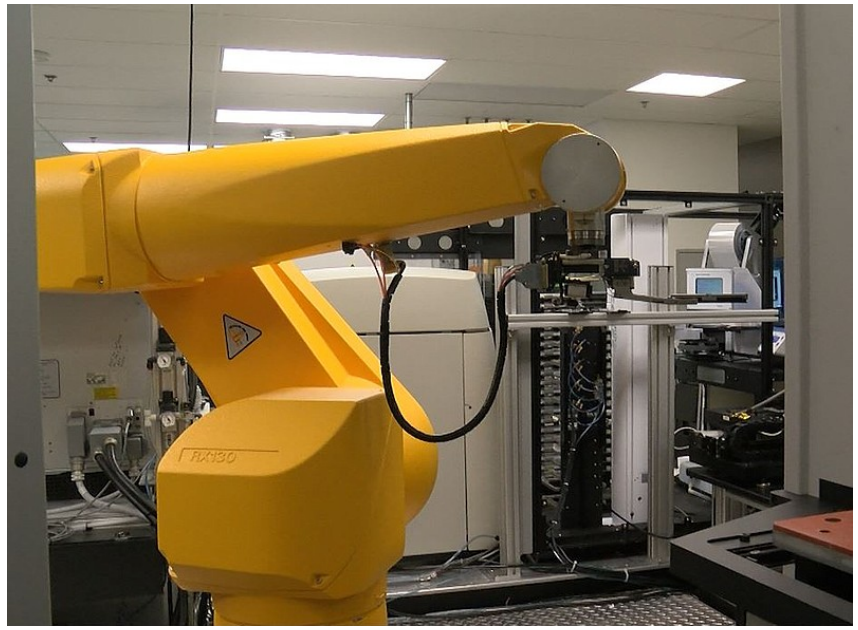
Repurposing of existing drugs for COVID-19:
Riva L et al (Calibr) Nature 2020
Bakowski MA et al (Calibr) Nat Commun 2021

Robotic technology for high-throughput screening facilitates the discovery and development of new therapeutics.

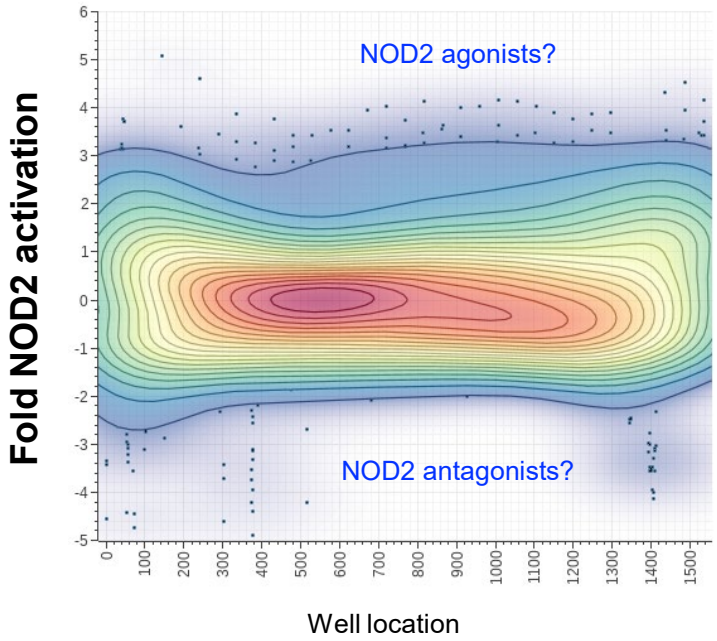
Exploration of novel NOD2 agonists with Calibr - Scripps Research



Kristen Johnson, Group Leader
@ Calibr – Scripps Research



Robotic HT screen of > 130,000 compounds

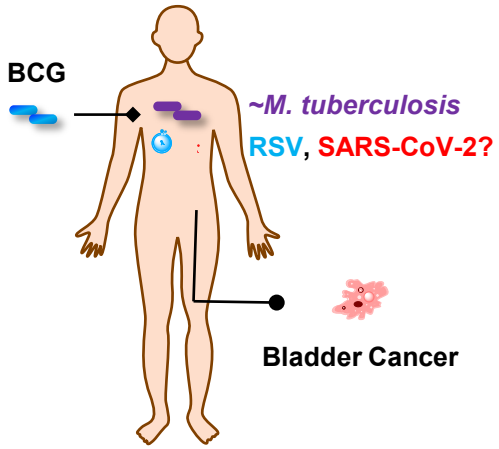


"Hits" from NOD2 activity screening

HTS at Calibr has revealed synergistic activity of existing drugs and other potential NOD2 agonists for immunotherapy.

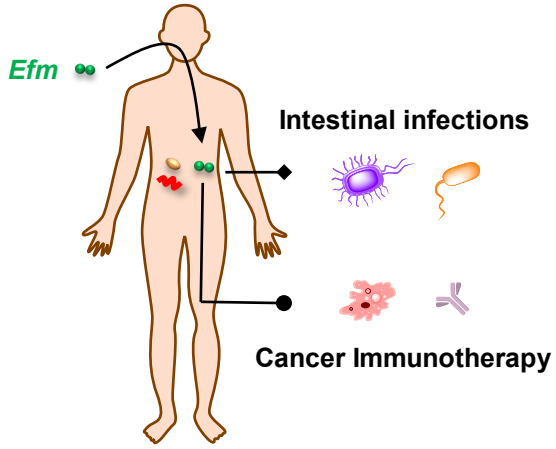
Summary and Future Directions on Microbiota Studies @ Scripps Research

Bacillus Calmette Guérin (BCG) vaccine - NOD2 activation



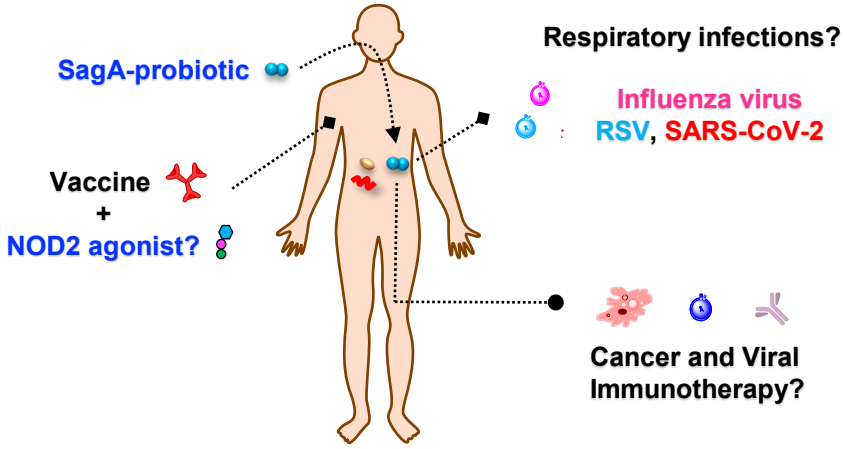
BCG vaccine turns 100: Singh AK, Netea MG, Bishai WR JCI 2021
COVID-19 clinical trails: Gong W et al Expert Rev Vaccines 2021
Bladder cancer therapy: Steinberg GD et al Nat Rev Urol 2021
NOD2 activation: Kleinnijenhuis J et al (Netea lab) PNAS 2012

SagA-*Enterococcus* - NOD2 activation



Rangan K et al Science 2016
Pedicord V et al Science Immunology 2016
Griffin M et al Science 2021

SagA-probiotics or novel NOD2 agonists?



New opportunities for therapeutic development at Scripps Research

Mechanistic analysis of specific microbiota mechanisms can inspire new therapeutic approaches.

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Thank you all for joining us!

Take Home Messages

- **Microbiota are important for host physiology, disease and response to therapy.**
- **New innovative approaches are need to dissect microbiota functions.**
- **Understanding fundamental microbiota mechanisms can reveal unique opportunities for new diagnostics and therapeutics.**

Photo Credit: Ridvan Arda