# Integrative Data Analysis for the Discovery of Novel Drug Targets for Infectious and Immune-related Diseases

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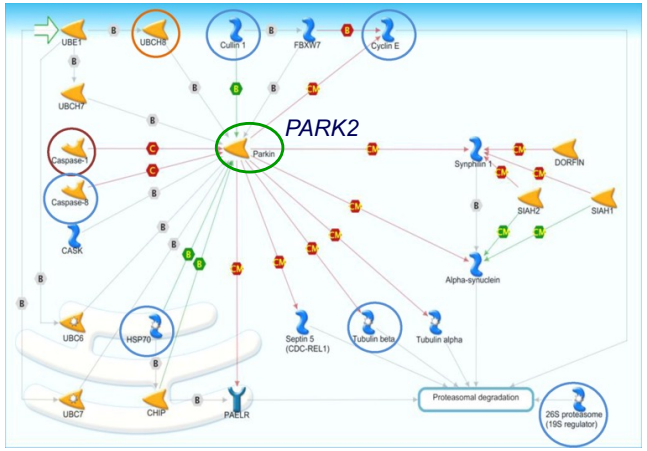
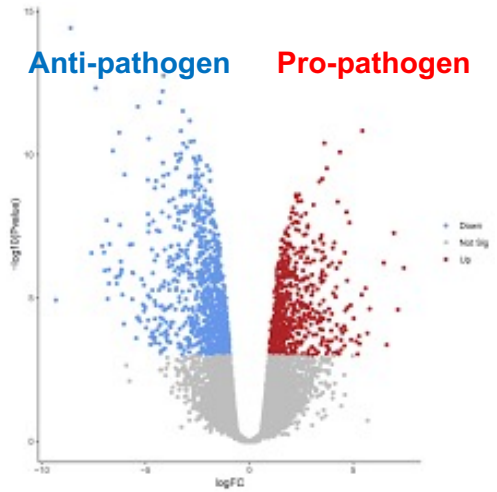
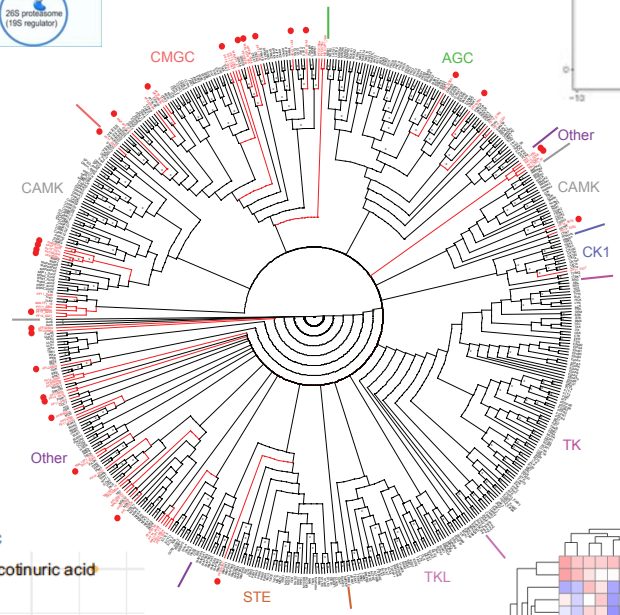
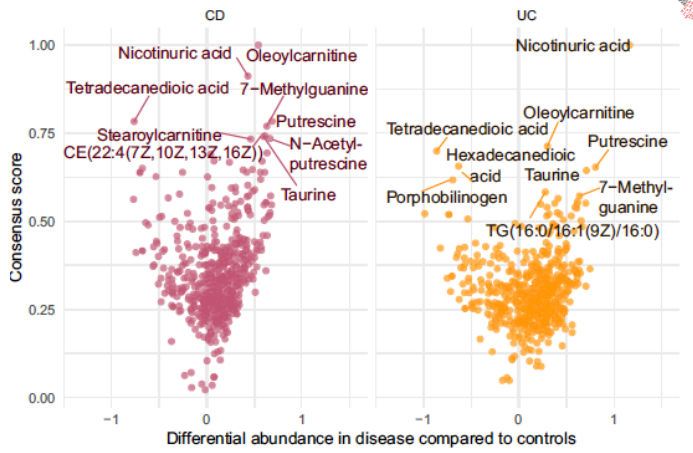
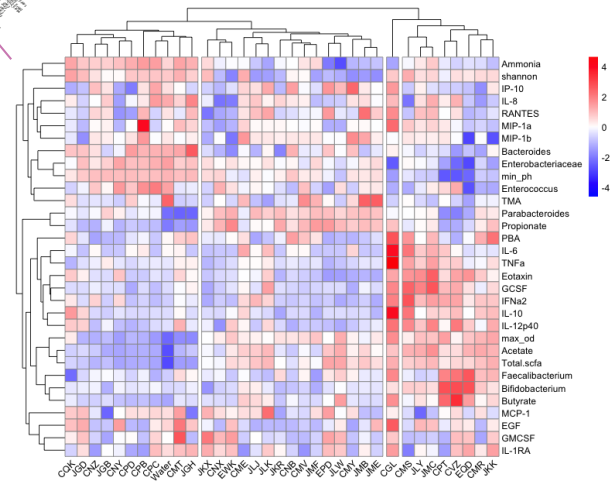
Former affiliations: GSK , Kaleido Biosciences, Novasenta, Dalhousie U., Simon Fraser U., McGill U.



# Outline

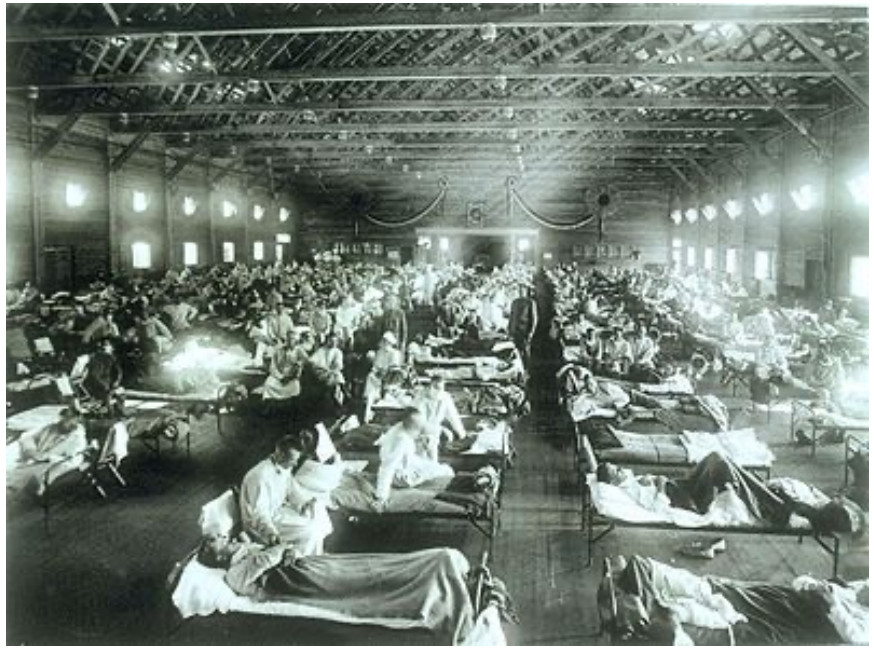
1. Targeting human host factors for infectious and neural degenerative diseases

2. The lung microbiome in respiratory diseases

3. Multi-omics data analysis of human protein-metabolite interactions

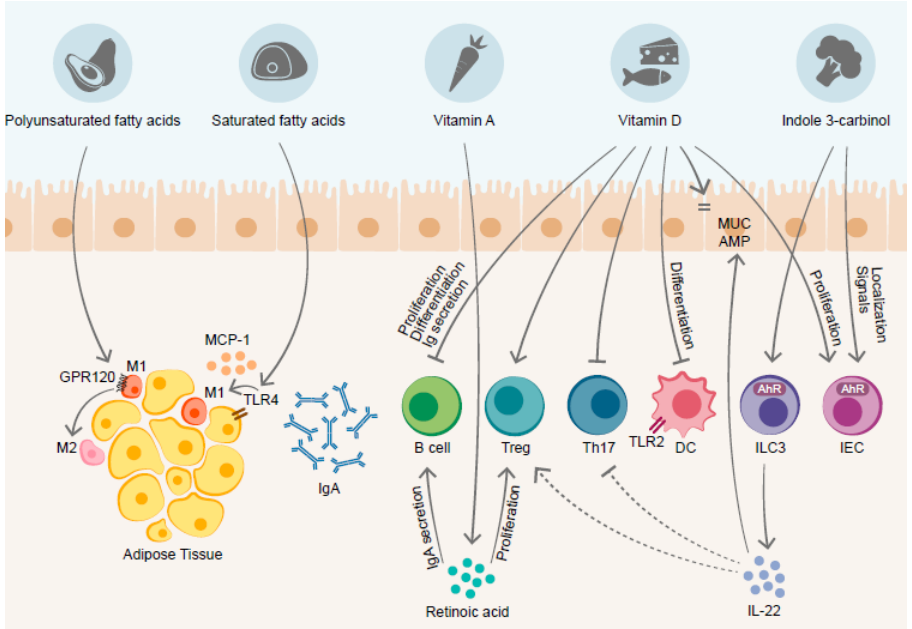
# Host-microbe Interactions: The Immune System Balancing Act

- Microbiome and pathogens interact with the host in different ways.
- Complex immune pathways have evolved to orchestrate an effective defense against a wide range of ***pathogens*** while still promoting colonization of ***beneficial microbes*** for dietary energy and immune homeostasis.



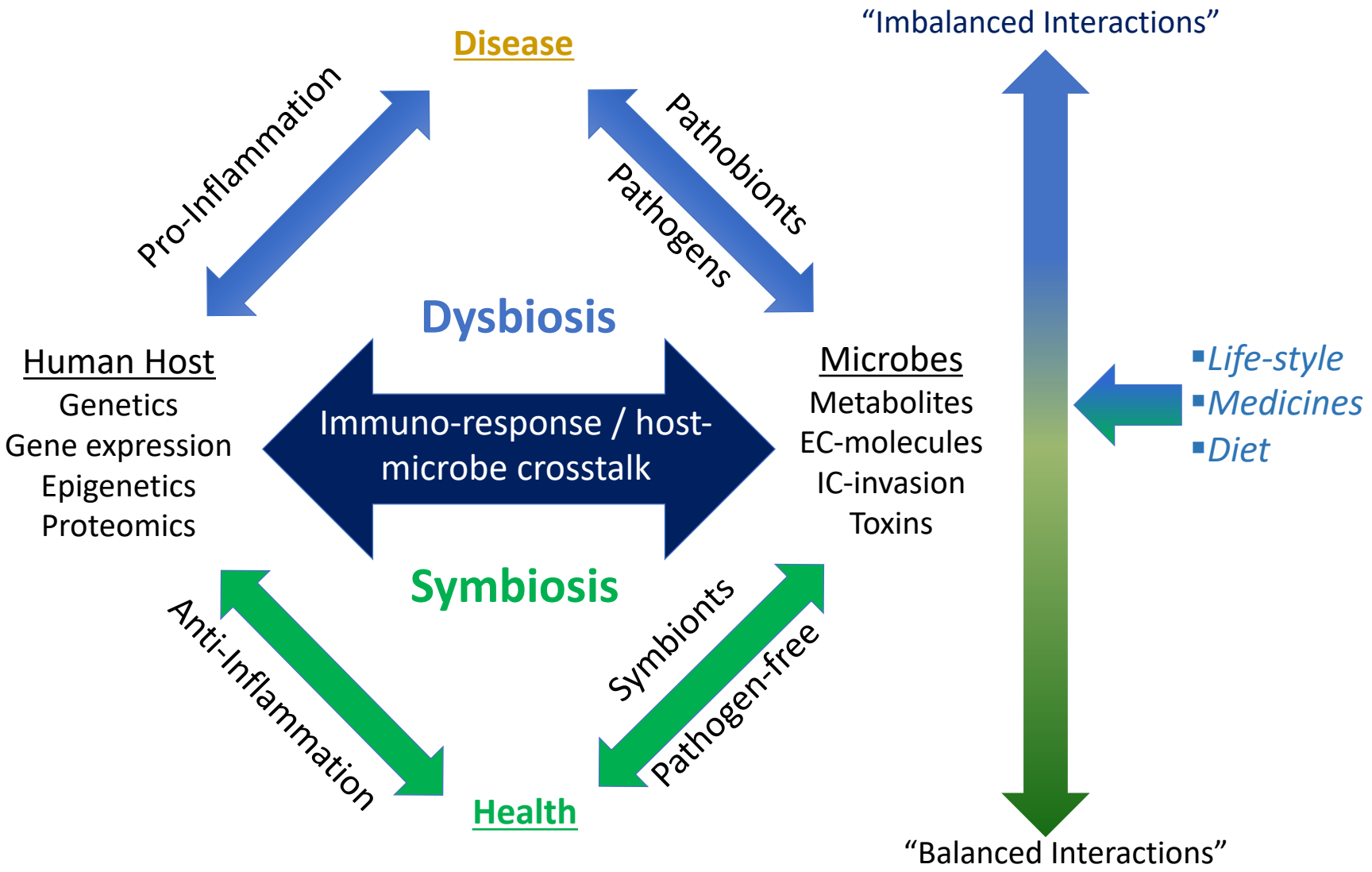
**1918 Flu Pandemic**

**Immune response**




**Microbial conversion of food stuffs & GI tract immune homeostasis**

# Host – Microbe Interactions in Health and Disease







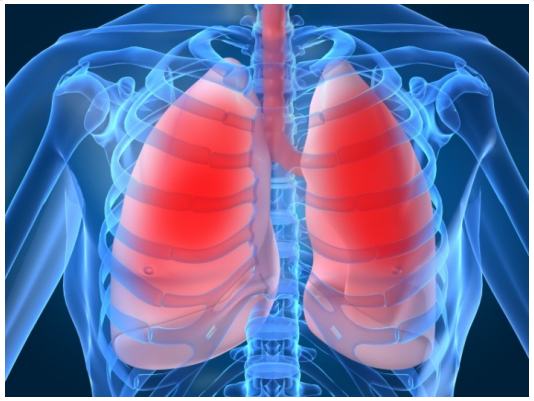
- *Life-style*
- *Medicines*
- *Diet*

Systems biology and multi-omics datasets have an important role in adding to our understanding of host-microbe interactions.

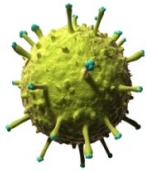
# The Rationale for Host Defense Targets

- Human targets less likely to have evolve resistance compared to highly mutable pathogen-specific targets.
- Potential to mitigate collateral tissue damage caused by overactive immune response to infection.
- Potential for broad applicability across multiple, genetically diverse pathogens.
- Broader range of human drug targets and chemical matter in pharma inventories.
- Compounds modulating human targets are active at lower doses (therefore less toxic) than direct-acting anti-pathogen drugs.
- Minimize collateral damage to the microbiome.

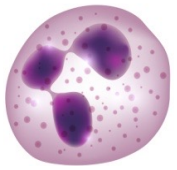
Severe respiratory infections
(viral & bacterial)



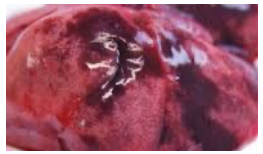
Opportunities for intervention



**Viral/bacterial infection & replication**

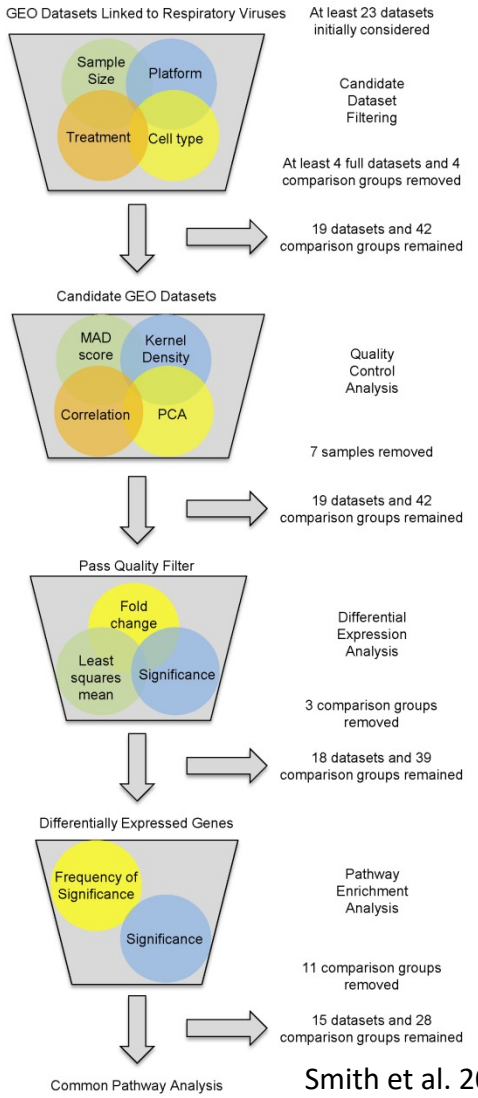


**Overactive immune response**



**Tissue injury**

# Host Response to Respiratory Viral Infections

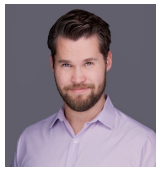



OPEN ACCESS Freely available online PLoS one

**Identification of Common Biological Pathways and Drug Targets Across Multiple Respiratory Viruses Based on Human Host Gene Expression Analysis**

Steven B. Smith[1,2], William Dampier[3], Aydin Tozeren[3], James R. Brown[4]*, Michal Magid-Slav[2]

2012

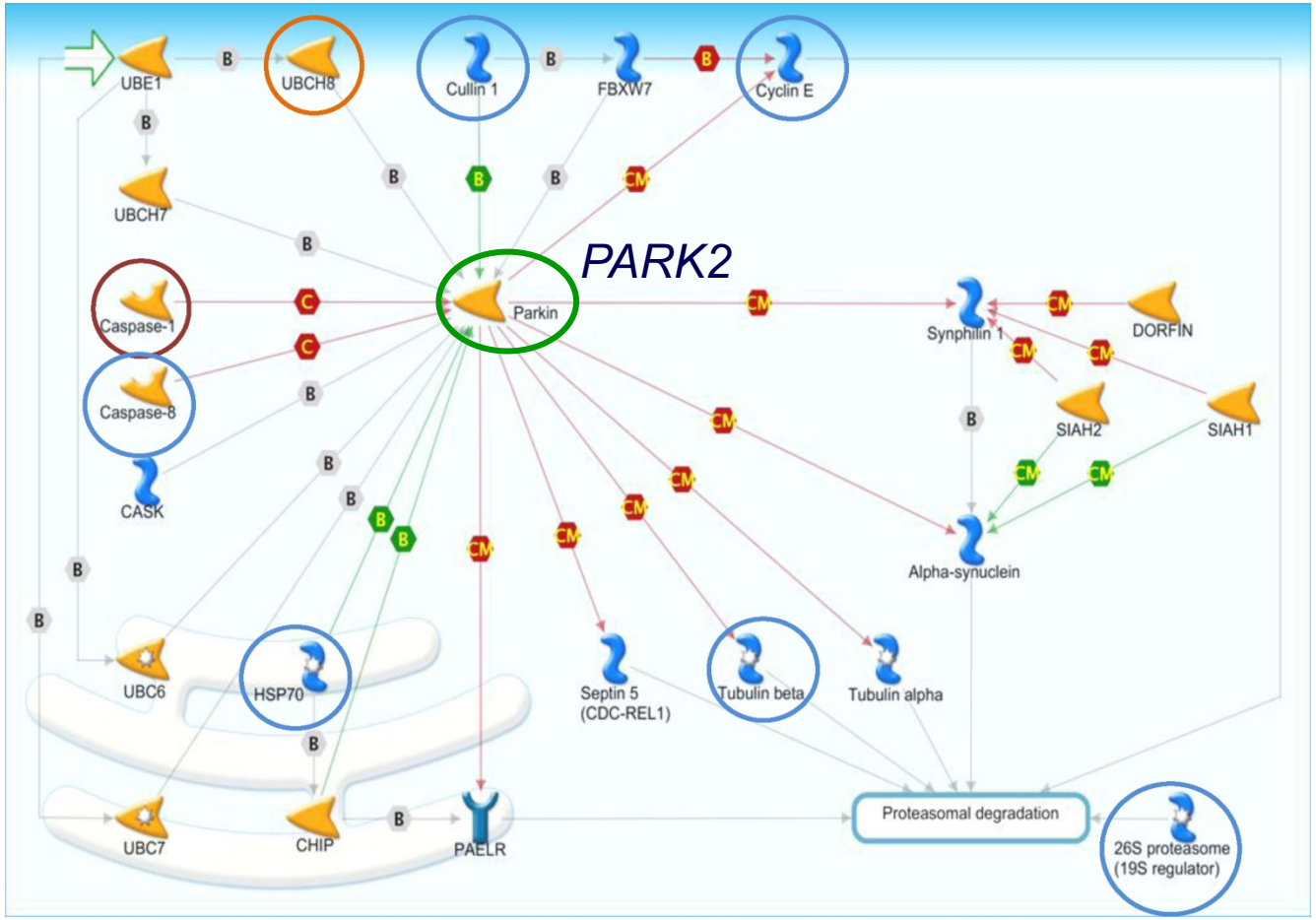


**Steve Smith (M.Sc. Student; Data Scientist, Labcorp)**
Will Dampier
Aydin Tozeren

- Analysis of human gene expression studies across seven common respiratory tract viruses
  - Respiratory synovial virus (RSV); Metapneumonia virus; Influenza A virus; Coronavirus (SARS); Rhinovirus; Coxsackievirus; Cytomegalovirus
  - Public RNA-array datasets with matched infected and un-infected human cell-types
  - Extensive QC criteria
  - Performed pathway enrichment and druggable target analyses
- 67 pathways in common among all seven viruses
- Multiple novel anti-viral and tissue damage targets (from Drug Bank and literature)
  - IL1B – Antagonists such as Canakinumab
  - TNF – Antagonists such as Pranlukast
  - CASP1 – Antagonists to reduce inflammatory damage
  - MMP9 – Antagonists to modulate NLRP3 inflammasome

Smith et al. 2012 ***PLoS One.*** e33174

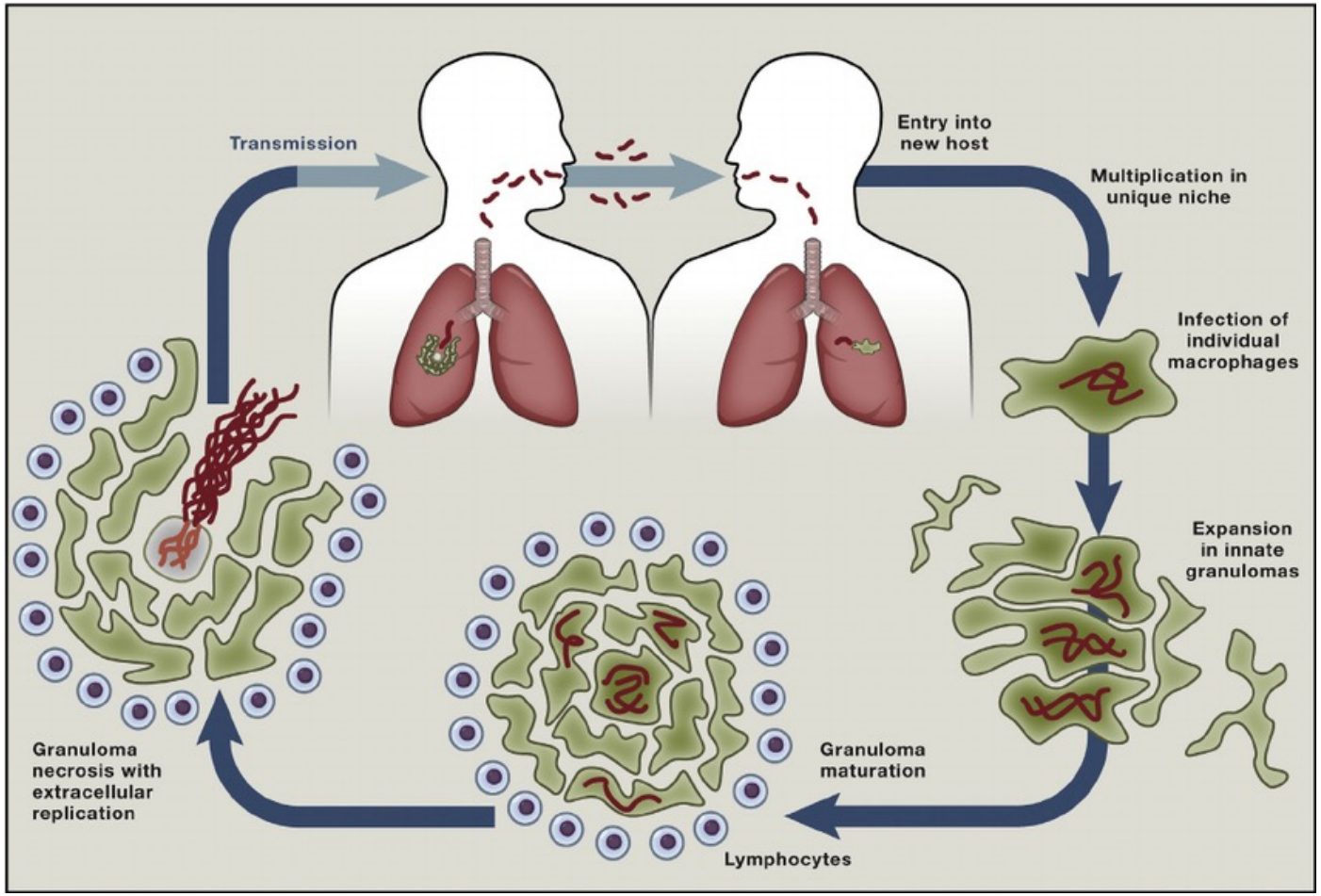
# Novel Pathways for Infectious Diseases




- *PARK2* (now called *PRKN*) encodes parkin RBR E3 ubiquitin protein ligase, a component of the Parkin-Ubiquitin Proteasomal System (Parkin-UPS) pathway.
- Mutations in *PARK2* are known to cause Parkinson disease and autosomal recessive juvenile Parkinson disease.
- Pathway enriched across 5 of 7 viruses based on human mRNA microarray analysis.
- In humans, *PARK2* gene variants are also associated with susceptibility to leprosy, typhoid and paratyphoid fever (Ali et al 2006 Clin. Exp. Immunol. 144:425).

Smith et al. 2012 ***PLoS One.*** e33174

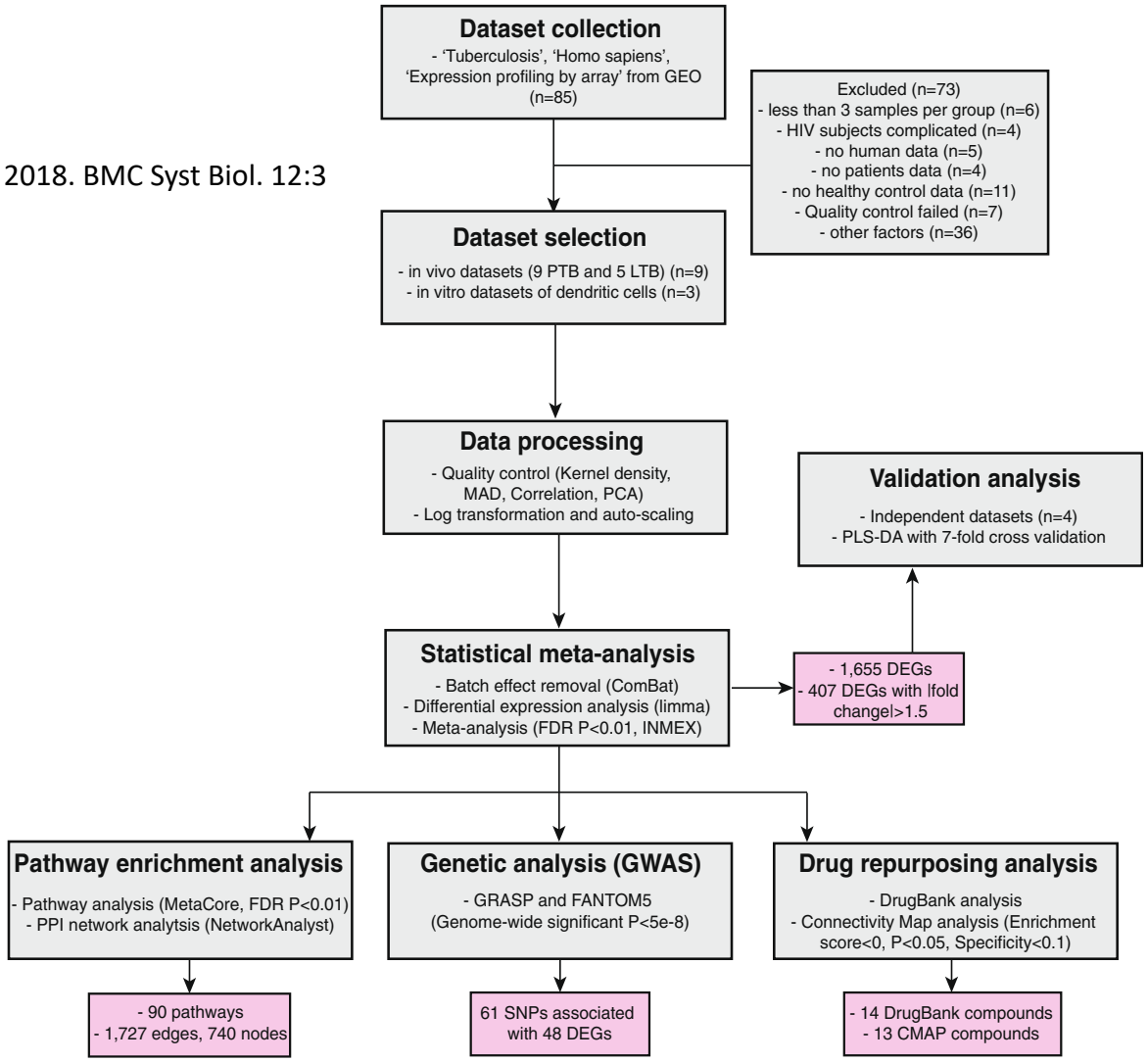
# Tuberculosis (TB) Infection Interactions with Host Immunity




- Globally TB is one of the most prevalent infectious diseases (WHO).
  - 1.8 billion people infected
  - In 2022, 10.6 million fell ill and 1.6 million died
  - High unmet medical need
- The bacterium *Mycobacterium tuberculosis* (MTB) is the causative agent of TB.
- Intra-cellular pathogen of lung macrophages.
- Latent MTB can be a long term infection requiring several months of treatment with multiple antibiotics:
  - Increase in multidrug-resistant (MDR) TB strains
  - Urgent need for new therapies
- Similar to viruses, MTB proliferation depends upon:
  - Evasion and/or subversion of host immune responses
  - Manipulation of the macrophage microenvironment

# Meta-analysis of Human Gene Response to *M. tuberculosis*

Wang et al. 2018. BMC Syst Biol. 12:3





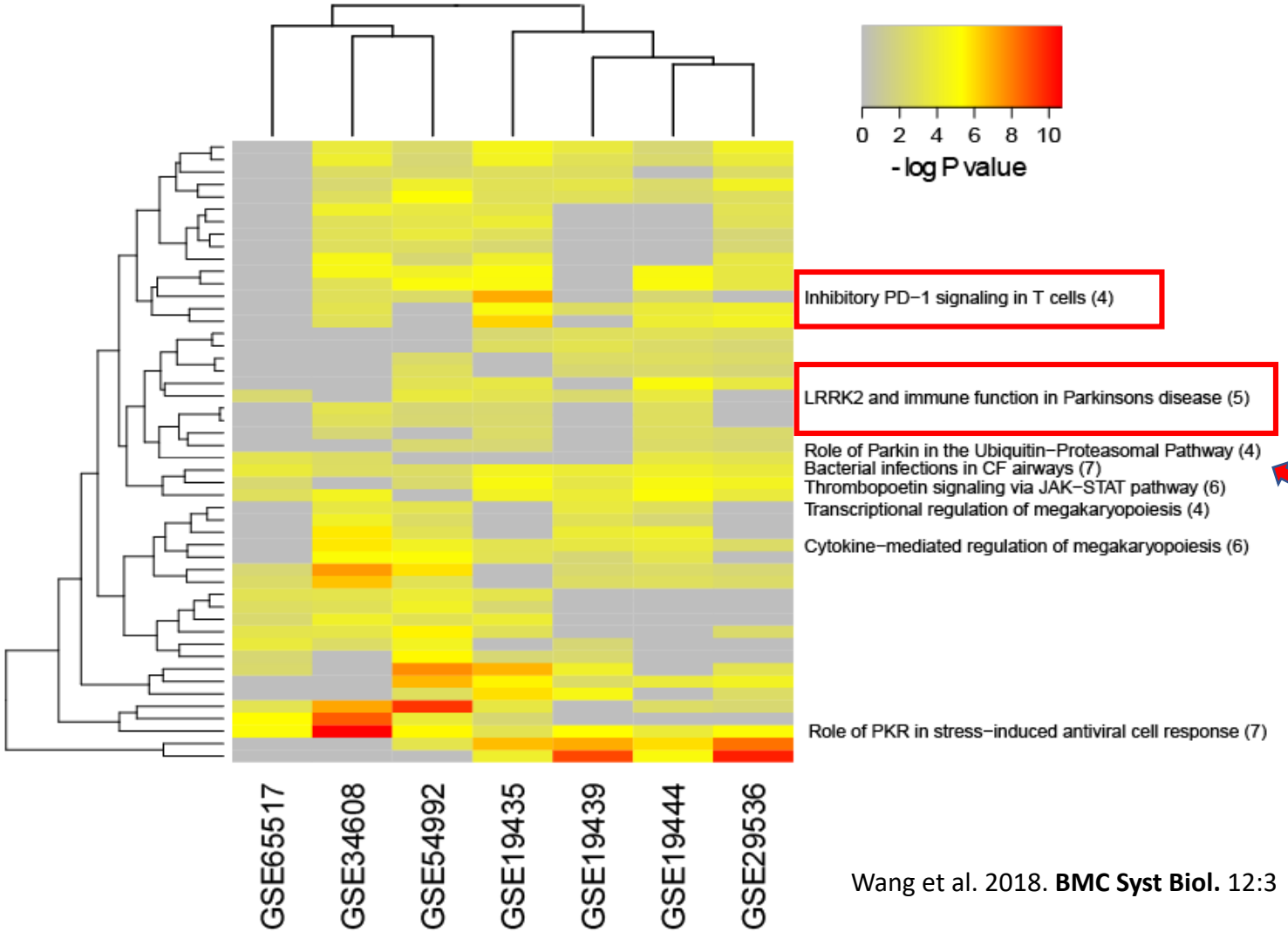
Dr. Seda Arat
Co-op Graduate Student;
Comp. Toxicologist, Pfizer



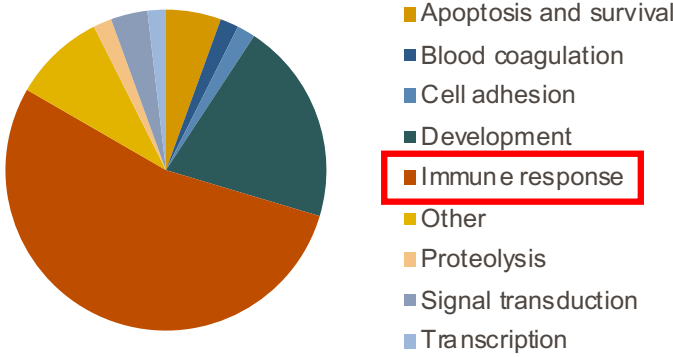
Dr. Zhang Wang,
Early Talent PDF;
CB Scientist GSK;
Professor Southern China Normal U.


- Human transcriptome meta-analysis of 7 published human transcriptome during active pulmonary TB infection (PTB) datasets
  - Health control groups
  - No co-occurring infections
  - Pass QC and sample size criteria
- Complete re-analysis of RNA-seq datasets for differentially expressed genes (DEGs)
- Meta-analysis of individual studies then looking at overlapping gene sets
- Pathway enrichment
- Targets reviewed for genetic (GWAS) associations
- Drug repurposing analysis

# Enriched Human Pathways in PTB Infections



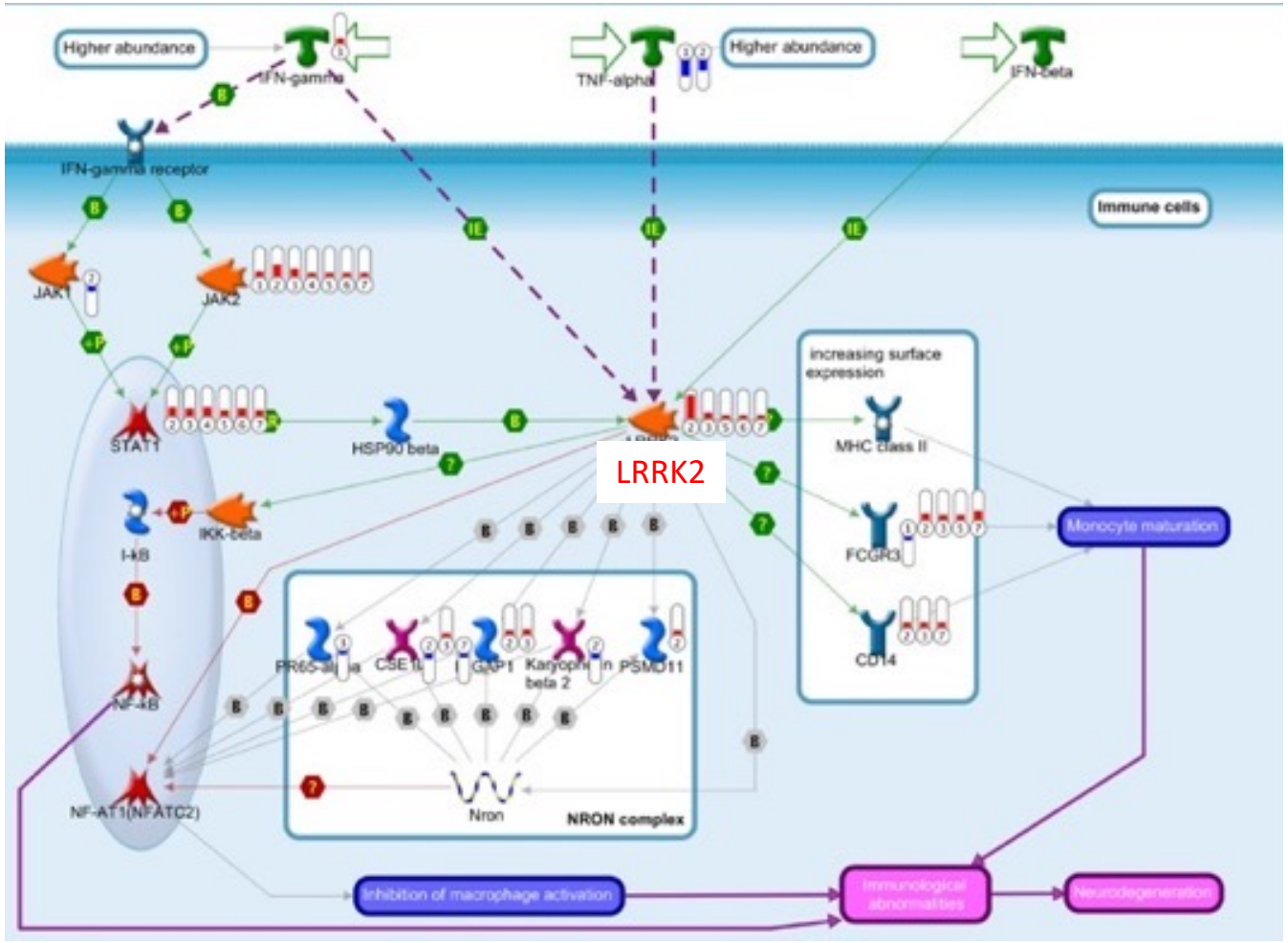

- 54 pathways found enriched for 4 or more out of 7 datasets




- Parkin-Ubiquitin Proteasomal System, involved in the progression of Parkinson disease

Wang et al. 2018. **BMC Syst Biol.** 12:3

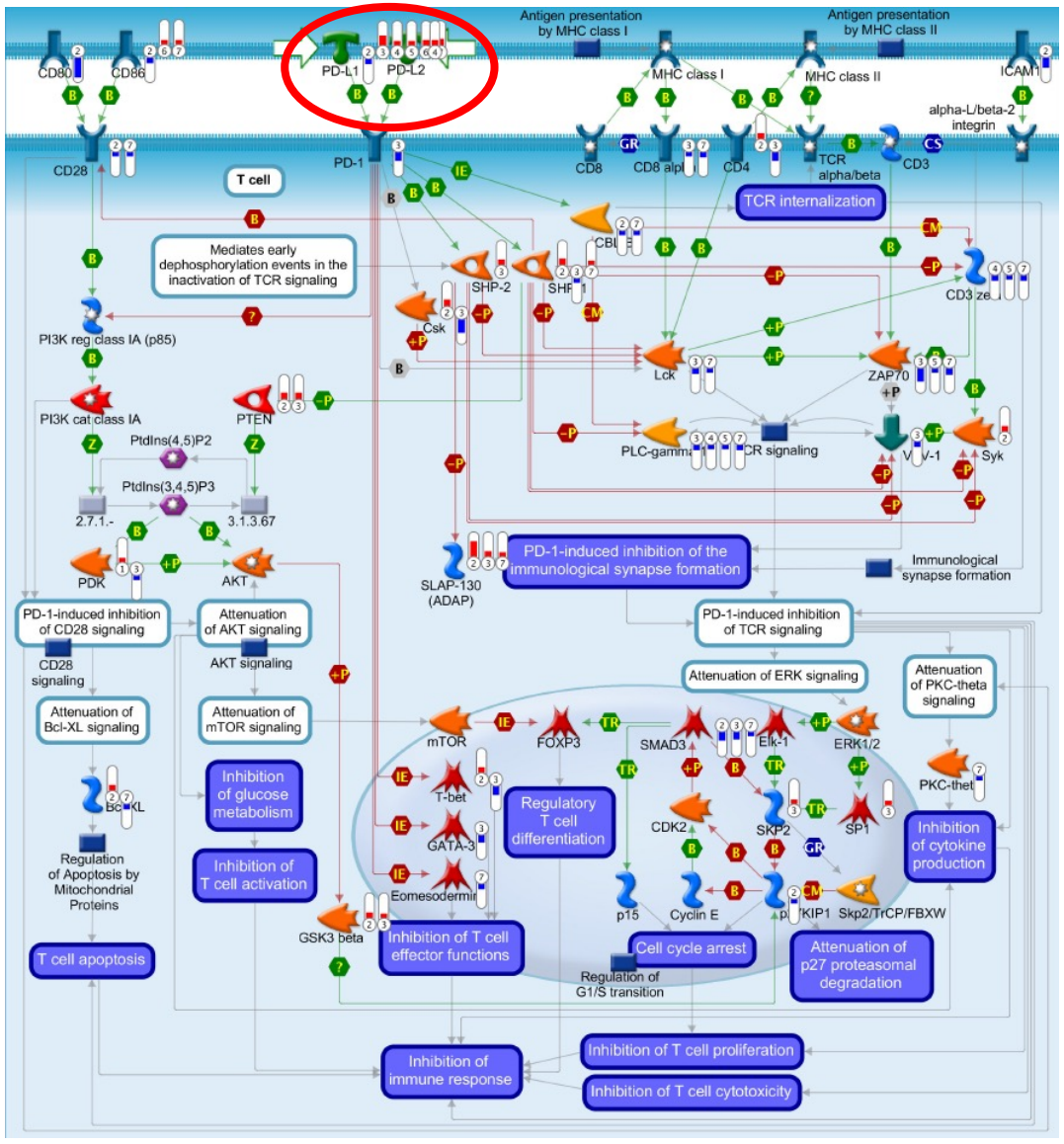
# LRRK2 in Parkinson's Disease (PD) and Tuberculosis




Wang et al. 2018. BMC Syst Biol. 12:3

- Several Parkinson's Disease core pathways are modulated in TB.
  - 1.38-fold risk of Parkinson's Disease in TB patients independent of other clinical factors (Shen et al. 2016. Medicine [Baltimore] 95:e2883).
- **58 genetic variants associated with PD** proximal to 407 Differential Expressed Genes (DEGs) in TB.
- **LRRK2** (leucine rich repeat kinase 2) mutations associated with PD; considered a potential target.
  - LRRK2 has wide immune regulatory functions and associates with the mitochondria
  - LRRK2 highly expressed in the lung and linked to gut-brain immunity (Peter & Strober. 2023. J. Parkinsons Dis.)
- GSK/Crick collaboration support LRRK2 as a potential TB target
  - LRRK2 deficiency in mice resulted in a significant decrease in *M. tuberculosis* burdens early during the infection (Hartlova et al. 2018. EMBO J. 37).

# Inhibitory PD-1 Signaling in T-cells




- Pathway significantly enriched in 4 PTB datasets. ***PD-L1*** gene significantly up-regulated in 5 PTB datasets.
- The PD-1/PD-L1 pathway has been shown to inhibit T cell effector function during PTB infections (Yin et al. 2014. **Tuberculosis** 94:131), suggesting *Mtb* might exploit PD-1/PD-L1 pathway to evade host immune response.
- Overcoming T-cell exhaustion is the basis of cancer immuno-therapy and might be a strategy for TB.
  - Potential to test PD-1 check point inhibitors clinically used for immuno-oncology (i.e., Pembrolizumab [Keytruda]) for activity against active PTB.

Wang et al. 2018. BMC Syst Biol. 12:3

# Potential Drug Repurposing For TB Therapy

| Targets and compounds proposed in this study | | |
|---|---|---|
| Compounds | Targets/ Pathways | Evidence |
| *LRRK2* inhibitor | *LRRK2* pathway | *LRRK2* pathway significantly upregulated in TB. *LRRK2* genetically associated with susceptibility of *M. leprae* infection. Cormobidities between TB and Parkinson's disease. |
| *PD-L1* inhibitor (Atezolizumab) | *PD-1/PD-L1* pathway | *PD-1/PD-L1* significantly upregulated in TB, and inhibit TB-specific T-cell and macrophage functions. |
| Carfizomib | *PSMB8*, *PSMB9*, *PSMB10*, *PSMB2* | *PSMB8*, *PSMB9* significantly upregulated in TB, with strong genetic association with TB infection. |
| Intraveneous Immunoglobulin (IVIg) | *FCGR2A*, *FCGR3A*, *C5* | *FCGR2A*, *FCGR3A*, *C5* significantly upregulated in TB. Efficacy of IVIg in reducing bacterial load in TB infection. |
| Disopyramide | *SCN5A*, *ORM1* | Top compound in CMAP analysis. *SCN5A* regulates spatial and temporal calcium signaling during *Mtb* phagocytosis. |
| Flunarizine | *HRH1*, *CACNA1G*, *CACNA1H*, *CACNA1I*, *CALM1* | Top compound in CMAP analysis. Potential efficacy in restricting *Mtb* growth. |

- Drug repurposing hypotheses – two methods.
- 407 DEGs searched for associations with known drugs listed in the Drug Bank database https://go.drugbank.com/ .
  - 19 drug-target links identified involving 14 drugs and 16 differentially expressed genes (DEGs).
- Connectivity MAP (L1000 CMAP https://clue.io/ ) analysis utilizes the anti-correlation relationships between gene expression (RNA-seq) signatures in diseases and drug perturbations.
  - 13 drugs with significantly anti-correlated signatures to the PTB signature

Wang et al. 2018. BMC Syst Biol. 12:3

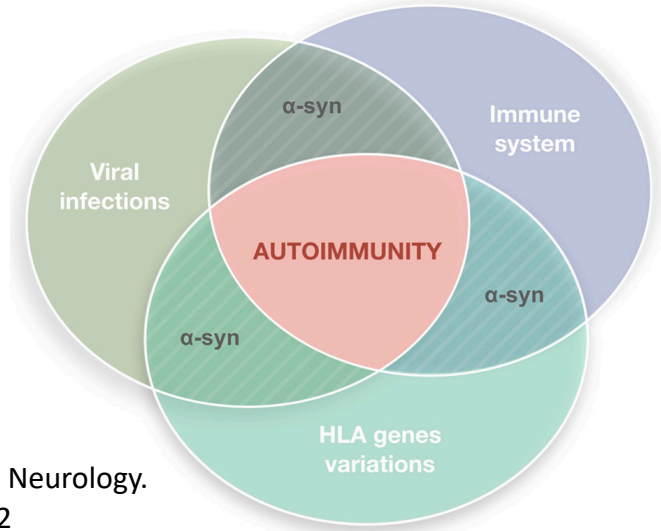
# Parkinson Disease (PD) and Alzheimer's Disease (AD): Associations with Neuro-Inflammation and Viral Infections

- Increasing evidence on the role of neuro-inflammation in neurodegenerative diseases (Lim et al. 2015 Microbes and Infection 17:549)
- Potentially peripheral factors could trigger CNS inflammation (Itagaki et al. 1989. J Neuroimmunol 1989;24:173e82; Cribbs et al. 2012. J Neuroinflammation 9:179)
- Infectious pathogens are often detected in the brains of Alzheimer's Disease (AD) patients (Miklossy et al. Expert Rev Mol Med 2011;13:e30; Xinhua et al. Neurology online October 26, 2016)
- Herpes simplex virus type 1 (HSV1) and other Herpesviridae family members including cytomegalovirus (CMV), Epstein-Barr virus (EBV), or human herpes virus 6 (HHV-6), can infect neurons and been associated with AD (Zhou L, Miranda-Saksena M, Saksena NK.. Virology Journal. 2013;10:172.; Carbone et al. 2014 Neurobiol Aging. 35:122-9)

*Hypothesis: Comparisons of gene expression profiles in AD/PD patients to those of patients infected with CMV, EBV or HHV-6 pathogens might reveal specific neuro-inflammation pathways*

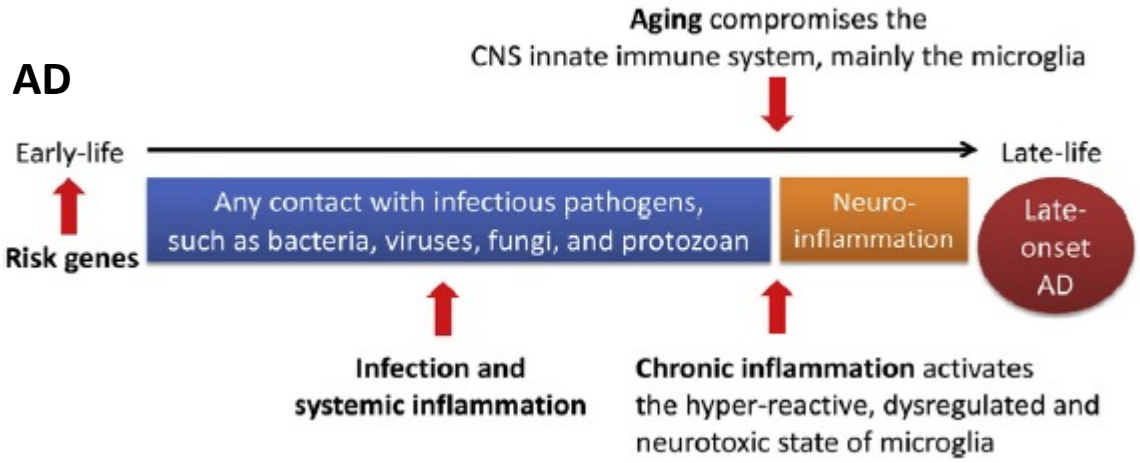
**PD**

Autoimmune dysfunction in the etiology of Parkinson's disease (PD).



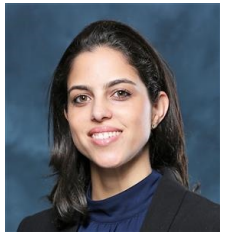

Caggiu et al. 2019. Frontiers in Neurology. doi: 10.3389/fneur.2019.00122

**AD**




Lim et al. 2015 Microbes and Infection 17:549

# Identifying Neuro-inflammation Targets



Dr. Carol Sa, Early Talent PDF
Sr. Data Scientist J&J

**Alzheimer's Disease**
GSE63036

**Parkinson's Disease**
GSE99039

**Healthy vs. Diseased**
Gene Expression Signatures

Find Common Genes & Pathways

**Target Selection**
GWAS
Proteomics
Literature
Tractability

**Bacterial pathogens**
- *Pseudomonas aeruginosa*
- *Streptococcus pneumoniae*

**Viruses**
- Influenza A
- Respiratory Syncytial
- Rhino
- SARS-corona
- Metapneumo.
- Coxsackie
- Cytomegalovirus
- Epstein Bar virus 2x
- Herpes virus 6

GSE81246
GSE20200
GSE45829
GSE40396

**Tuberculosis**
- *Mycobacterium tuberculosis*

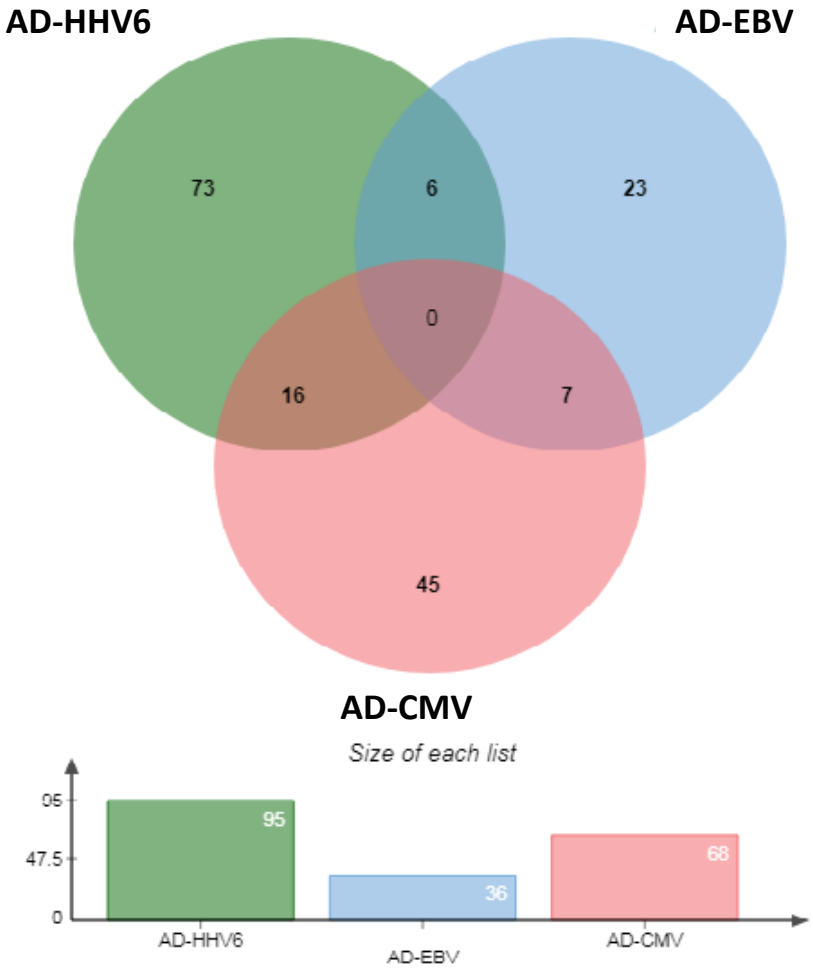
**Human Host Response to Infections**
Gene Expression Signatures

Sa et al. 2019. Scientific Reports (Nature) 9:8795

- Systems biology analysis to find common AD/PD and viral host response targets
- Published GEO datasets involving patients and human blood samples used in all comparisons

# CMV, EBV and HHV6 Shared Gene Signatures with AD

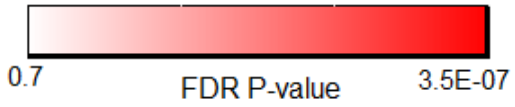
## Differentially Expressed Genes




## Significantly Enriched Pathways

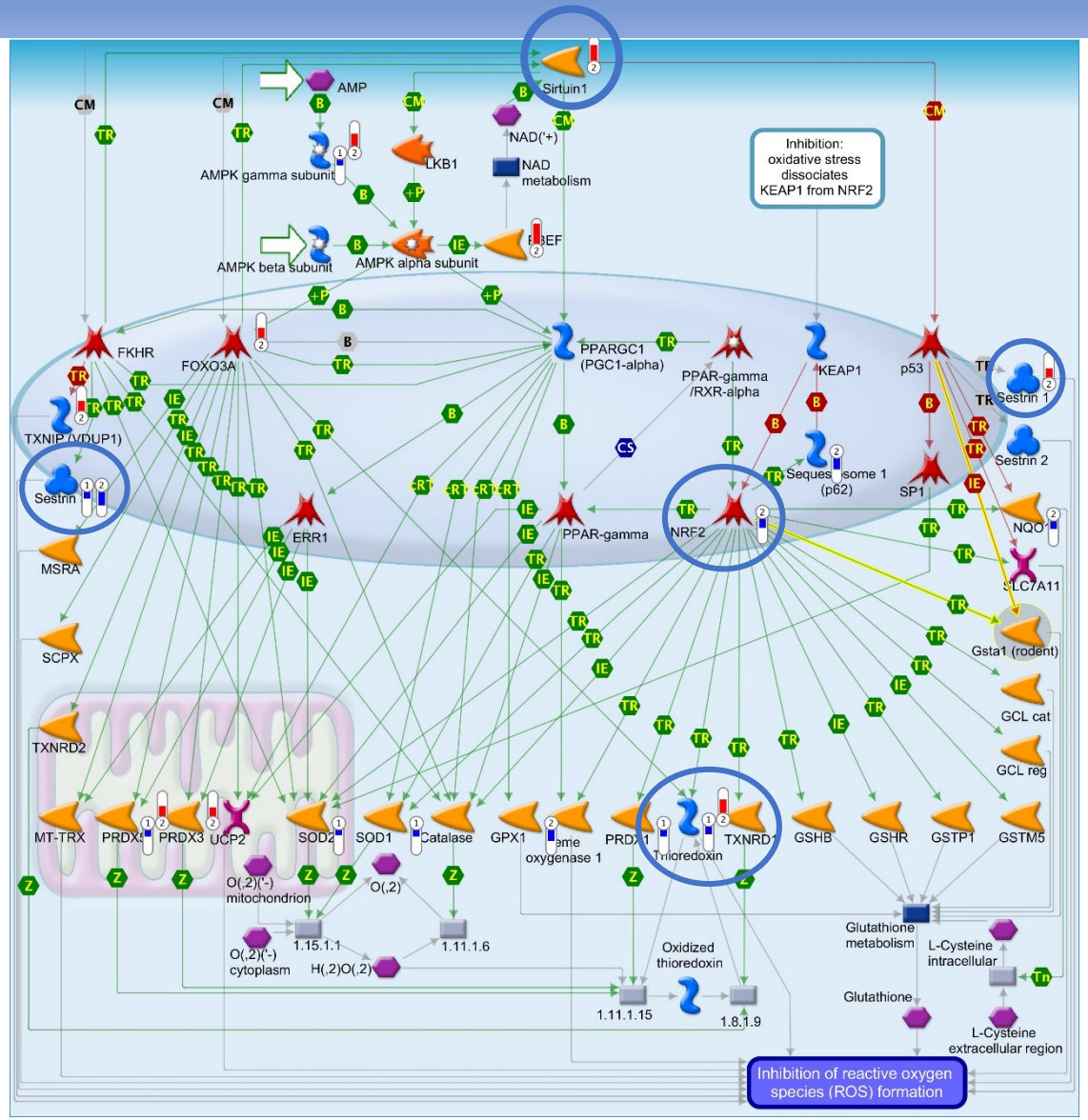
| HHV6 host response | EBV host response | CMV host response | Alzheimer's Disease | |
|---|---|---|---|---|
| 6.2E-07 | 3.9E-02 | 8.6E-04 | 6.8E-03 | Antigen presentation by MHC class II |
| 6.0E-05 | 1.8E-02 | 3.7E-03 | 1.9E-02 | Role of IAP-proteins in apoptosis |
| 2.6E-02 | 4.9E-02 | 2.2E-03 | 1.0E-02 | Role of Sirtuin1 and PGC1-alpha in activation of antioxidant defense syster |
| 4.9E-03 | 3.3E-01 | 2.5E-02 | 3.6E-02 | Cigarette smoke-induced oxidative stress and apoptosis |
| 3.2E-02 | 3.2E-01 | 1.7E-04 | 3.0E-02 | Regulation of G1/S transition |
| 3.7E-03 | 7.2E-01 | 2.1E-01 | 1.1E-03 | HSP70 and HSP40-dependent folding in Huntington's disease |
| 1.4E-02 | 3.3E-01 | 7.2E-02 | 1.1E-03 | Regulation of degradation of deltaF508-CFTR in CF |
| 1.6E-02 | 4.2E-01 | 2.3E-01 | 1.1E-03 | CREB1-dependent transcription deregulation in Huntington's Disease |
| 2.1E-02 | 5.2E-02 | 1.4E-01 | 2.6E-02 | Possible regulation of HSF-1/ chaperone pathway in Huntington's disease |

**Role of Sirtuin 1 and PGC1-alpha (PPARG coactivator 1 alpha) in activation of antioxidant defense system**




Sa et al. 2019. Scientific Reports (Nature) 9:8795

# Oxidative Stress in Alzheimer's Disease

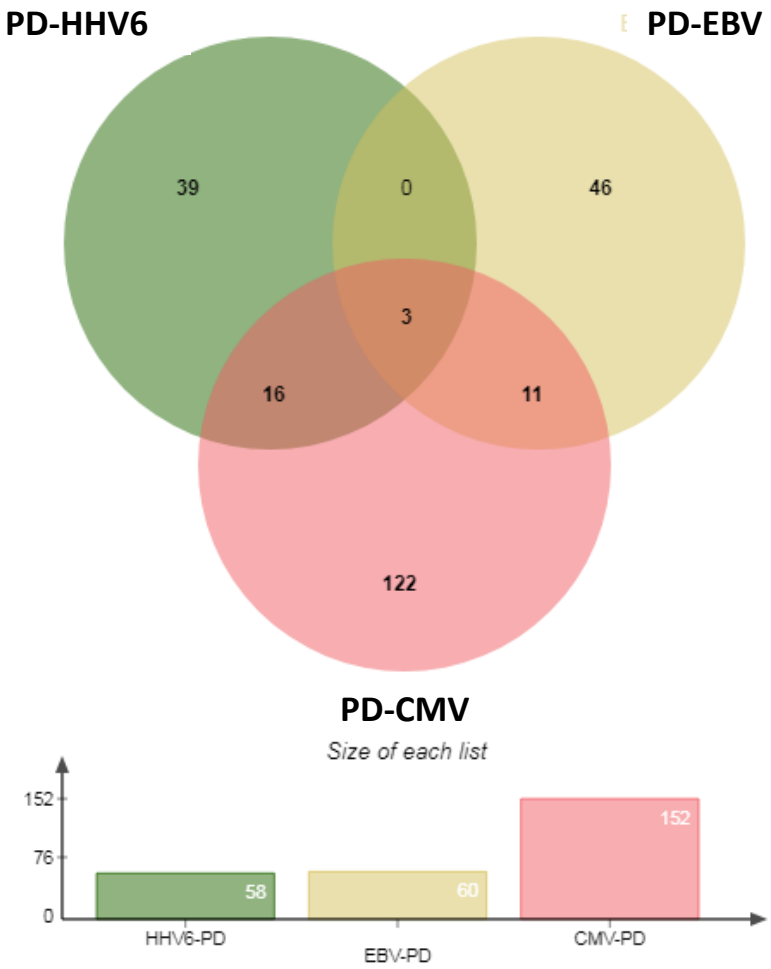



- "Role of Sirtuin 1 and PGC1-alpha (PPARG coactivator 1 alpha) in activation of antioxidant defense system" was the top pathway significant for all 3 viruses and AD.
- *SESN3* (sestrin 3) and *TXN* (thioredoxin), which play important roles in this pathway, ranked among the top genes associated with CMV/EBV, and CMV/HHV-6 host responses, respectively.
- *TXN* has been suggested to be an early biomarker of AD (Arodin et al. 2014. Alzheimer's Dis. 39:787).
- SNP rs3911569 near *SESN3* associated with 5-fold increased risk for AD (Herold et al. 2016 Mol Psychiatry 21:1608-12).
- Findings support the "mitochondrial cascade hypothesis" which postulates the co-occurrence of AD-related mitochondrial dysfunction (Wang et al. 2013 Cell Metab 17:685-94).

Sa et al. 2019. Scientific Reports (Nature) 9:8795

# CMV, EBV and HHV6 Shared Gene Signatures with PD

## Differentially Expressed Genes

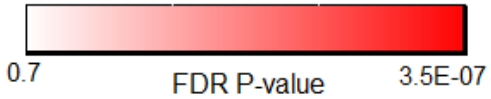



## Significantly Enriched Pathways

| HHV6 host response | EBV host response | CMV host response | Parkinson's Disease | |
|---|---|---|---|---|
| 3.7E-02 | 1.9E-02 | 1.5E-03 | 3.5E-07 | Cytoskeleton remodeling_Reverse signaling by Ephrin-B |
| 1.6E-02 | 1.7E-02 | 2.7E-03 | 2.6E-06 | Immune response_Platelet activating factor/ PTAFR pathway signaling |
| 1.1E-03 | 2.7E-02 | 7.3E-03 | 2.7E-06 | CHDI_Correlations from Replication data_Cytoskeleton and adhesion module |
| 4.2E-02 | 2.0E-02 | 1.8E-03 | 3.3E-0( | LRRK2 in neurons in Parkinson's disease |
| 2.1E-02 | 2.0E-02 | 6.1E-04 | 4.3E-06 | Role of integrins in eosinophil degranulation in asthma |
| 6.0E-07 | 3.6E-02 | 1.8E-03 | 4.4E-06 | Immune response_Antigen presentation by MHC class II |
| 2.0E-03 | 2.7E-03 | 5.1E-03 | 5.0E-06 | Signal transduction_mTORC2 downstream signaling |
| 2.1E-05 | 3.5E-03 | 3.8E-02 | 2.6E-05 | Macrophage and dendritic cell phenotype shift in cancer |
| 1.0E-03 | 4.8E-02 | 6.5E-04 | 3.9E-05 | Development_VEGF signaling via VEGFR2 - generic cascades |
| 6.7E-05 | 2.2E-02 | 5.0E-02 | 1.1E-04 | Neutrophil resistance to apoptosis in COPD and proresolving impact of lipid mediators |
| 5.1E-04 | 3.8E-02 | 2.5E-05 | 2.9E-04 | Immune response_IL-3 signaling via ERK and PI3K |
| 7.6E-06 | 2.6E-02 | 2.9E-07 | 3.7E-04 | Immune response_IL-3 signaling via JAK/STAT, p38, JNK and NF-kB |
| 2.3E-04 | 5.1E-03 | 4.1E-06 | 5.5E-04 | Immune response_B cell antigen receptor (BCR) pathway |
| 7.4E-06 | 3.4E-02 | 3.2E-04 | 6.0E-04 | SLE genetic marker-specific pathways in B cells |
| 1.1E-04 | 1.7E-02 | 7.0E-03 | 8.3E-04 | Immune response _IFN gamma signaling pathway |

**Reverse signaling by Ephrin-B**
(involved in neural regeneration)

**LRRK2 Pathway**

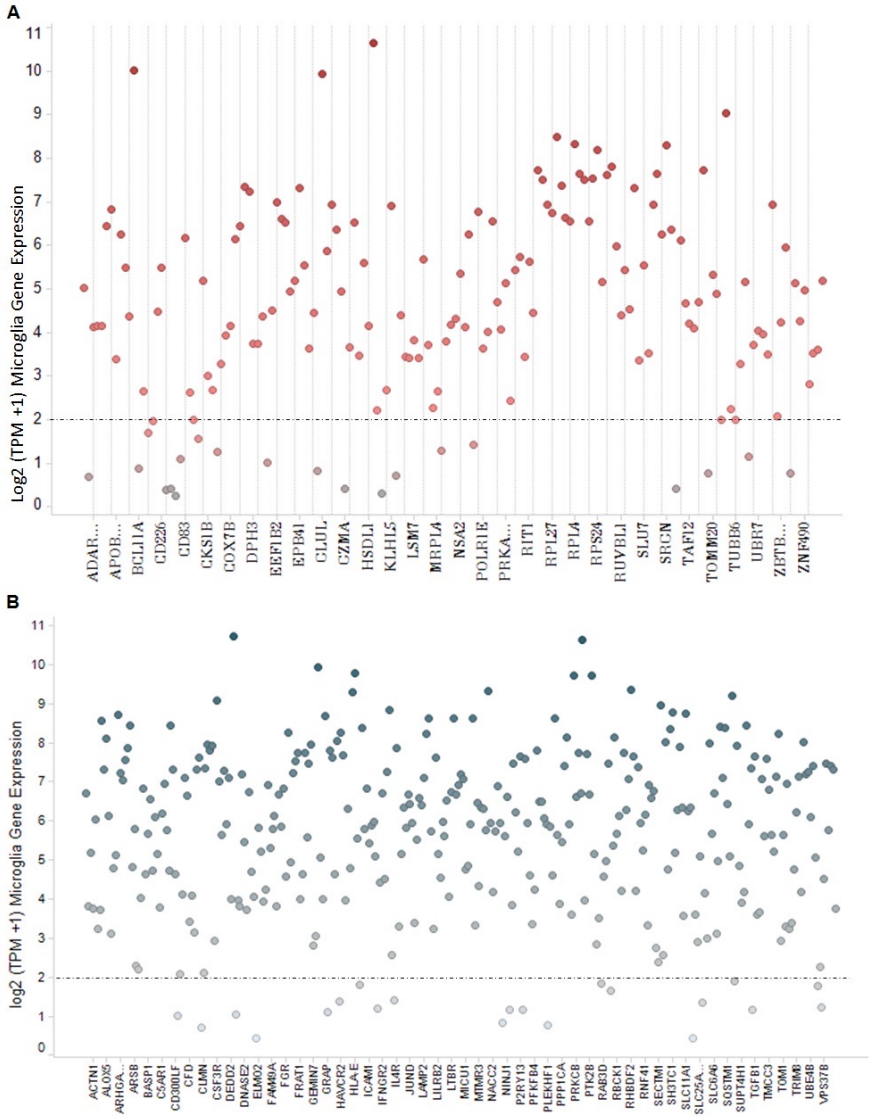



Sa et al. 2019. Scientific Reports (Nature) 9:8795

# Gene Expression Levels in Brain Microglia Cells

Alzheimer's Disease

Parkinson's Disease




- Microglia cells function as macrophages in the central nervous system and fulfill the role of immunity surveillance in the brain
- Using public datasets, we confirmed that the majority of significant genes are also actively expressed in human microglia ($log_2$ transcripts per million reads > 2)
  - 86.3% in AD
  - 93.6% in PD
- Supports sampling of the blood as a surrogate for direct microglia gene expression profiling

Sa et al. 2019. Scientific Reports (Nature) 9:8795

# Translating Targets into Therapeutic Opportunities

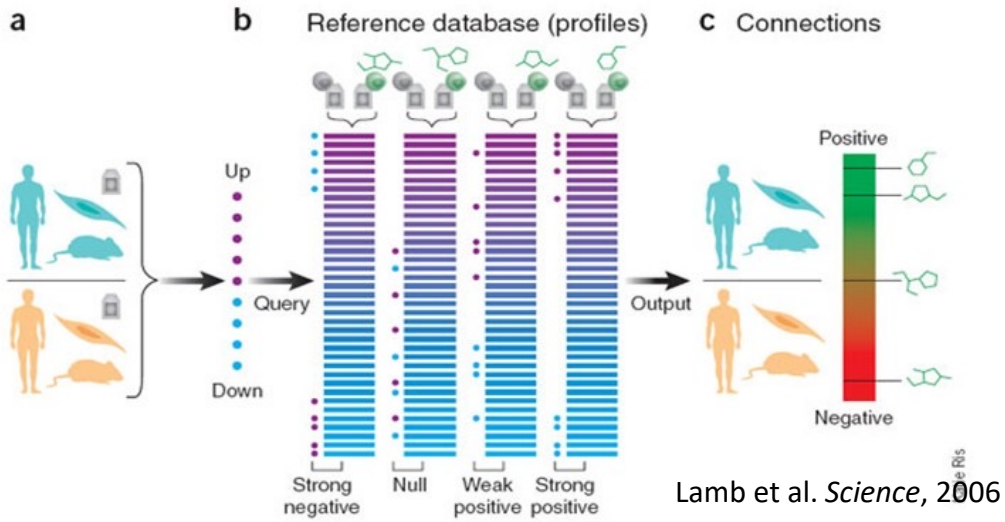
- Three strategies for prioritizing potential targets for further *in vitro* / *in vivo* validation studies:

1. Genetic evidence for target being associated with the primary disease indication using human genetic data (i.e. UKBB, 23andMe, FinnGen, GWAS catalogue).
2. Availability of potential tool compounds or antibodies for reversing disease gene expression signatures using CMAP (L1000).
    1. GSK has internal CMAP data on lead compounds in multiple cell-types.
3. Availability of potential tool compounds or antibodies for modulating specific targets using GSK compound database and public DrugBank.

23andMe

biobank uk

FINNGEN




Lamb et al. *Science*, 2006

DRUGBANK

gsk do more feel better live longer

Sa et al. 2019. Scientific Reports (Nature) 9:8795

# Genetic Evidence and Drug Repositioning

- 19 viral associated DEGs had proximal SNPs associated with neurodegenerative diseases in the GWAS catalog.
- CMV CMAP: 16 drugs identified with a significantly anti-correlated signature to the CMV signature ($P < 0.05$, Specificity < 0.1).
  - 8 compounds (highlighted) with literature evidence of neuro protection to AD or PD.
- EBV CMAP revealed 24 compounds; 4 compounds had neurological indications including PD.
- HHV-6 CMAP had 16 compounds; 3 compounds with neuro-indications.
- In-house CMAP found more than 30 GSK compounds with unique targets.

| Compound | Mechanism of Action | Indication | Compound Score | Enrichment Score | P-Value |
|---|---|---|---|---|---|
| quinostatin | PI3-Kinase/mTOR inhibitors | Oncology | 1 | -0.87 | 0.0337 |
| cortisone | Corticosteroid Hormone Receptor Agonists | Anti-inflammatory | 0.5 | -0.88 | 0.0285 |
| quinethazone | Sodium/chloride tranporter inhibitor | Antihypertensive | 0.5 | -0.84 | 0.0492 |
| metrifonate | Cholinesterase inhibitor | Neuro protection[1,2] | 0.37 | -0.95 | 0.0054 |
| cicloheximide | Protein synthesis inhibitor | Antibiotics | 0.33 | -0.99 | 0.0002 |
| anisomycin | MAP kinase activator | Neuro protection[3] | 0.33 | -0.97 | 0.0023 |
| molindone | Dopamine receptor antagonist | Neuro protection[4] | 0.33 | -0.94 | 0.0059 |
| hydroflumethiazide | Na-Cl cotransporter inhibitor | Antihypertensive | 0.33 | -0.94 | 0.0077 |
| pronetalol | Adrenoreceptor blocker (beta) | Neuro protection[4] | 0.33 | -0.93 | 0.0089 |
| picotamide | Eicosenoid receptor antagonist | Anti-inflammatory | 0.33 | -0.93 | 0.0103 |
| mephenytoin | Sodium channel blocker | Antihypertensive | 0.33 | -0.89 | 0.0231 |
| dipivefrine | adrenergic agonist | Neuro protection[4] | 0.33 | -0.87 | 0.0343 |
| etamsylate | Prostaglandin synthesis inhibitor | Anti-inflammatory | 0.33 | -0.85 | 0.0422 |
| mebeverine | Phosphodiesterase inhibitor | Neuro protection[5] | 0.33 | -0.85 | 0.045 |
| prasterone | Estrogen receptor (ER) agonists Androgen receptor (AR) agonists | Neuro protection[6] | 0.33 | -0.85 | 0.0467 |
| pirenzepine | Muscarinic M1 receptor antagonist | Neuro protection[4] | 0.3 | -0.95 | 0.0045 |

Sa et al. 2019. Scientific Reports (Nature) 9:8795

# Outline

1. Targeting human host factors for infectious and neural degenerative diseases

2. The lung microbiome in respiratory diseases

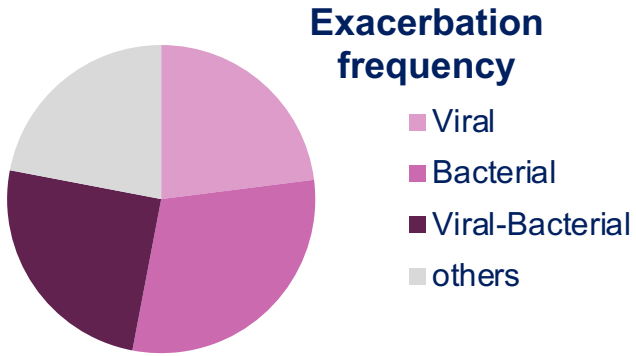
3. Multi-omics data analysis of human protein-metabolite interactions

# COPD and the Lung Microbiome

- Chronic obstructive pulmonary disease (COPD): lung disease characterized by chronic obstruction of lung airflow that interferes with normal breathing and not fully reversible (WHO).

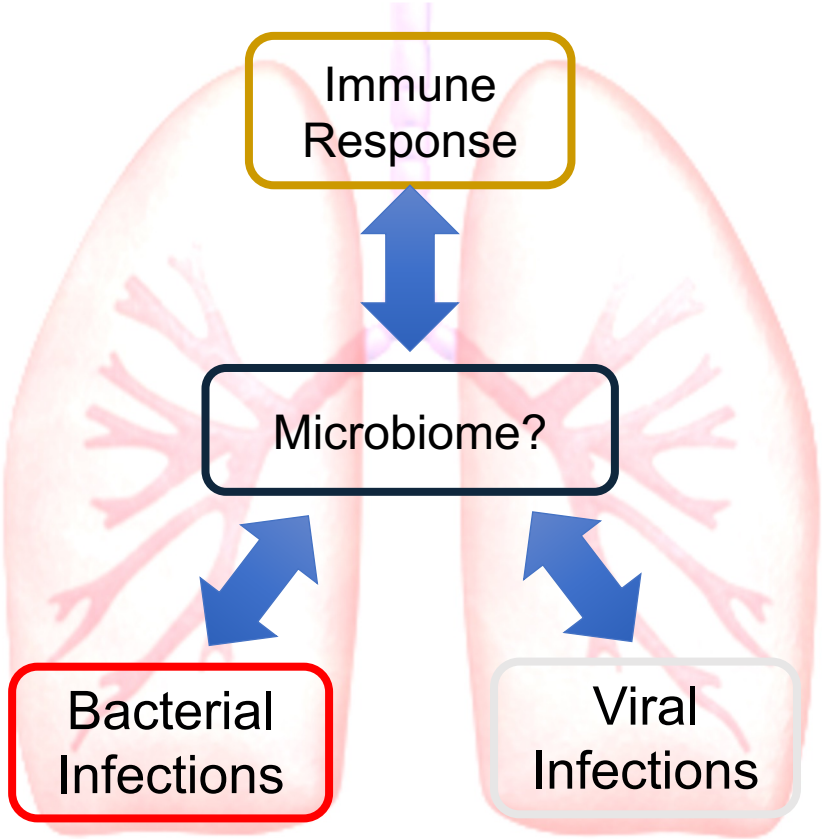
- Globally, about 300M people with COPD and is the 5th leading cause of death in 2022, projected to be 4th by 2030 (Mathers et al. 2006. PLOS Med. 3:e442; Lozano et al. 2012. Lancet 380:2095)

- COPD exacerbations are heterogenous and not all the same.



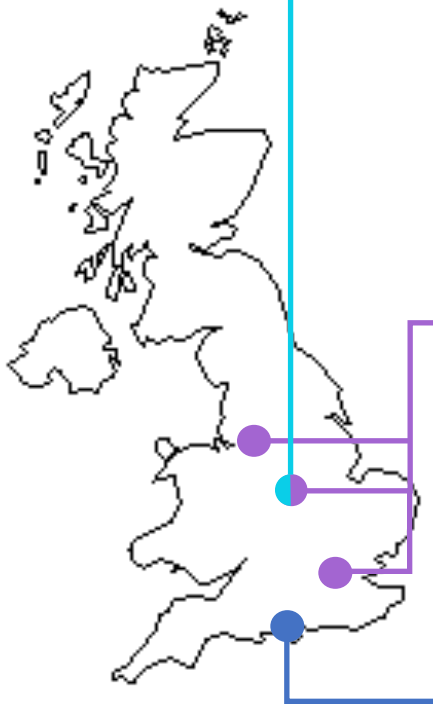

- 78% of COPD exacerbations associated with viral/bacterial infections (Papi et al. 2006 **Am J Respir Crit Care Med**)

- Need for precision medicine strategies for COPD.




The healthy lung is not sterile!

# GSK Sponsored COPD-Microbiome Studies



1) COPD-BEAT (Wang et al. 2016. **European Resp. J. 47:1082)**
   n = 87 COPD subjects; sample no. = 476 sputum
   single site study : University of Leicester
   samples: stable, exacerbation, treatment, recovery

2) COPD-MAP (Wang et al. 2017. **Thorax.** 73:331)
   n = 285 COPD subjects; sample no. = 716 sputum
   3x sites: Imperial, Leicester, Manchester
   samples: stable + exacerbation

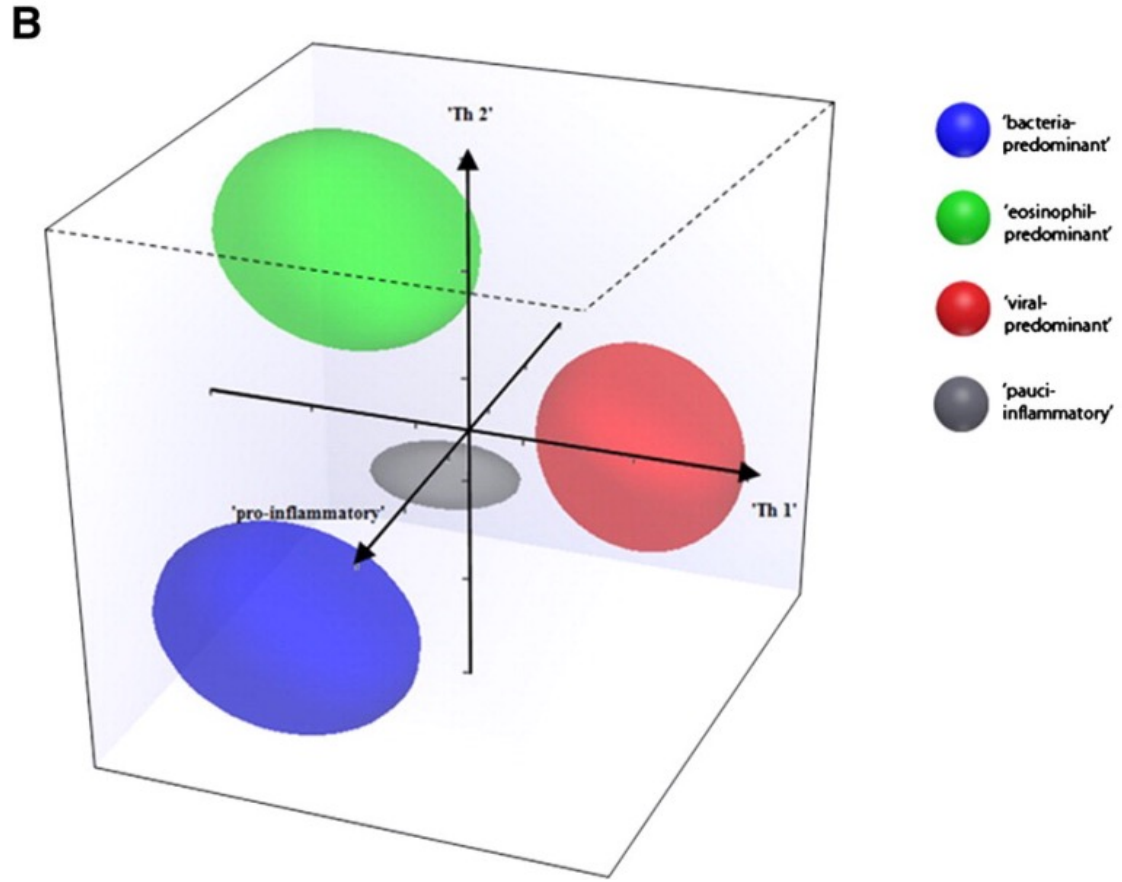
3) AERIS (GSK Rx & Vx) (Mayhew et al. 2018. **Thorax.** 73:422)
   n = 101 COPD subjects; sample no. = 584
   single site study: University of Southampton
   samples: monthly visits (stable) + exacerbation

# Four Potential Exacerbation Driver Phenotypes

**Acute Exacerbations of Chronic Obstructive Pulmonary Disease**

Identification of Biologic Clusters and Their Biomarkers

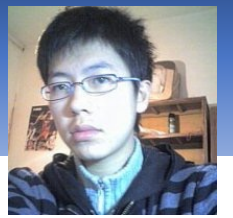
Mona Bafadhel[1,2], Susan McKenna[1], Sarah Terry[1], Vijay Mistry[1,2], Carlene Reid[1], Pranabashis Haldar[2], Margaret McCormick[3], Koirobi Haldar[2], Tatiana Kebadze[4], Annelyse Duvoix[5], Kerstin Lindblad[6], Hemu Patel[7], Paul Rugman[3], Paul Dodson[3], Martin Jenkins[3], Michael Saunders[3], Paul Newbold[3], Ruth H. Green[1], Per Venge[6], David A. Lomas[5], Michael R. Barer[2,7], Sebastian L. Johnston[4], Ian D. Pavord[1], and Christopher E. Brightling[1,2]



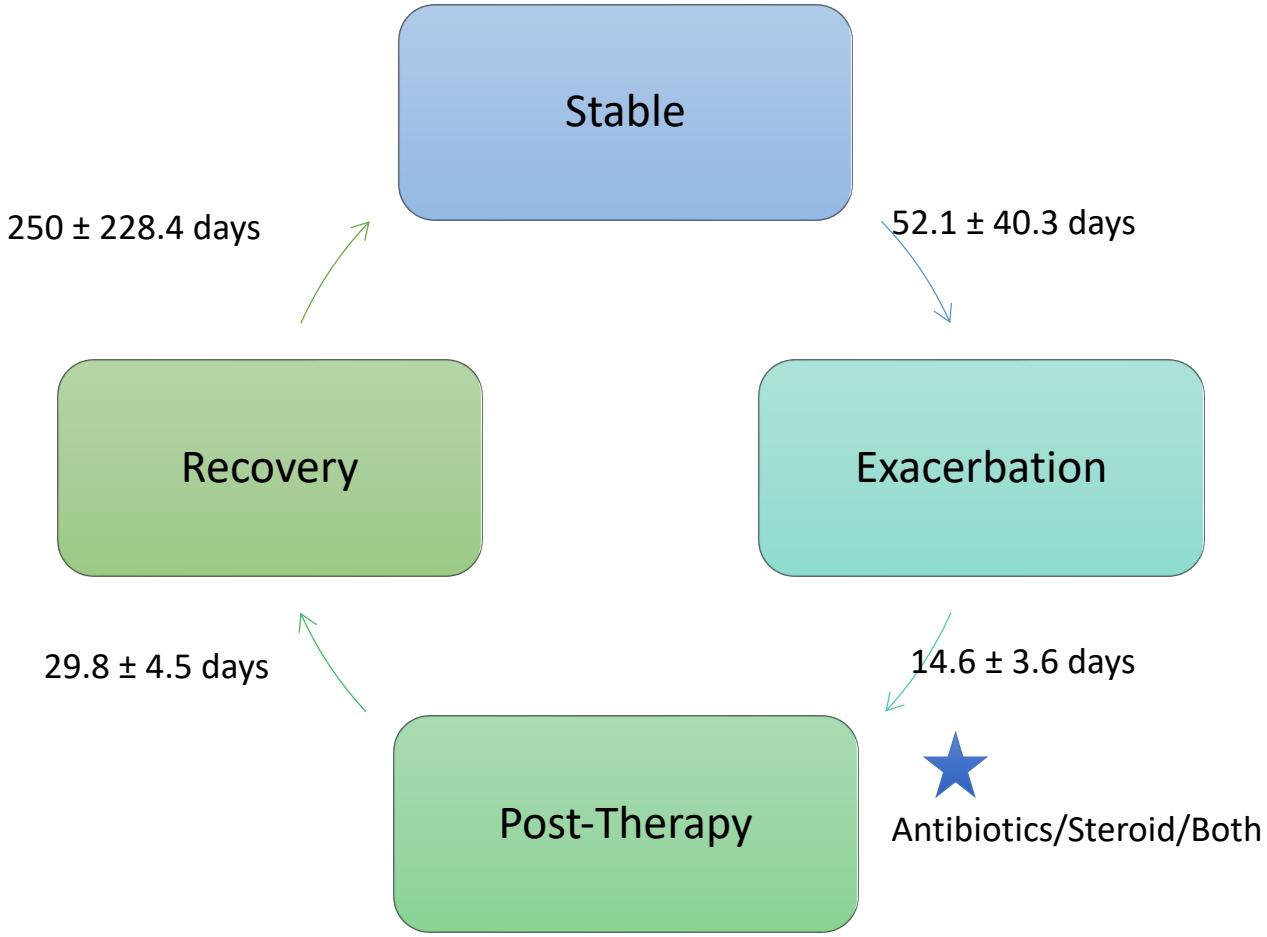

- **Bacterial (B):** positive bacterial pathogen (HI, MC, SP, SA, PA) on routine culture, or total aerobic CFU >= $10^7$ cells (micro_culture1)
- **Viral (V):** positive sputum viral PCR
- **Eosinophil (E):** eosinophil percent >= 3% nonsquamous cells
- **Pauciinflammatory (Pauci):** others, limited changes in the inflammatory profile
- *What changes occur clinically in the lung microbiome across COPD exacerbation phenotypes and treatment regimens?*

Bafadhel et al. 2011 **Am J Respir Crit Care Med** 184: 662

# GSK/U. Leicester COPD Microbiome BEAT Study



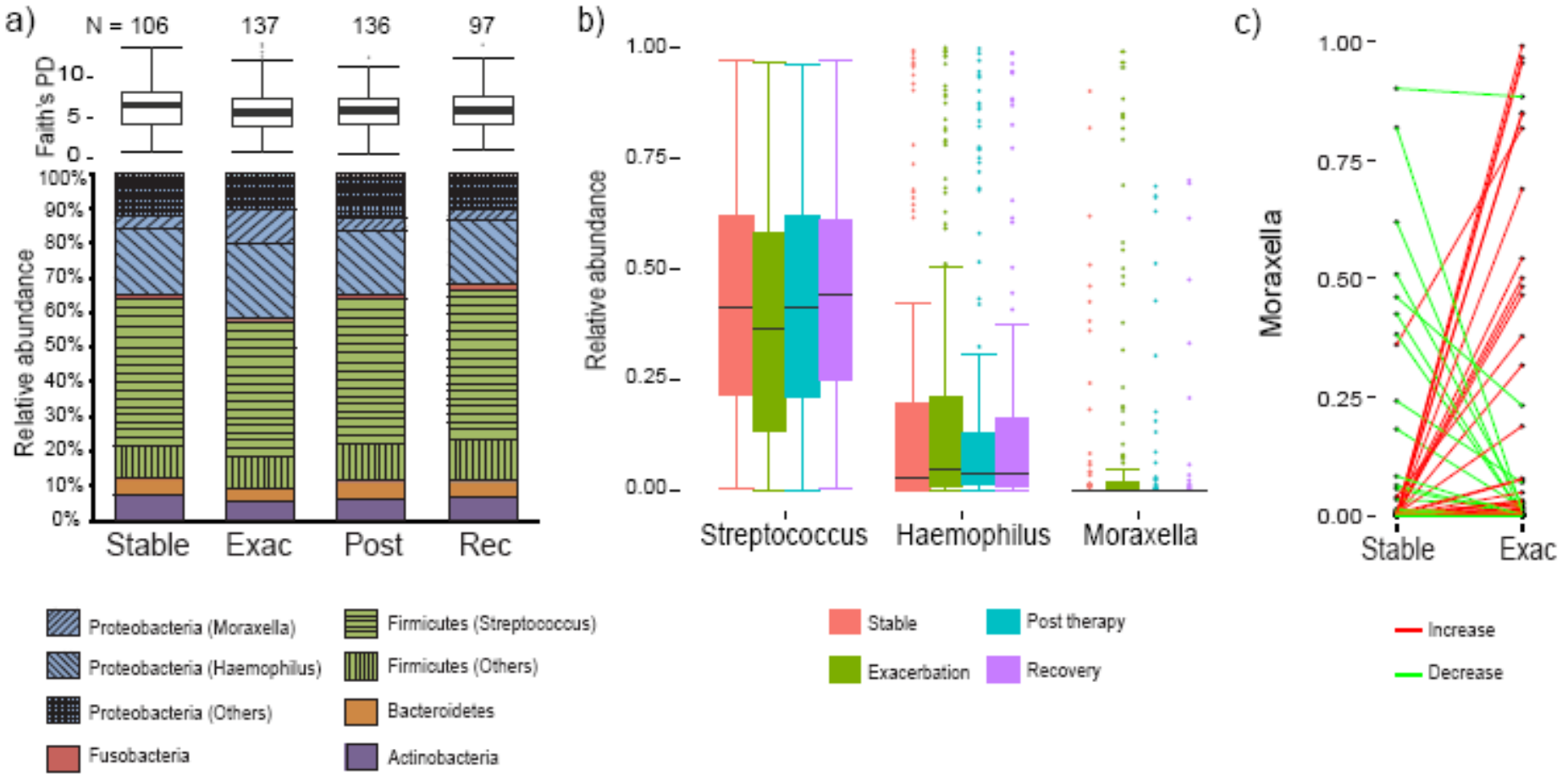
Dr. Zhang Wang, Prof. South China Normal University
Former GSK CB analyst & Early Talent PDF




Wang et al. 2016. **European Resp. J.** 47:1082

- Clinical, viral, bacterial and sputum cell-type data previously published this patient cohort (2008 –10). (Bafadhel et al. 2011. Am J Respir Crit Care Med 184: 662)
- Lung sputum samples collected at stable, exacerbation, post-therapy and recovery time-points:
  - 87 patients
  - 139 visit series
  - 476 sputum samples
- 16S rRNA amplicon sequencing carried out.

# Microbiome Dynamics During Exacerbation Events



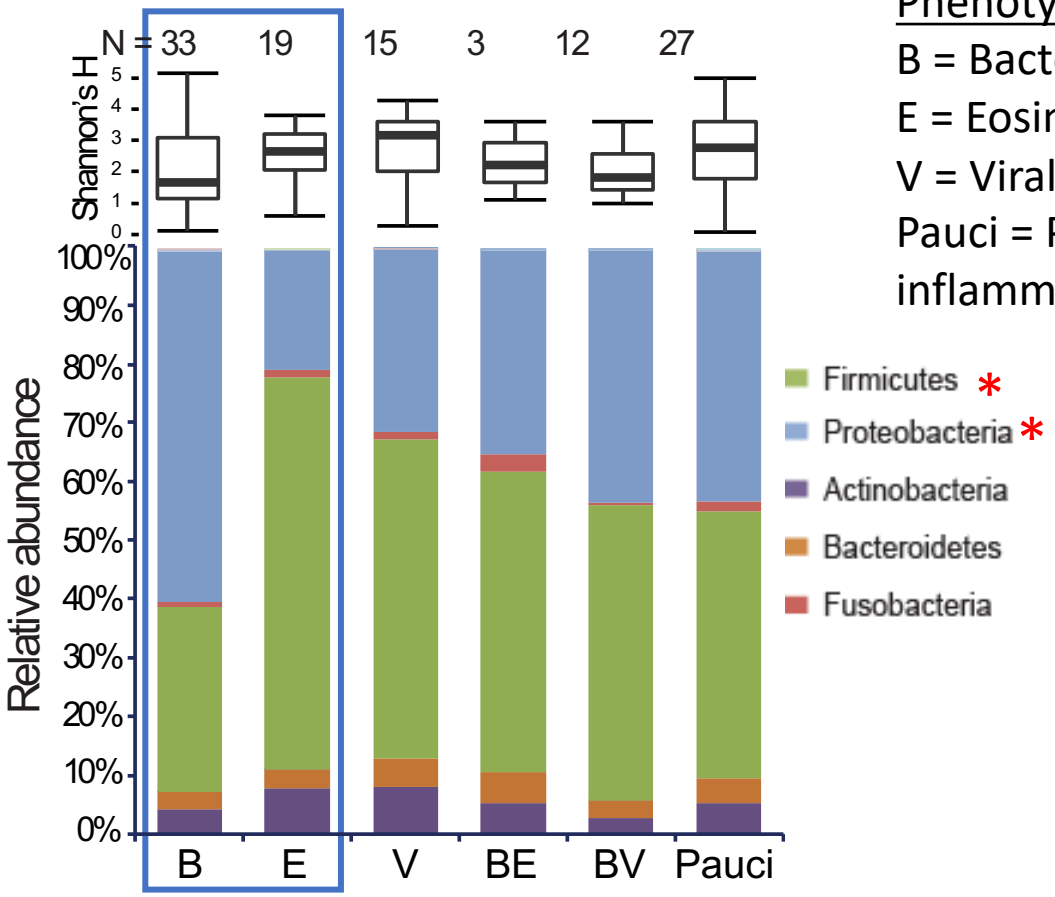

a. Overall reduced alpha diversity during exacerbations

b. Increased ratio of Proteobacteria : Firmicutes driven by increased *Moraxella* sp and decreased *Streptococcus* sp.

c. Subset of 36 out of 87 patients show an increase in *Moraxella* sp.

Wang et al. 2016. **European Resp. J.** 47:1082

# Bacterial vs Eosinophilic Driven Exacerbation Events




**Phenotypes**

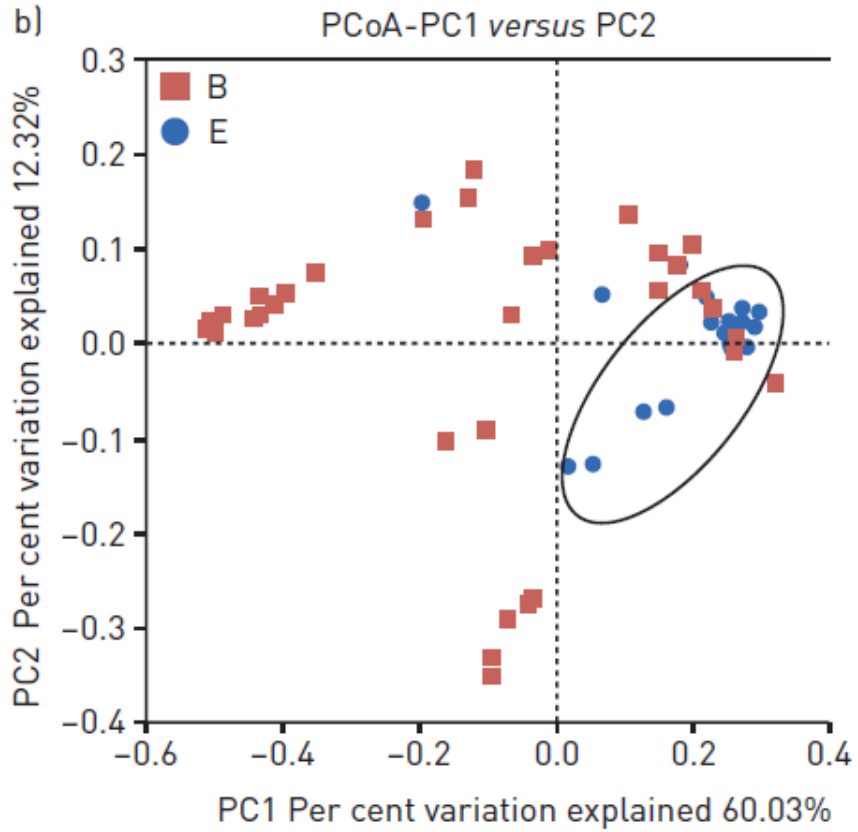
B = Bacterial

E = Eosinophil

V = Viral

Pauci = Pauci-inflammatory

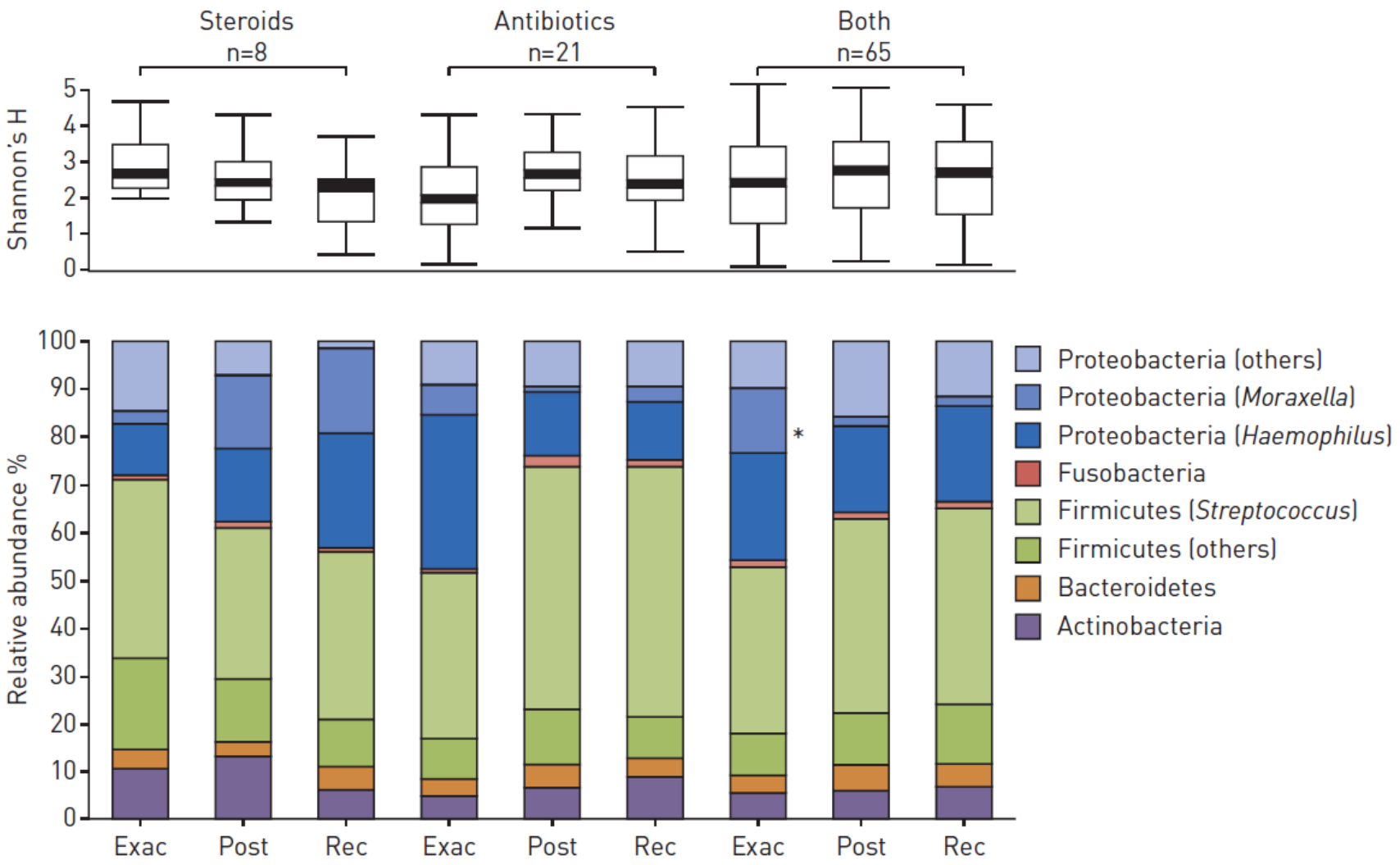
* ANOVA FDR Corrected P < 0.05




- Beta diversity plot of **bacteria (red squares)** and **eosinophil (blue circles)** exacerbations

Wang et al. 2016. **European Resp. J.** 47:1082

# Standard of Care Alters the Microbiome

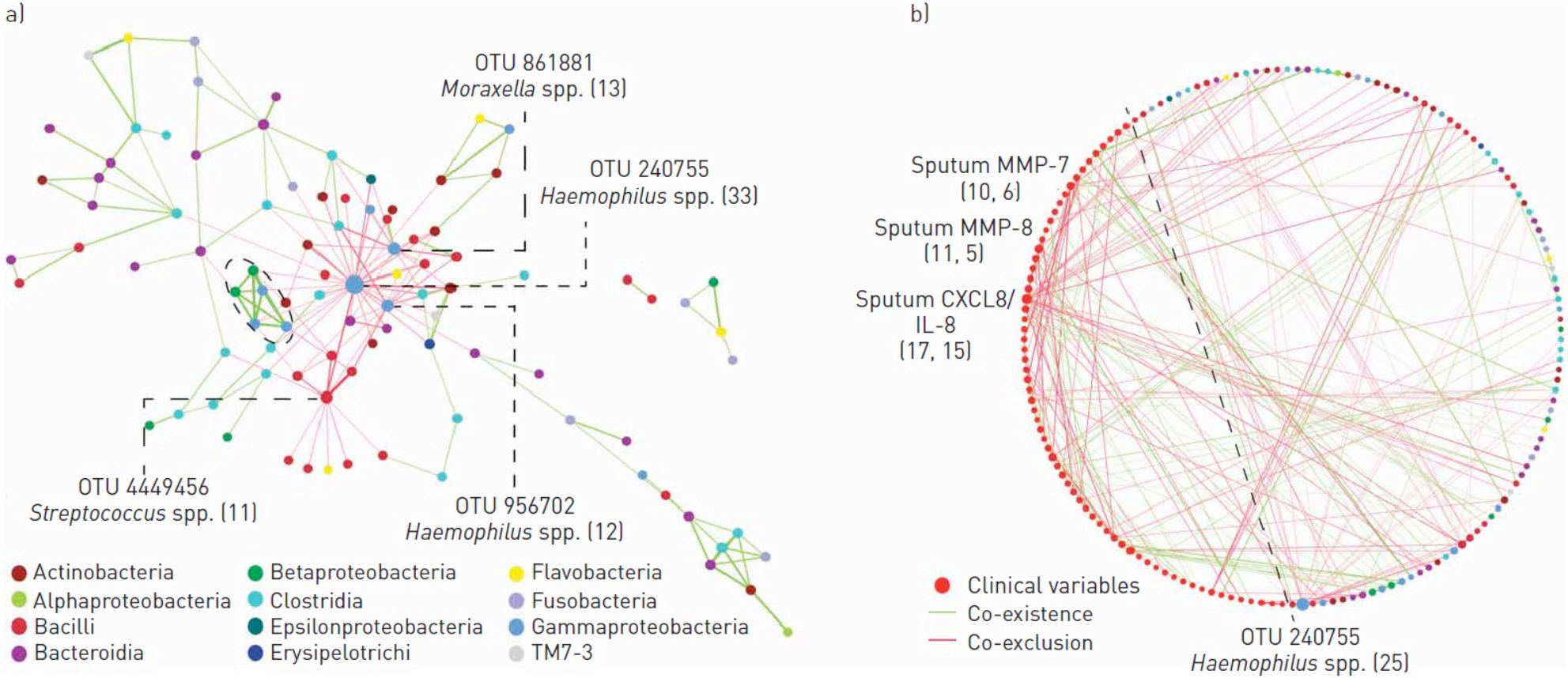



- Steroids and antibiotics have opposite effects on microbiome diversity and composition.
- Steroids decreased diversity and increased proteobacteria genera (i.e., *Moraxella* & *Haemophilus*).
- Antibiotics increased overall diversity by decreasing Proteobacteria.

Wang et al. 2016. **European Resp. J.** 47:1082

# Microbiota Interactions: Self and The Human Host

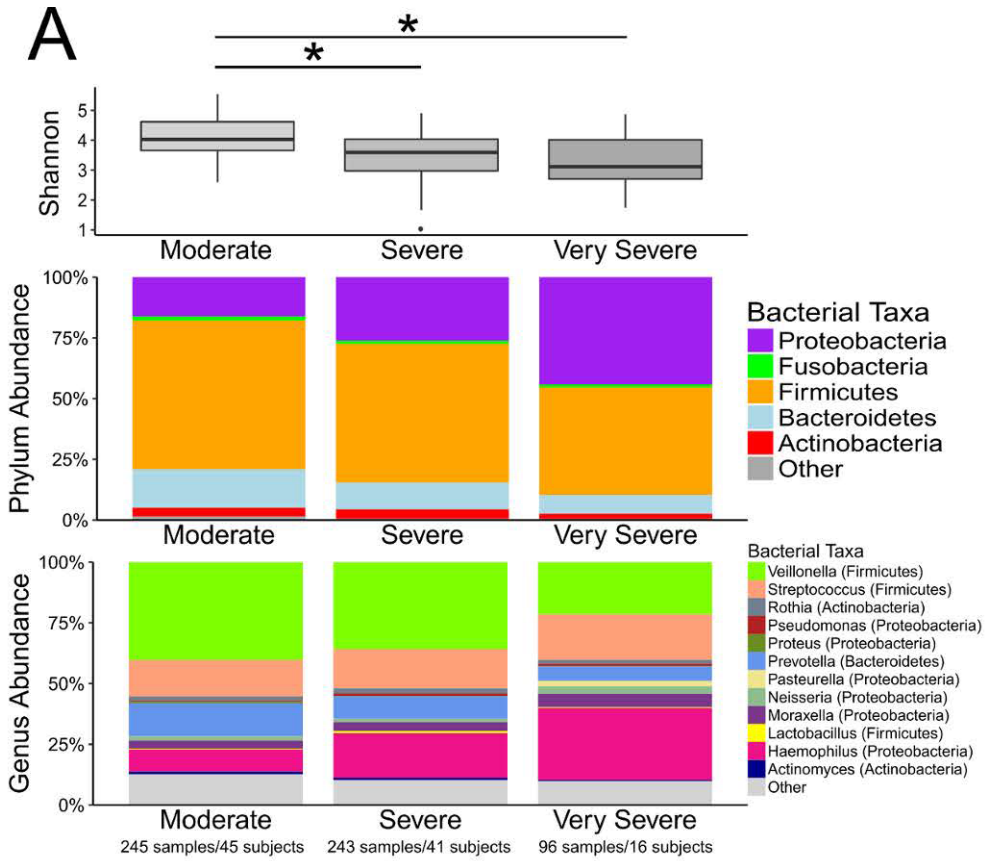
- Bacterial co-existence / co-exclusion suggest *Haemophilus* sp. and *Moraxella* sp. as potential keystone species.
- Sputum IL-8, a pro-inflammatory cytokine, had the highest degree of connectivity being negatively correlated with 15 bacterial OTUs.



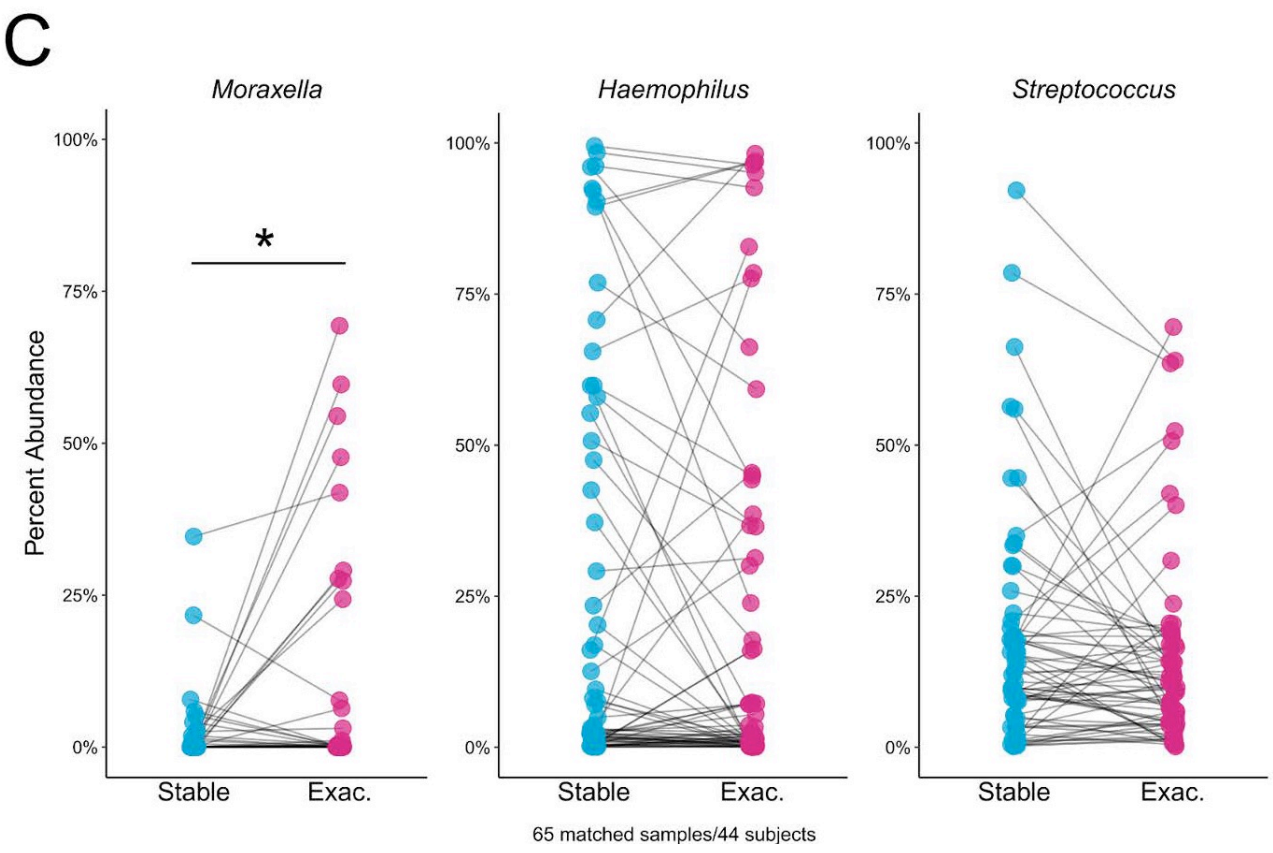

Wang et al. 2016. **European Resp. J.** 47:1082

# AERIS: Monthly Stable + Exacerbation Time Series

- Severe exacerbations associated with an increase in Proteobacteria, decrease in Bacteroidetes and Firmicutes and decreased Shannon diversity.

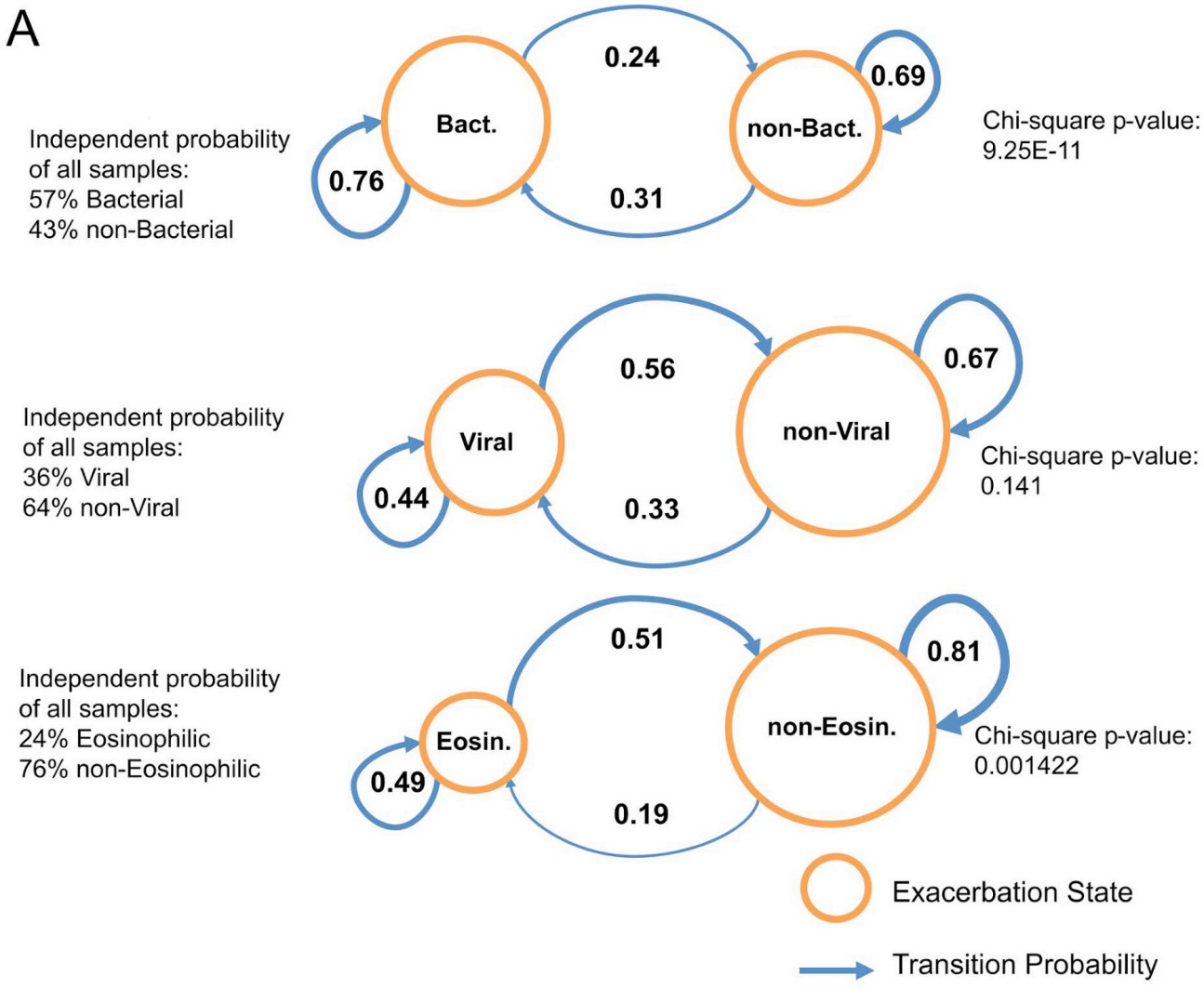



- Within subjects, *Moraxella* relatively increased from stable to exacerbation states.




Mayhew et al. 2018. **Thorax.** 73:422

# AERIS: Probability of Phenotype Transitions




- Markov model shows significantly non-random transition probabilities for bacterial and eosinophilic phenotypes but not viral phenotype.
- For bacterial and eosinophilic exacerbations, the phenotype of the next exacerbation for an individual is more likely to repeat the prior exacerbation phenotype than expected by chance.
- Respiratory viral infections can often proceed worsening bacterial infections.
- Opportunities for precision medicine strategies for COPD treatments based on bacterial vs eosinophilic phenotypes.

Mayhew et al. 2018. **Thorax.** 73:422

# Outline

1. Targeting human host factors for infectious and neural degenerative diseases

2. The lung microbiome in respiratory diseases

3. Multi-omics data analysis of human protein-metabolite interactions

# Metabolites: The Currency of Microbiome Crosstalk with Human Signaling Pathways

## Commensal bacteria make GPCR ligands that mimic human signalling molecules

Louis J. Cohen[1,2], Daria Esterhazy[3], Seong-Hwan Kim[1], Christophe Lemetre[1], Rhiannon R. Aguilar[1], Emma A. Gordon[1], Amanda J. Pickard[4], Justin R. Cross[4], Ana B. Emiliano[5], Sun M. Han[1], John Chu[1], Xavier Vila-Farres[1], Jeremy Kaplitt[1], Aneta Rogoz[3], Paula Y. Calle[1], Craig Hunter[6], J. Kipchirchir Bitok[1] & Sean F. Brady[1]

48 | NATURE | VOL 549 | 7 SEPTEMBER 2017

## A Forward Chemical Genetic Screen Reveals Gut Microbiota Metabolites That Modulate Host Physiology

Haiwei Chen,[1] Phu-Khat Nwe,[2] Yi Yang,[1] Connor E. Rosen,[1] Agata A. Bielecka,[1] Manik Kuchroo,[3] Gary W. Cline,[4] Andrew C. Kruse,[5] Aaron M. Ring,[1] Jason M. Crawford,[2,6] and Noah W. Palm[1,7,*]

Cell *177*, 1217–1231, May 16, 2019

OPEN ACCESS Freely available online

PLOS | BIOLOGY

## Production of α-Galactosylceramide by a Prominent Member of the Human Gut Microbiota

Laura C. Wieland Brown[1,2☯], Cristina Penaranda[3☯], Purna C. Kashyap[4], Brianna B. Williams[1], Jon Clardy[2], Mitchell Kronenberg[5], Justin L. Sonnenburg[4], Laurie E. Comstock[6], Jeffrey A. Bluestone[3*], Michael A. Fischbach[1*]

July 2013 | Volume 11 | Issue 7 | e1001610

(Natural Killer Cell Agonists)

PLOS | ONE

RESEARCH ARTICLE

## Human gut bacteria as potent class I histone deacetylase inhibitors *in vitro* through production of butyric acid and valeric acid

**Samantha Yuille, Nicole Reichardt, Suchita Panda, Hayley Dunbar, Imke E. Mulder***

4DPharma Research Ltd., Aberdeen, United Kingdom

PLOS ONE | https://doi.org/10.1371/journal.pone.0201073 July 27, 2018

MucosalImmunology www.nature.com/mi

ARTICLE

## A screen of Crohn's disease-associated microbial metabolites identifies ascorbate as a novel metabolic inhibitor of activated human T cells

Yu-Ling Chang[1,2], Maura Rossetti[2], Hera Vlamakis[3], David Casero[2], Gemalene Sunga[2], Nicholas Harre[2], Shelley Miller[2], Romney Humphries[2], Thaddeus Stappenbeck[4], Kenneth W. Simpson[5], R. Balfour Sartor[6], Gary Wu[7], James Lewis[8], Frederic Bushman[9], Dermot P. B. McGovern[10], Nita Salzman[11], James Borneman[12], Ramnik Xavier[3], Curtis Huttenhower[3] and Jonathan Braun[2]

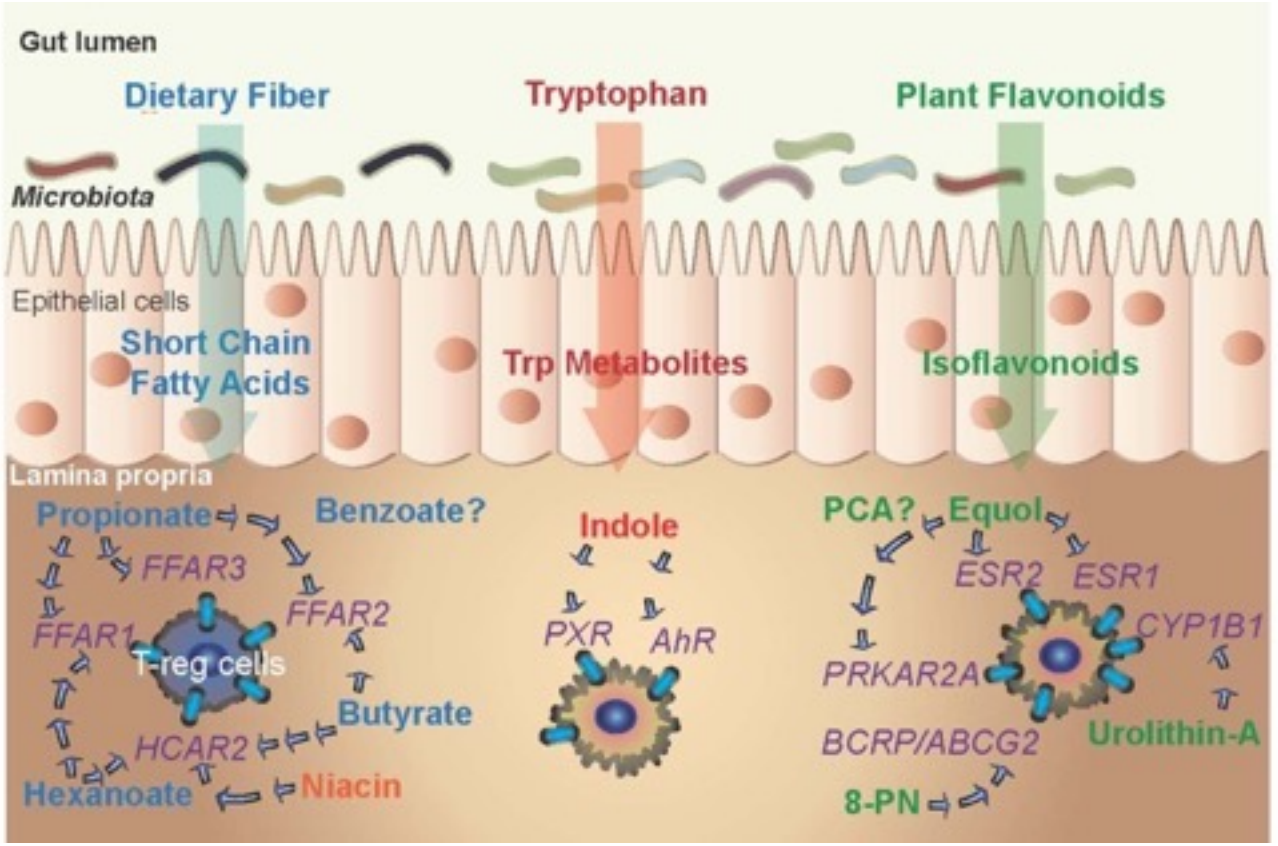
Mucosal Immunol. 2018 Apr 25. doi: 10.1038/s41385-018-0022-7.



34

# The Apothecary Within: Targeting Human-Microbial Crosstalk

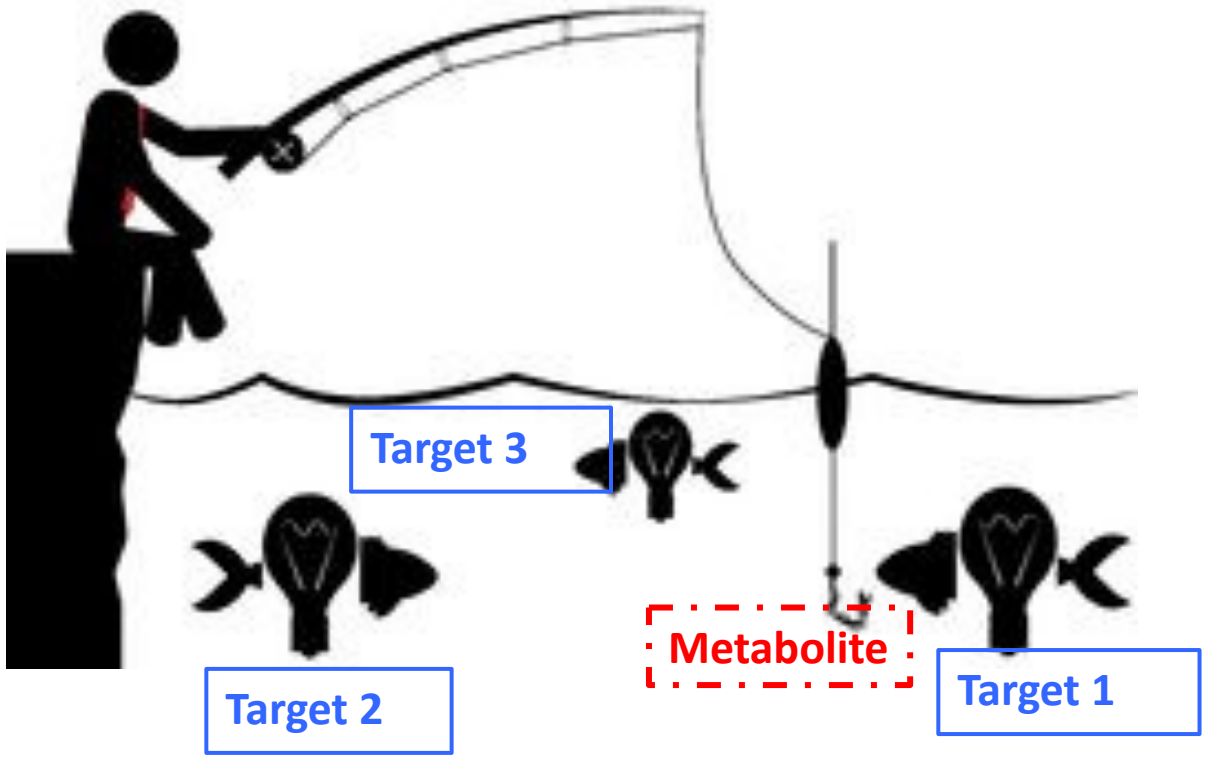
- Microbiome metabolism of dietary fibers generates many diverse metabolites with positive immuno-modulatory effects.
- Metabolites are advantageous starting points for drug discovery:
  - Known modulators of host immunity (i.e., Cohen et al. 2017. **Nature** 549:48).
  - Well-tolerated as endogenous molecules.
  - Evolutionary optimized metabolite-receptor pairing for selectivity and specificity.
  - Many successfully launched drugs have "metabolite-like" properties (Dobson et al. 2009 **Drug Discovery Today** 14:31).
- Challenge: Low-throughput of current experimental approaches to identify potential metabolite ligand-receptor linkages.
- *Can we accelerate the discovery of useful metabolite-protein ligand pairings via in silico hypothesis generation?*



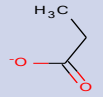
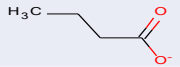
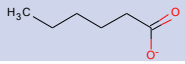
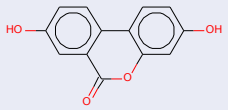
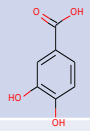
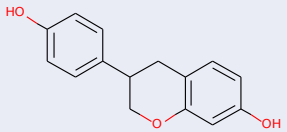
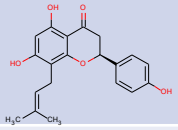
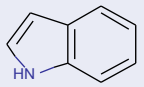

Saha et al. 2016. **Drug Discovery Today** 21:692

# Finding Human Target – Metabolite Ligand Pairings

- ***Question: How do we find the human targets of endogenous metabolites?***
- ***Answer: The experiment has already been done … at least, partially!***
- ***The hypothesis*** – In public and pharma compound-assay databases, there are likely many "metabolite-mimics" with specific annotated human target interaction data
- *"Fish" for drug targets in the "lake" of compound data using similarity to metabolite chemotypes as the "bait"!*



# Metabolites Reported As Immuno-modulators

| Microbial Metabolite | Structure | Known Target (if any) |
|---|---|---|
| Propionate |  | G-protein coupled receptors |
| Butyrate |  | G-protein coupled receptors |
| Hexanoate |  | G-protein coupled receptors |
| Benzoate |  | Unknown |
| Niacin |  | G-protein coupled receptors |
| Urolithin -A |  | Cytochrome P450s family 1B1 |
| Protocatechuic Acid (PCA) |  | Unknown |
| Equol |  | cAMP-protein kinase A<br>Estrogen receptors |
| 8-Prenylnaringenin (8-PN) |  | Unknown |
| Indole |  | Voltage Gated K+ channels |

CCC(=O)[O-]
SMILE String 2D Conversion

> 0.8 Tanimoto score

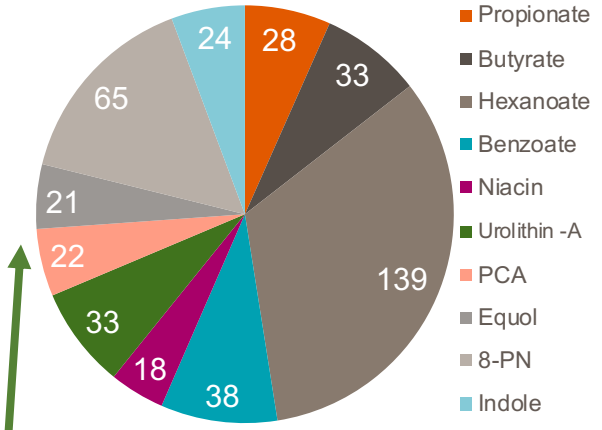
Search GSK 4.5 M compound collection

Retrieve metabolite-mimics

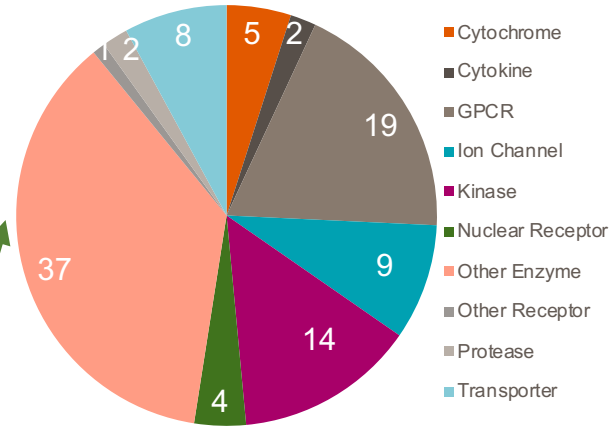
Retrieve associated human protein assay data

Output:
1. Cpds highly similar to microbial metabolites.
2. Their 2D chemical structures.
3. Putative protein ligands.

Dr. Somdutta Saha, Early Talent PDF
Bix Scientist, SpringWorks




421 “metabolite-mimics”




101 putative metabolite-receptor ligand relationships

Saha et al. 2016. Drug Discovery Today. 21:692

# The Human Microbiome Project 2 (HMP2)

- Inflammatory bowel disease (IBD) patients:
  - CD: Crohn's disease
  - UC: Ulcerative colitis
- Multi-omics longitudinal assays:
  - Human host genetics (though underpowered for GWAS)
  - RNASeq from biopsies
  - Metagenome, metatranscriptome, metaproteome & stool metabolome

| | Controls (nonIBD) | Crohn's disease (CD) | Ulcerative colitis (UC) | *Tot* |
|---|---|---|---|---|
| Participants | 26 | 49 | 30 | *105* |
| Metagenomic samples | 429 | 750 | 459 | *1638* |
| Metabolomic samples | 135 | 265 | 146 | *546* |
| RNAseq samples | 51 | 127 | 74 | *252* |


**Dr. Somdutta Saha,**
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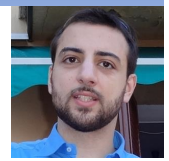
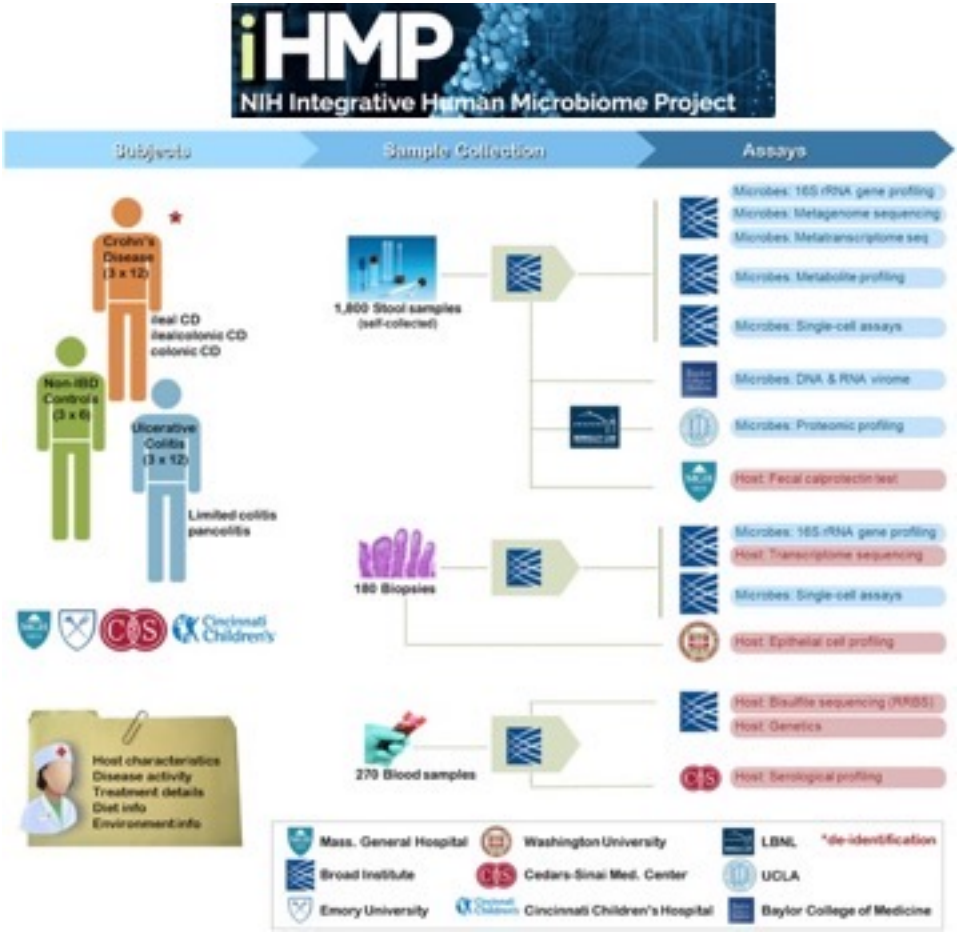
**Bix Scientist, SpringWorks**



**Dr. Andrea Nuzzo,**
**Early Talent PDF;**
**CB Manager at GSK**

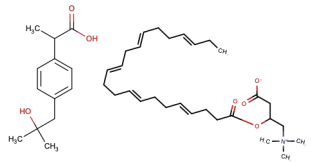




Cell Host & Microbe, 2014, 16:3, 276-289, https://doi.org/10.1016/j.chom.2014.08.014

# Computational and In vitro Validation Workflow

Metabolomics
(548 metabolites verified in HMDB, Human Metabolome Database)



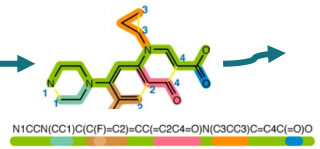
Machine learning (XGBoost)

Relevant metabolites

Genome-wide association studies

GWAS Catalog

biobank uk
Improving the health of future generations



Chemoinformatics
metabolite analogs
Tanimoto similarity ≥ 0.85
Tversky$_{\alpha=0.05}$ similarity ≥ 0.95

Analog cpds

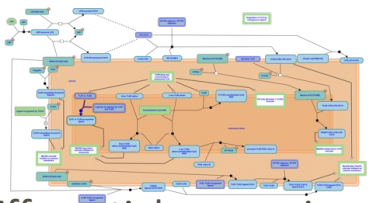
Functional assays databases

gsk

Cpd-target pair w/MOA

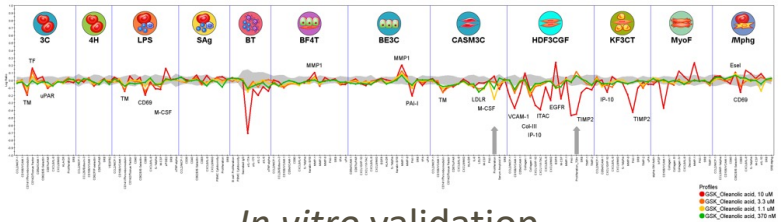
Metabolite-target pair evaluation

Human biopsy transcriptomics
(43870 transcripts)



Differential expression and pathway enrichment analysis

Relevant targets




*In vitro* validation

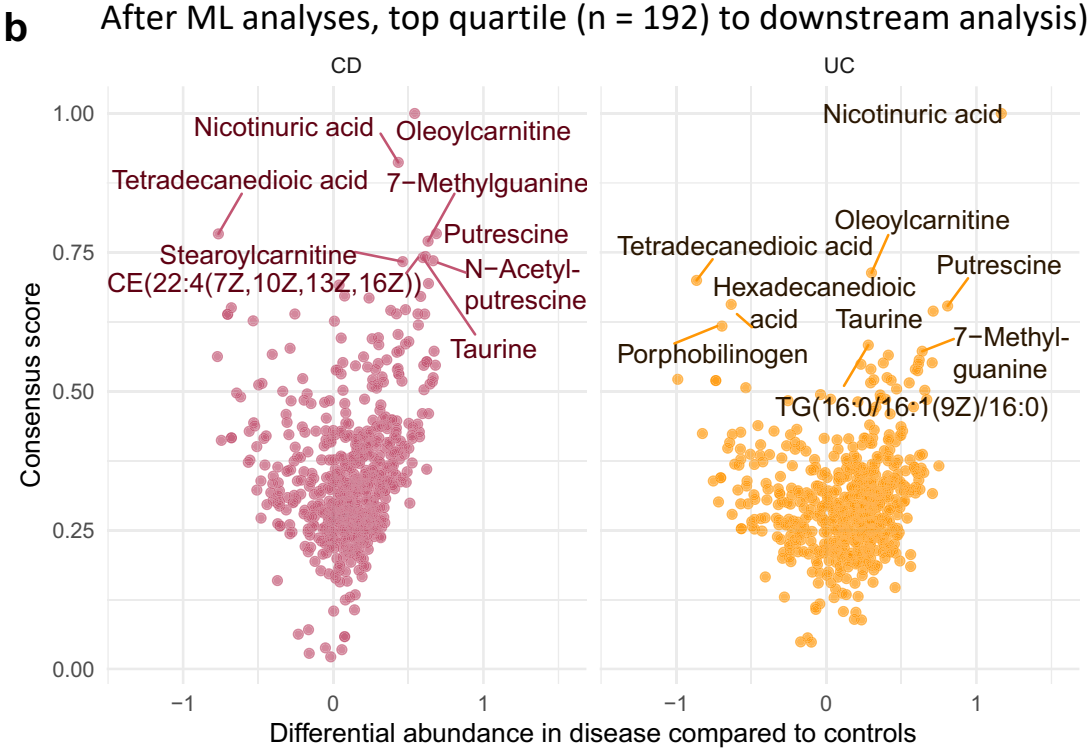
* identified in the Human Metabolome Database [HMDB]

Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

# Metabolomics and Transcriptomics in IBD Samples

## Metabolites

After ML analyses, top quartile (n = 192) to downstream analysis)



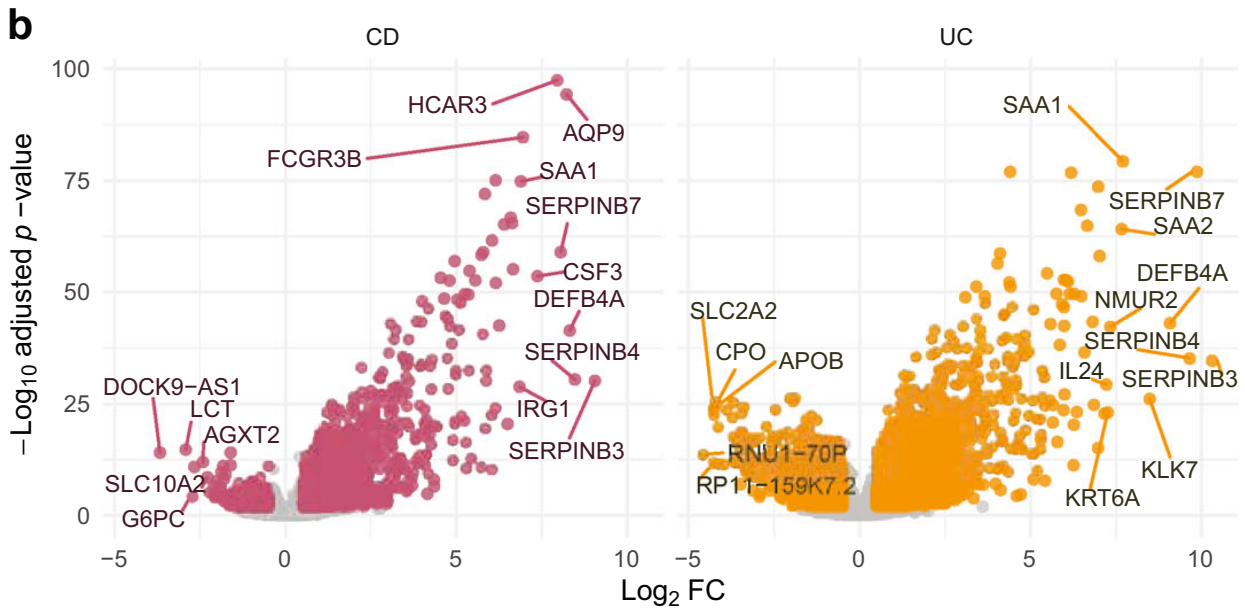

diagnosis

CD

UC

## Gene Expression

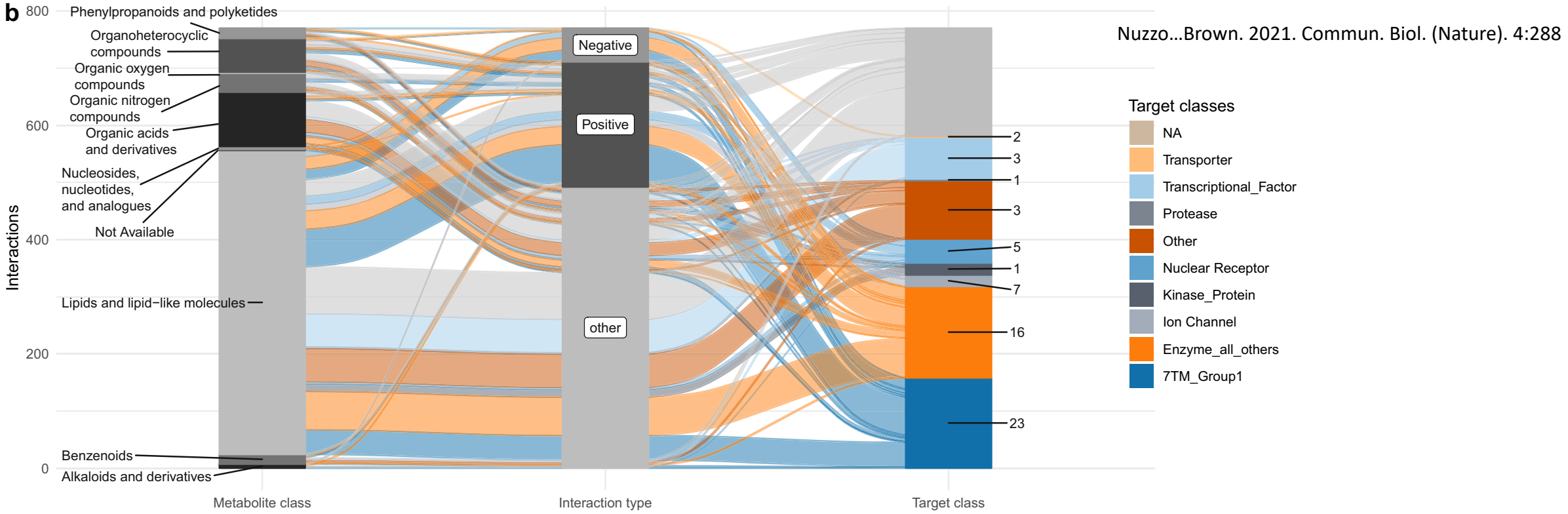
Total DEGs n = 2107 of which 820 DEGs shared between CD & UC




- Differential abundance of metabolites and gene RNA-seq in CD and UC patients compared to non-IBD subjects
- Prioritized known metabolites reported in the Human Metabolome Database.
- Gene transcripts were aligned to Genome Reference Consortium Human Build 37 (GRCh37).

Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

# Connecting Metabolites and Drug Targets

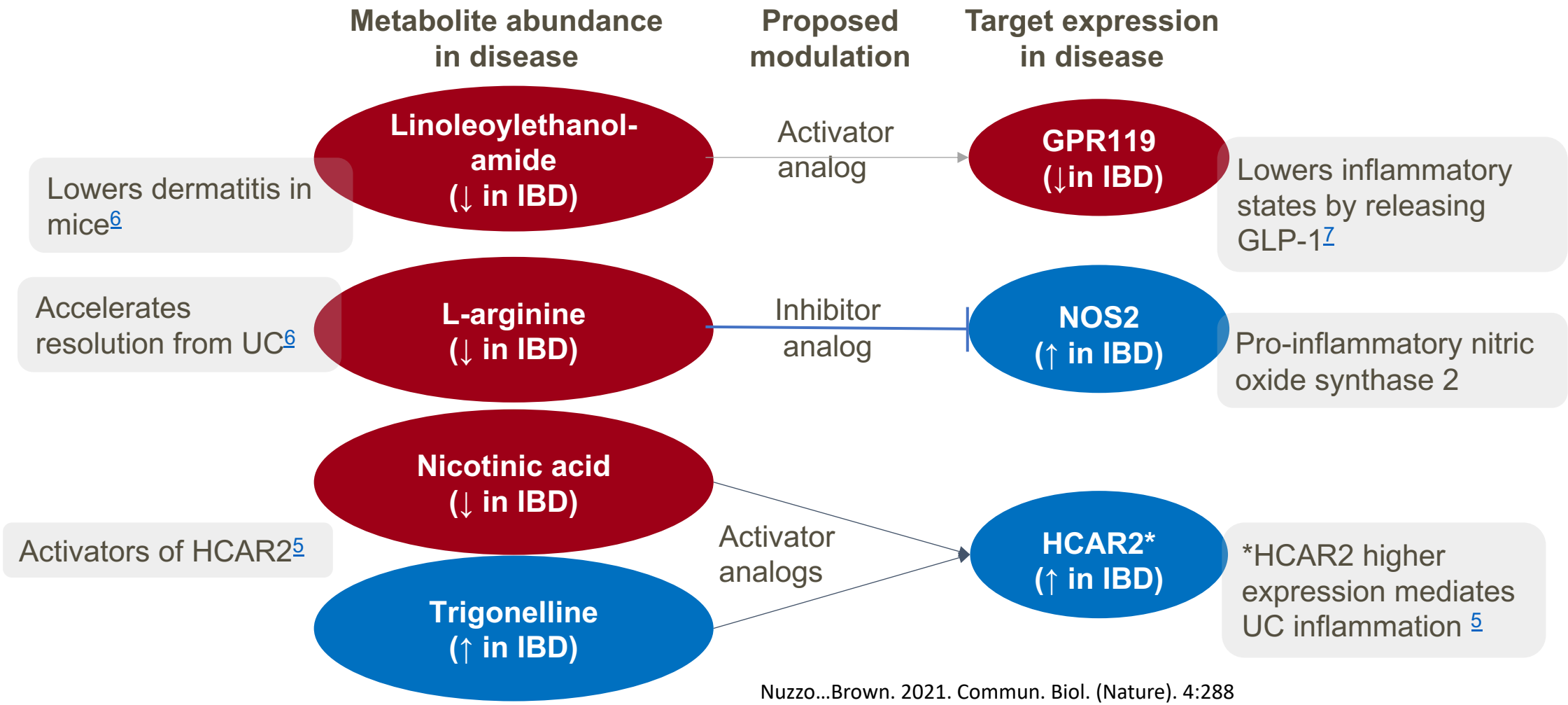



Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

- After filtering, 135 metabolites provisionally connected to 80 perspective proteins.
- Distribution of connections between metabolite classes, modulation type and drug target classes (numbers represent unique targets per drug target class [ *n* = 61]). Some genes and metabolites have multiple interactions)
  - Filtered for metabolite-protein pairs with high binding affinity (i.e., either pIC50 or pEC50 values ≥5.5)
  - Highly pleiotropic metabolites and targets (≥ 20 predicted interactions) were removed.

# Metabolite Co-directionality with Target Gene Expression

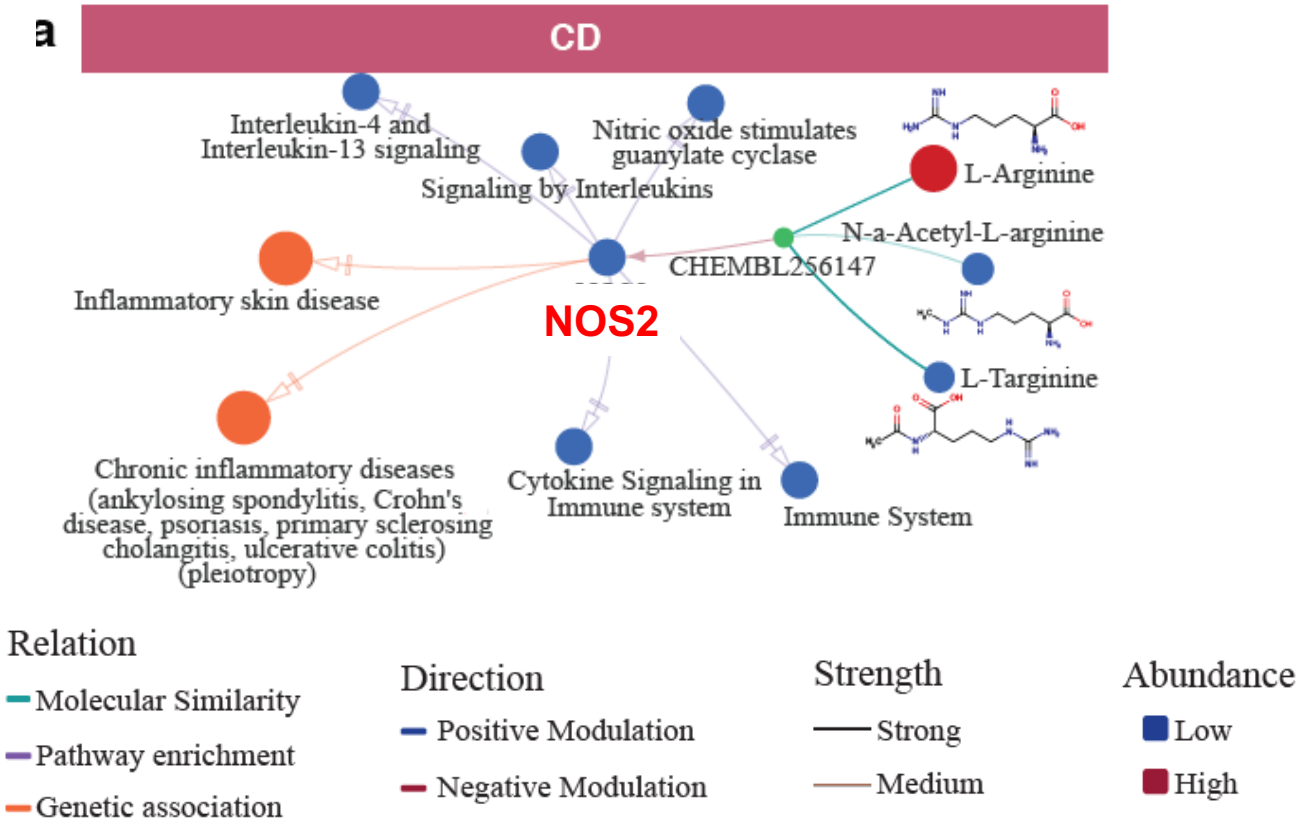
- Reversing transcriptomic disease signature using candidate modulators

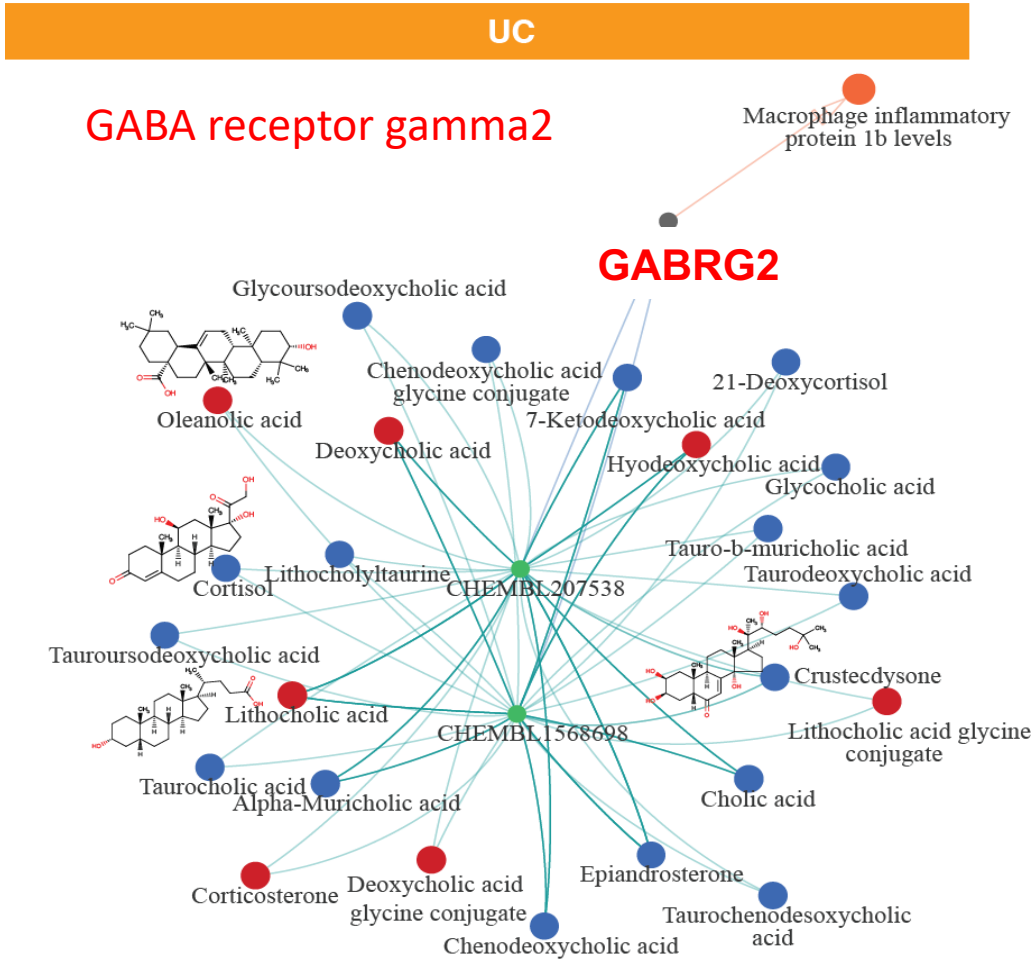



Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

# Linkages to Disease Genetics

- Metabolites passing thresholds and tractable targets with genetic evidence
- Retrieved 808 genes with genetic associations to IBD
- Identified 464 potential pairings between genetic targets with metabolite modulators, 13 with known modulation mechanisms



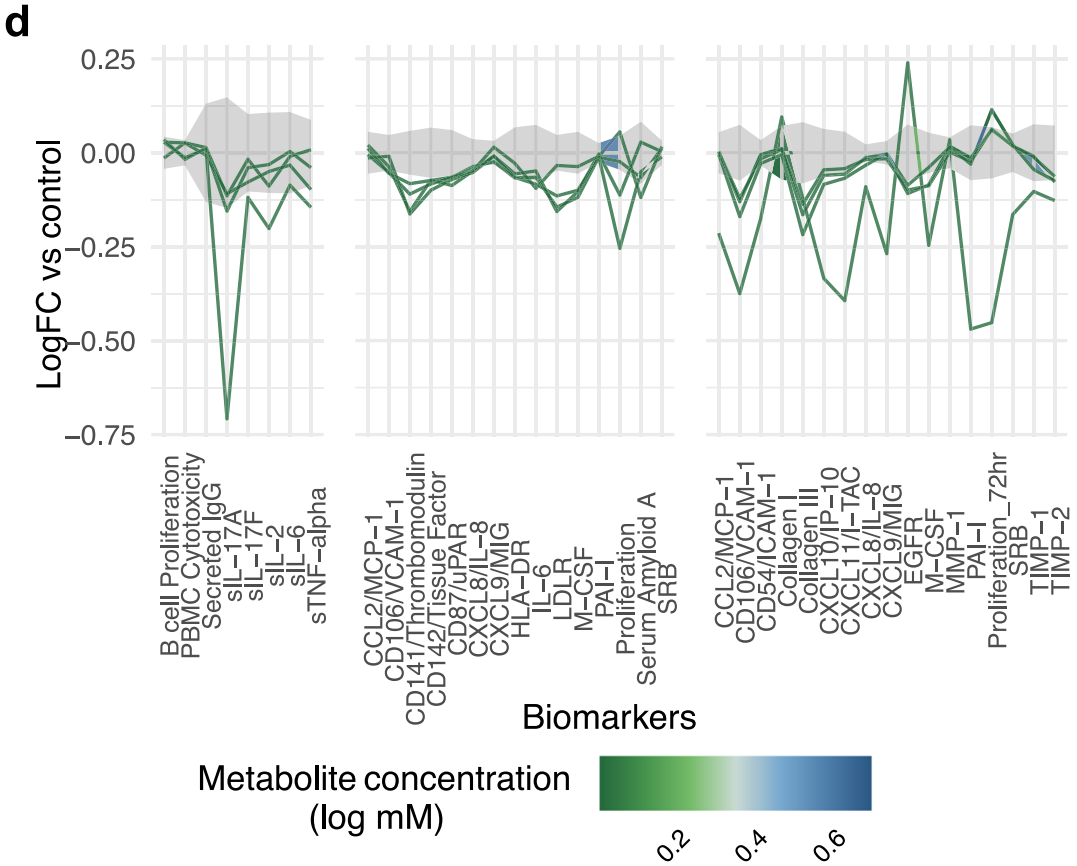



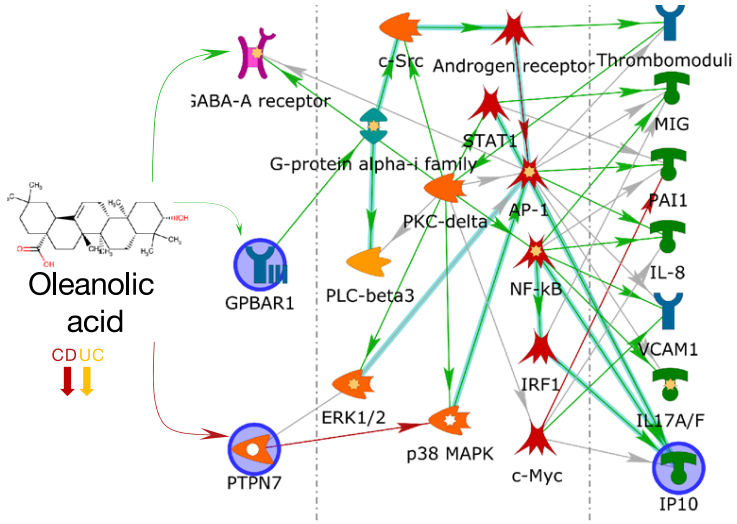

Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288

# in vitro Validation Assays for Selected Metabolites

- Selected 11 metabolites for profiling in human primary cell-based phenotypic assays (BioMAP® Diversity PLUS panel)
- 8 metabolites showed significant modulation of immune biomarkers in one or more cellular systems.
- Summary
  - 135 metabolites provisionally connected to 80 different targets in IBD
  - 983 potential metabolite-target interactions identified
  - Immuno-modulating metabolites and targets are potential starting points for drug discovery

Nuzzo…Brown. 2021. Commun. Biol. (Nature). 4:288




- Oleanolic acid (OA) showed activity in T-cell dependent B-cell activation (BT), coronary artery smooth muscle (CASM3C), fibroblasts (HDF3CGF) assays
- OA is a connected ligand of GABRG2, PTPN7 and GPBAR1

# Summary and Future Directions

- Multi-omics analyses of human-microbe interactions can assist in drug discovery:
  - New targets
  - Mechanism of action
  - Biomarkers
  - Drug repositioning
  - Precision medicine – Identify potential disease subtypes in patient populations
  - Find common targets and pathways across diverse disease etiologies
- Future areas
  - New frontier for AI enabled target discovery:
    - Growing number of Large language models (LLMs) trained on diverse chemical (compounds), biological (DNA, mRNA and proteins) and clinical datasets.
    - Understanding “feature” selection and the underlying drivers of AI model predictions are important for confidence-building as well as furthering biomedical insights and innovation.
    - Need to benchmark AI predictions with standardized multi-omics analytical approaches as well as biological results.
- For any computational method, it is essential to have experimental and clinical validation.
  - Use biological relevant datasets to improve future algorithms and pipelines.

# Acknowledgments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