Exploring Gut Microbiota as a Source of Potential Biomarkers: Initial Results from the ANAVEX®2-73 Alzheimer's Disease Clinical Study

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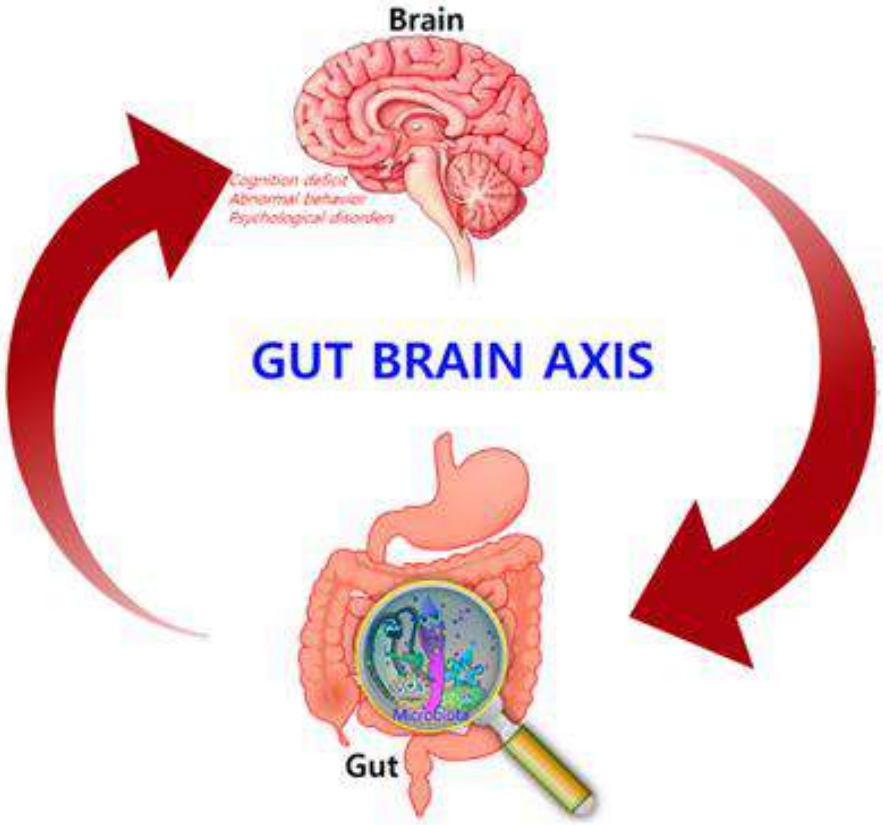
AAIC July 14-18, 2019

Disclosures

- MA is an employee and shareholder of Ariana Pharmaceuticals
- FP, AE and CW are employed by Ariana Pharmaceuticals
- CM is an employee and shareholder of Anavex Life Sciences

Brain-Gut-Microbiota Axis …

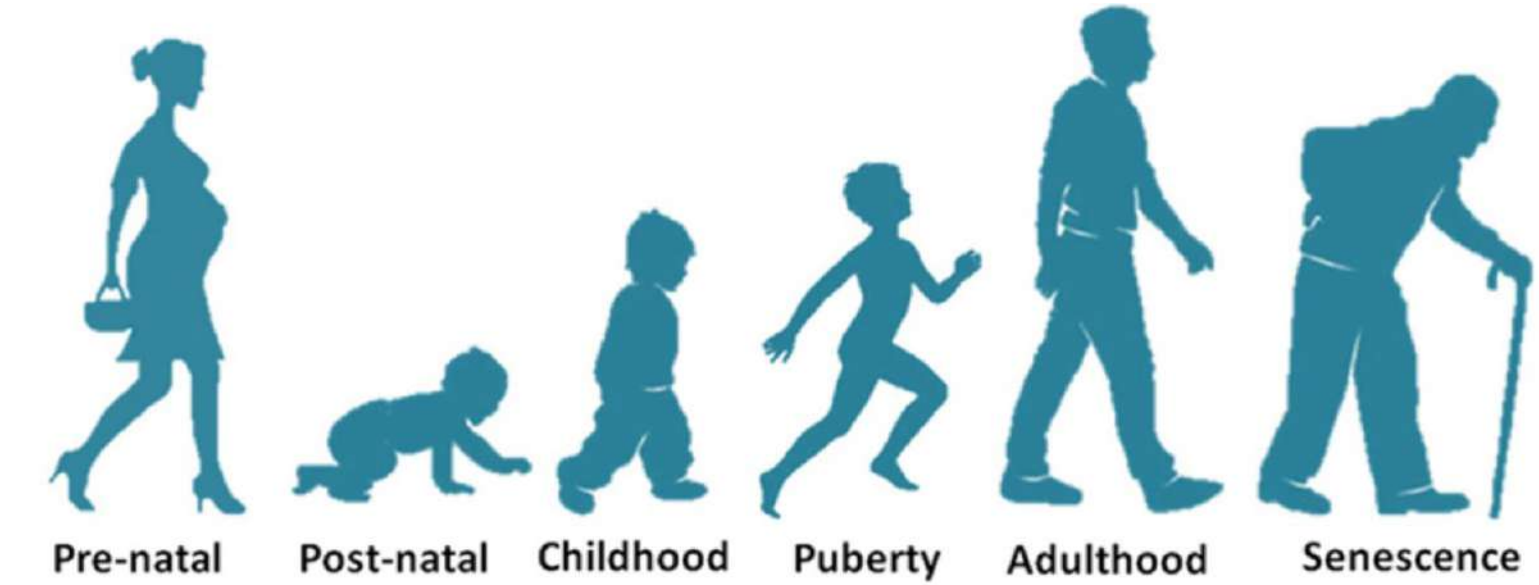
- Gut microbiota has been implicated in the maturation and modulation of the host immune response
- One of the hallmarks of aging comprises of decrease gut microbiota diversity. Disturbances in gut microbiota communities have been linked with several (age-related) neurological conditions, including depression, Alzheimer's disease, and Parkinson's disease (Calvani et al., 2018)
- More than 100 million years of mammalian–microbial coevolution have shaped a life-long interdependency



Giau et al. (2018)

Brain-Gut-Microbiota Axis: Gut microbiota modulating brain morphology and function from birth to old age

Disturbances of the Brain-Gut-Microbiota Axis in Alzheimer's Disease



Microbial diversity

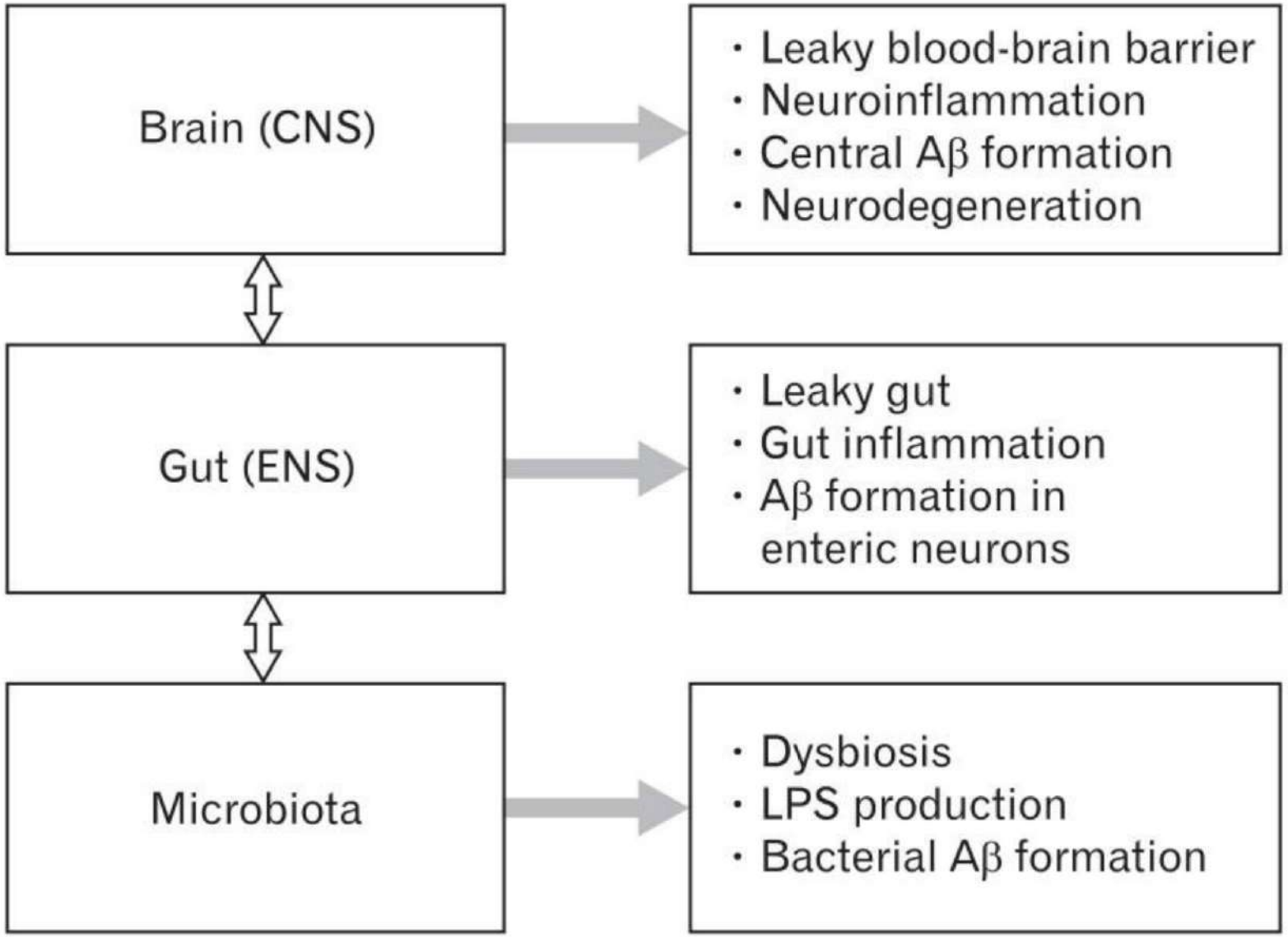
Neuronal proliferation
Neuronal migration
Dendritic arborization
BBB development
Microglia priming

Neuronal and microglia homeostasis

Neuronal loss
Synaptic loss
Loss of BBB integrity
Neurodegeneration
Microglia alterations

Calvani et al. (2018)

Disturbances of the Brain-Gut-Microbiota Axis in Alzheimer's Disease



Disturbances along the brain-gut-microbiota axis, including the central nervous system (CNS) and the enteric nervous system (ENS), contribute to the pathogenesis of Alzheimer's disease. The gut microbiota is known to upregulate local and systemic inflammation due to lipopolysaccharides (LPS) from pathogenic bacteria and synthesis of proinflammatory cytokines. Alterations in the gut microbiota composition may induce increased permeability of the intestinal barrier and the blood-brain barrier further enhancing inflammation at the gut, systemic and CNS levels. Amyloid beta (Aβ) formation takes place in the ENS and the CNS. In addition, a large amount of amyloids is secreted by the gut microbiota.

Kowalski and Mulak (2019)

Disturbances along the Brain-Gut-Microbiota Axis …

… including the CNS contribute to the pathogenesis of Alzheimer's disease

- Alterations in the gut microbiota composition
- induce increased permeability of the gut barrier and immune activation leading to systemic inflammation
- which in turn may impair the blood-brain barrier and promote neuroinflammation, neural injury, and ultimately neurodegeneration

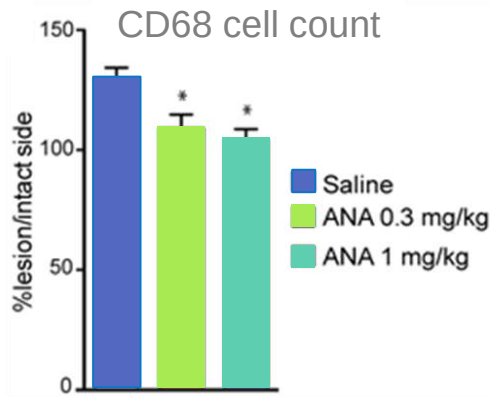
The gut microbiota is known to upregulate local and systemic inflammation from pathogenic bacteria and synthesis of proinflammatory cytokines

Giau et al. (2018)

SIGMAR1 *Restores* Homeostasis Caused by Neuro-inflammation

- Numerous studies demonstrate beneficial effects of SIGMAR1 (S1R) agonists on neuro-inflammation:
  - S1R expressed in microglia, modulate microglial activation and dampen neuroinflammation[1]
  - In rat microglial cultures, S1R agonist reduces the ability of microglia to release TNF-α, IL-10, and NO in response to ATP, MCP-1, and lipopolysaccharides (LPS)[2]
  - S1R ligands improved microglial cell survival during ischemia or Aβ exposure in primary microglia cultures[3]
- The S1R agonist ANAVEX®2-73 could potentially normalize neuroinflammatory processes by several different mechanisms:
  1. Reducing microglia over-activation[4]
  2. Reducing inflammatory cytokines[5]
  3. Increasing anti-inflammatory cytokines[6]
  4. Releasing protective factors, e.g. BDNF[7]
  5. Protect against inflammatory molecules[8,9]

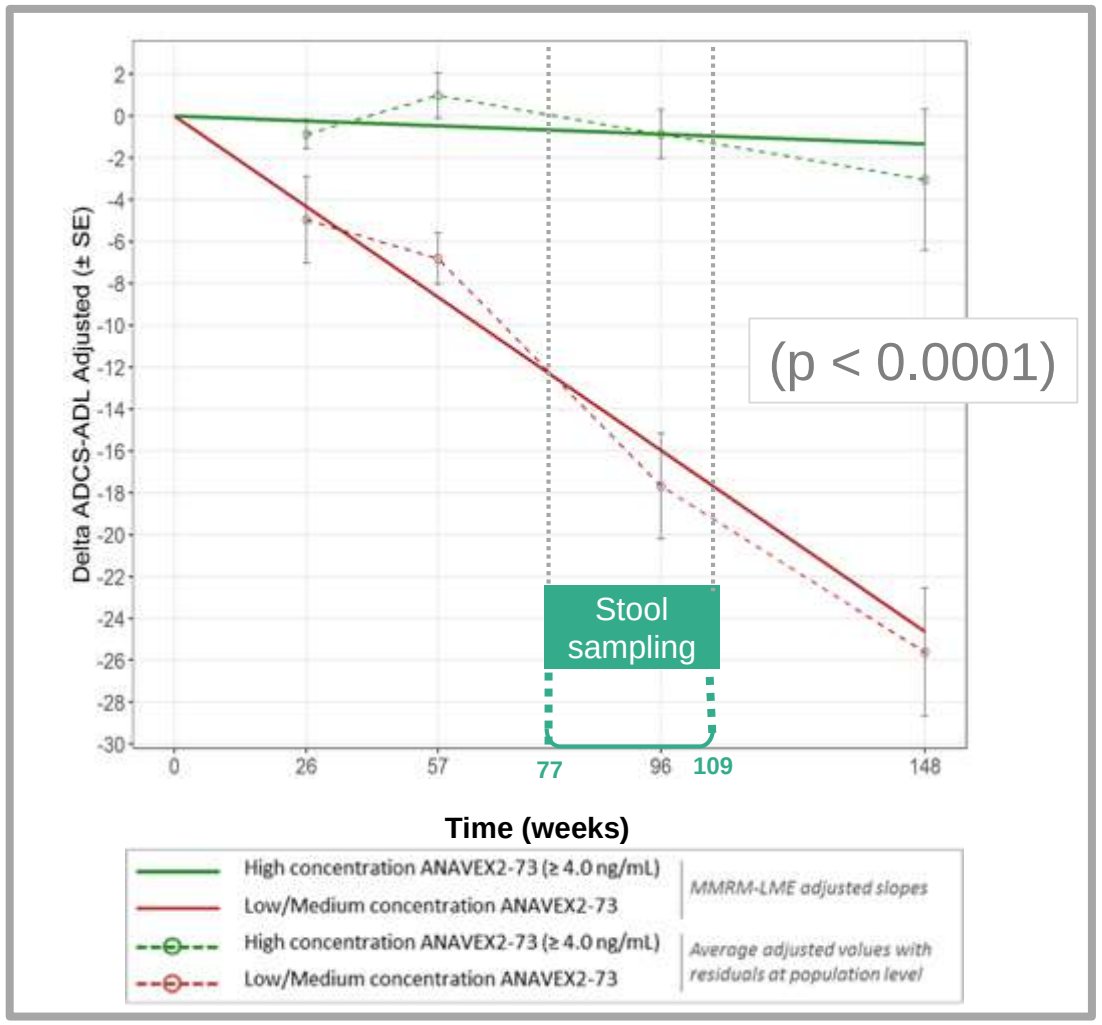
ANAVEX®2-73 reduces microglia over-activation



ANAVEX®2-73 significantly decreased the expression of CD68 (marker of activated microglia) in the substantia nigra in a model of Parkinson's disease[4]

1) Jia J et al 2018. Front Cell Neurosci. 2018 Sep 20;12:314; 2) Zhao J et al 2014. Invest. Ophthalmol. Vis. Sci. 55, 3375–3384
3) Behensky AA et al 2013. J. Pharmacol. Exp. Ther. 347, 458–467; 4) Cenci A et al 2016. Presented at World Parkinson Congress
5) Hall H et al 2018. Alzheimers Dement. Jun;14(6):811-823; 6) Allahtavakoli M et al 2011. Brain Res Bull. May 30;85(3-4):219-24
7) Cogram P et al 2016. Presented at Gordon Research Conference; 8) Lisak RP et al 2016. Poster presentation at ACTRIMS
9) Lisak RP et al 2017. Oral presentation at ECTRIMS

ANAVEX®2-73 Selective Sigma-1 Receptor (SIGMAR1) Agonist Demonstrated Improved ADCS-ADL Scores in Phase 2a AD Study through 148 Weeks



Source: Hampel H., Afshar M., Parmentier F. et al., CTAD 2018

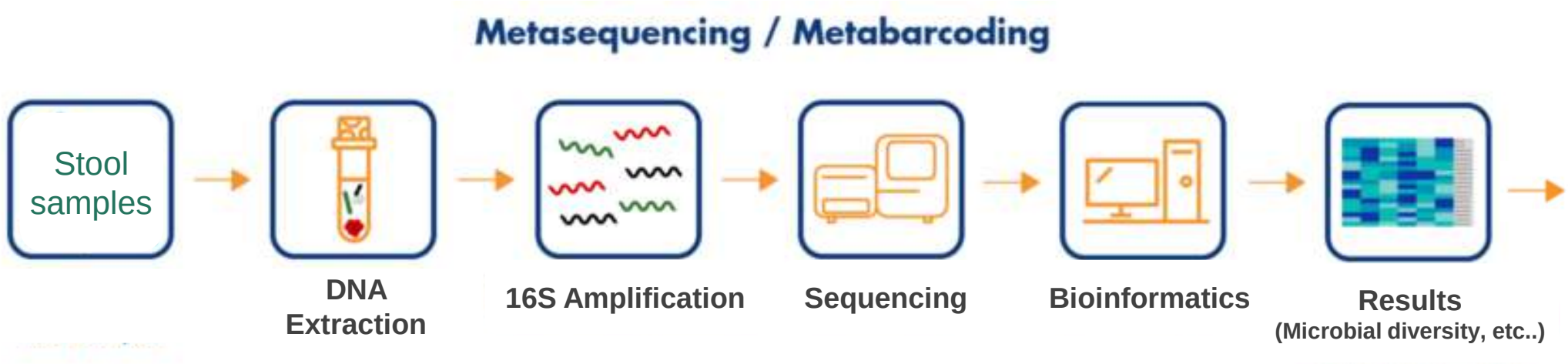
Patients Treated with Higher ANAVEX®2-73 Concentration Maintain ADCS-ADL* Performance vs Lower Concentration Cohort (88 % difference)

- High plasma concentration of ANAVEX®2-73 [>4.0 ng/ml] is correlated with the clinically administered dose
- In addition to concentration, the significant covariates identified in MMRM-LME model are:
  - **SIGMAR1** (p<0.0080),
  - **COMT** (p<0.0014)

Out of 21 patients in the extension study, microbiota analysis was performed on 16 patients who consented to stool sampling (1 patient withdrew from study, 4 patients did not consent)

** Alzheimer's Disease Cooperative Study Group - Activities of Daily Living Inventory (ADCS-ADL)*

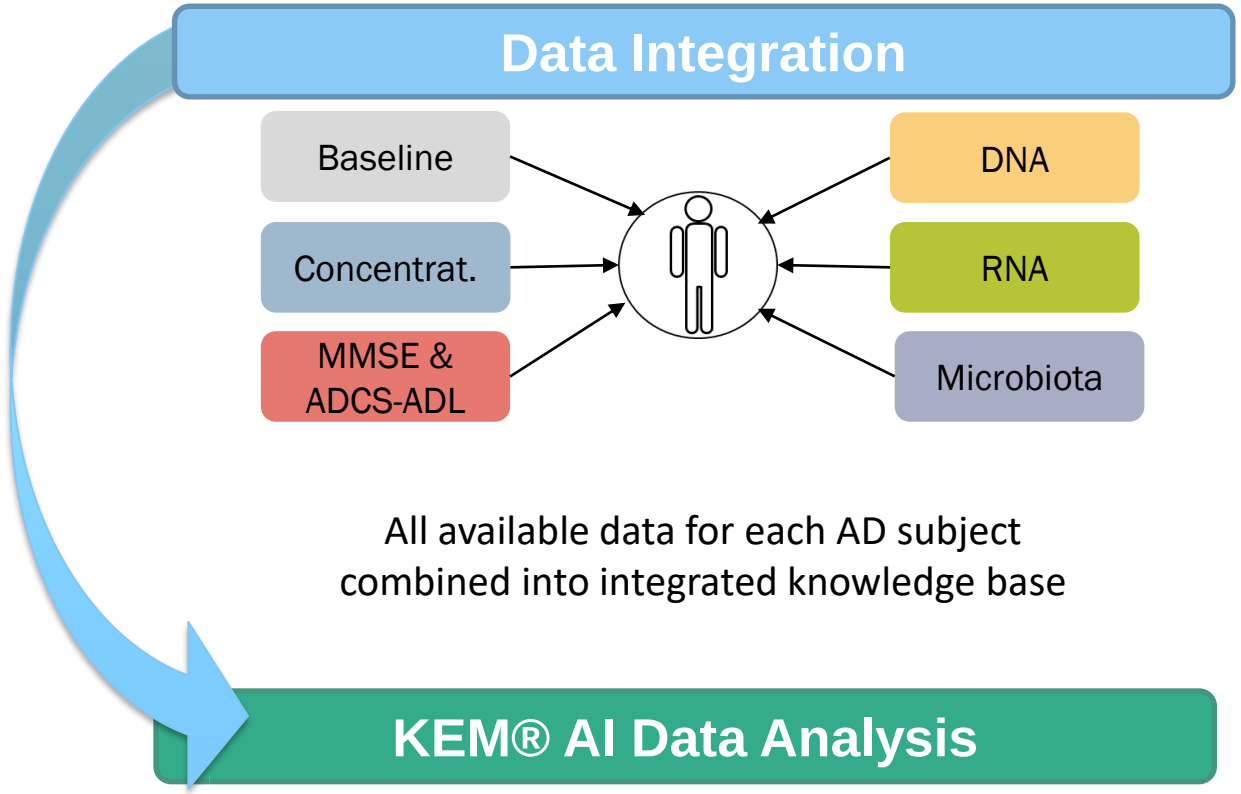
ANAVEX®2-73 Phase 2a AD Stool Sample Protocol Overview

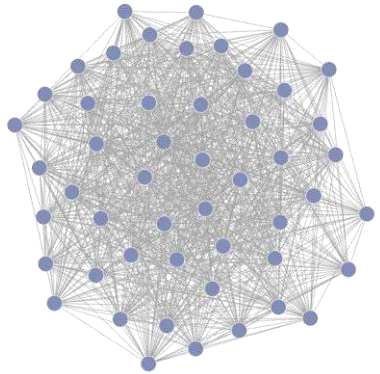


- 1 stool collection event per patient, between week 77 and week 109 (variability caused by patient's agreement and visit schedule)
- 16 patients consented to sampling
- Samples sent to stool analysis lab for sequencing
- Abundance of each microbiota genus/family/phylum is assessed using 16S meta-sequencing
- A dedicated bioinformatics pipeline was used for taxonomic classification of sequences; abundances measurement of operational taxonomic units (OTU) were mapped to phylums, families and genera of gut microbiota

Gut Microbiota Data Integrated and Analyzed using Artificial Intelligence Platform KEM®

Translation of Precision Medicine Paradigm from Oncology to Alzheimer's Disease



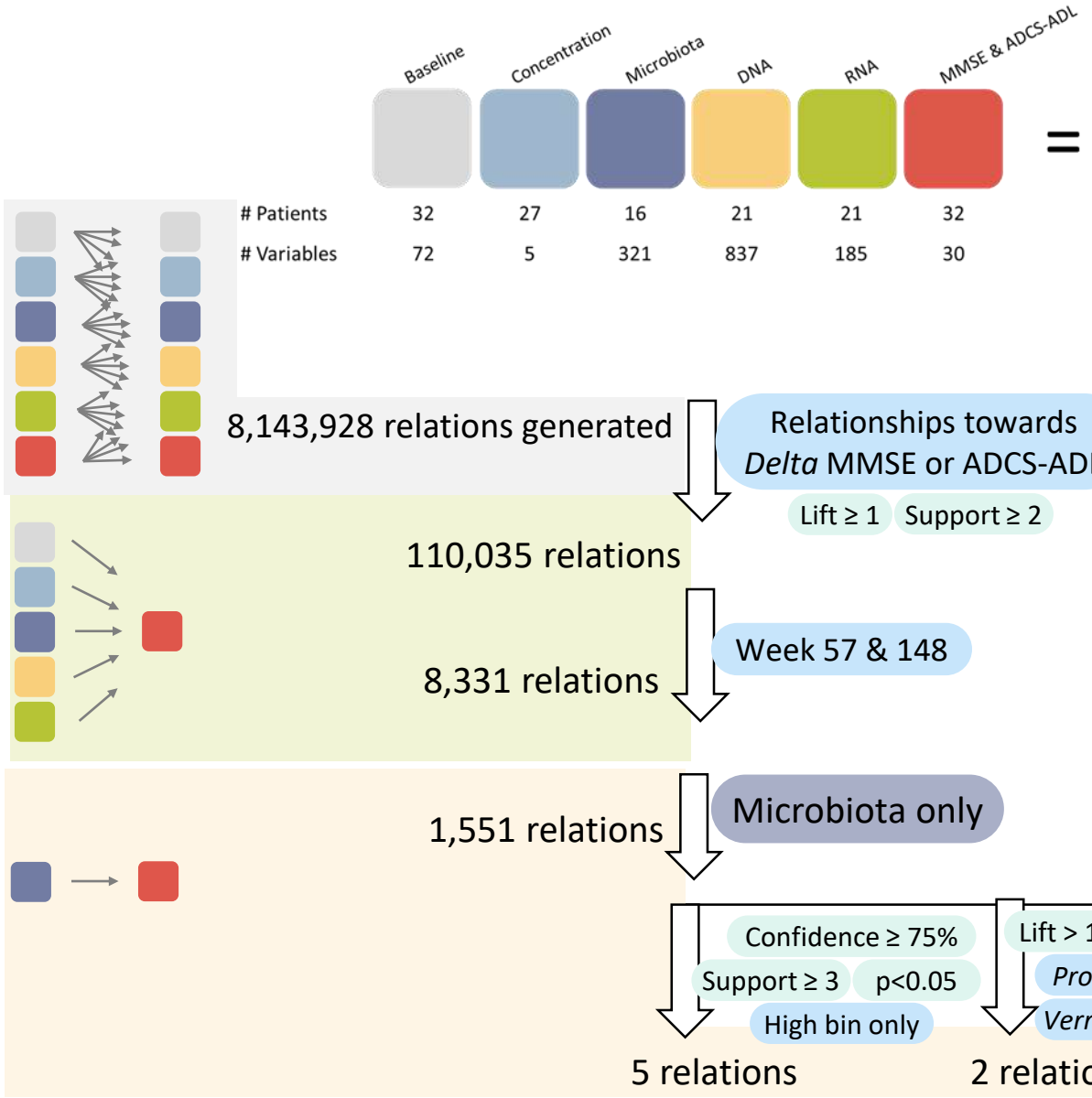


Systematic analysis of lattice generated by KEM® of all relations in knowledge base

Data-driven analysis

Systematic Generation of all Relationships in Knowledge Base and Stringent Filtering using KEM® Platform identifies Microbiota Markers of Response to ANAVEX®2-73

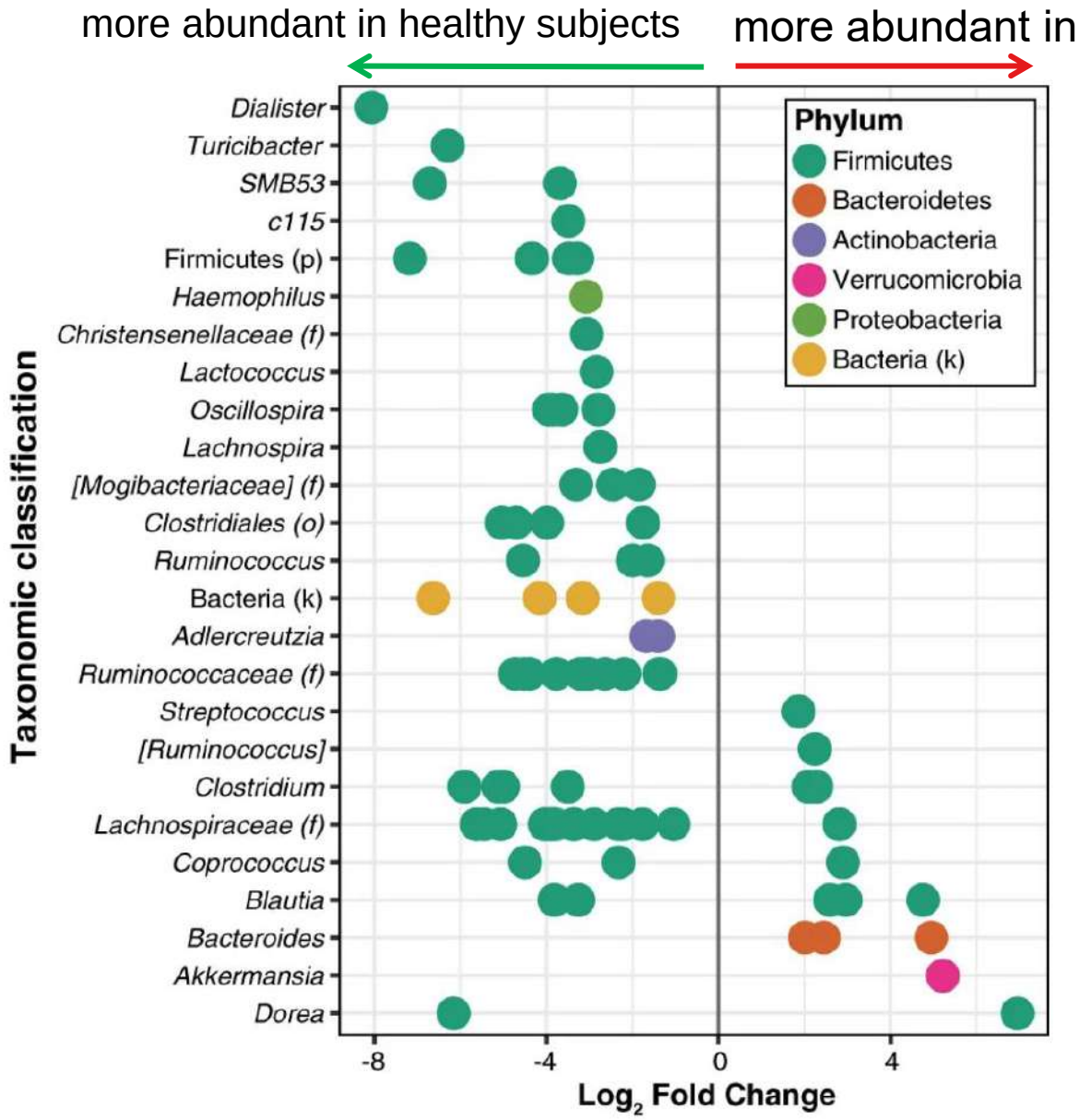
Data Analysis



KEM® identifies and ranks biomarkers relating to outcome derived from a small number of samples, avoiding overfitting

Stringent filtering identifies most relevant and powerful causal relations, revealing hidden relationships

AD is Associated with Changes in the Gut Microbiome Phyla and Genera

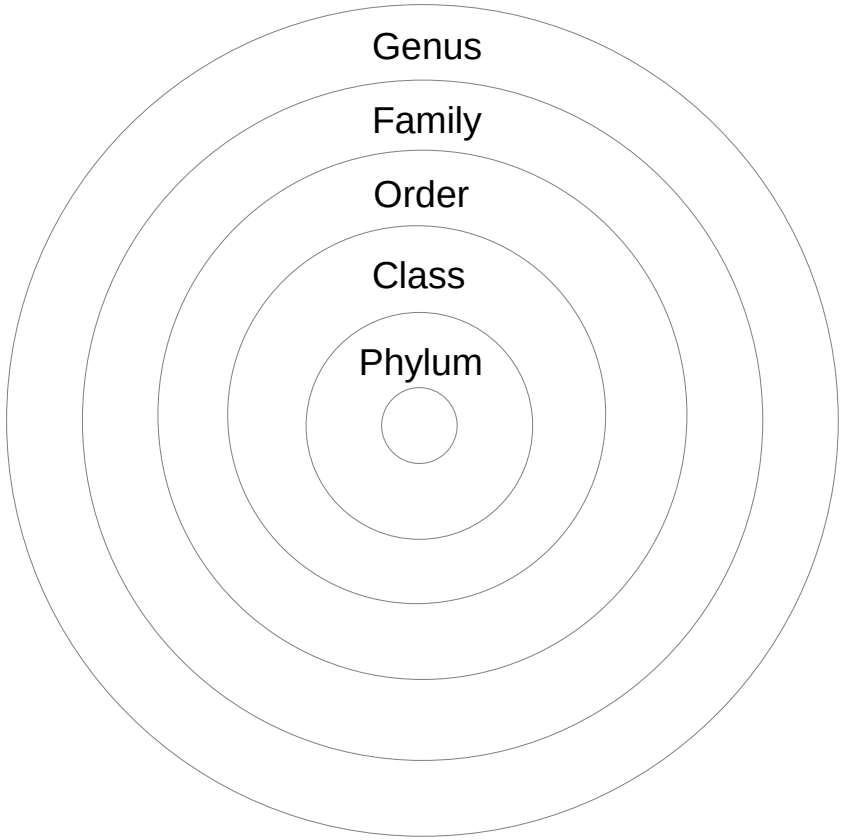


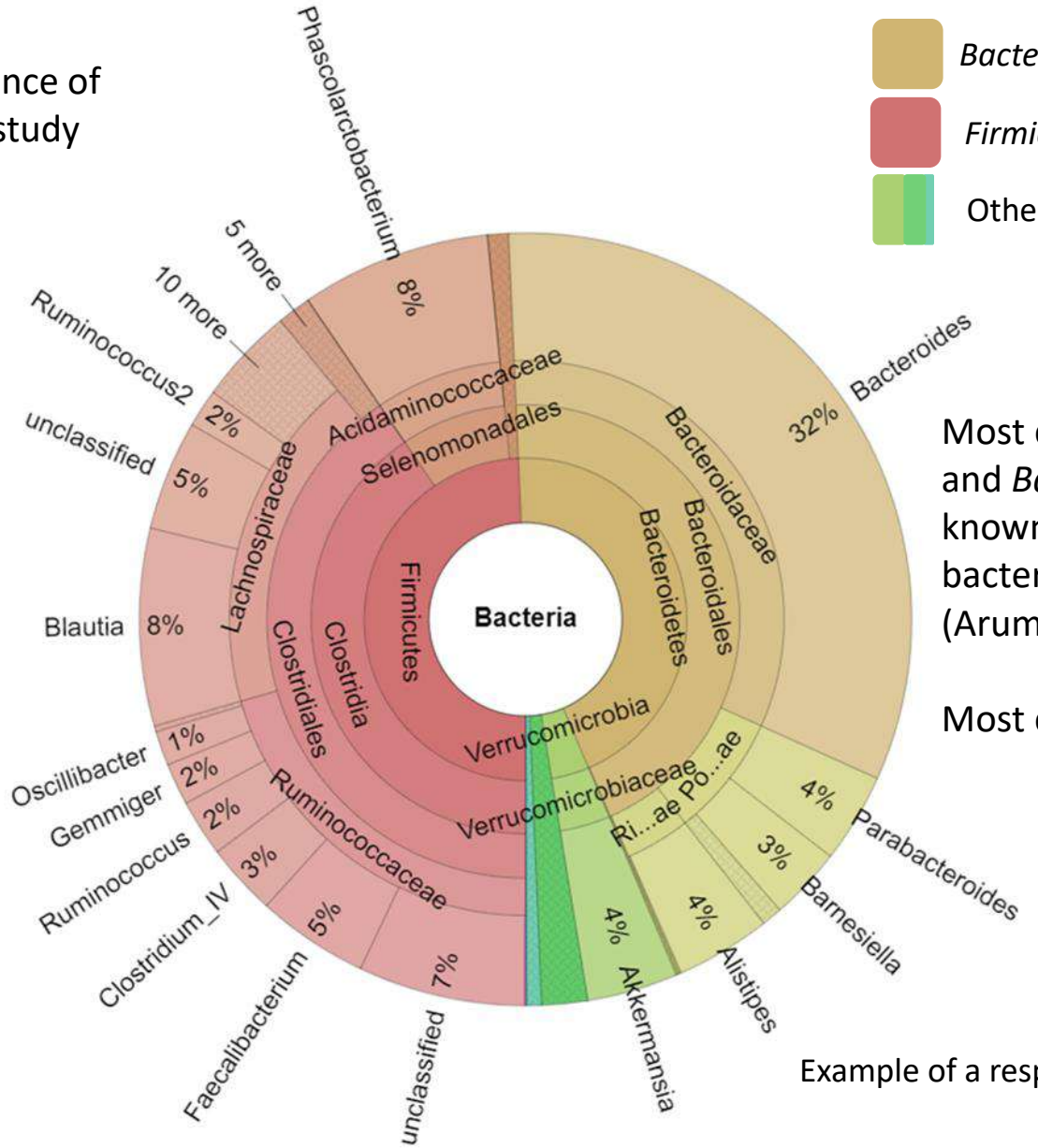
Differential abundance analysis identified 14 OTUs that were increased and 68 OTUs that were decreased in AD relative to Control participants ($p < 0.05$, FDR-corrected). Each point represents an OTU. Data plotted as log2 fold change; OTUs to the right of the zero line are more abundant and OTUs to the left of the zero line are less abundant in AD compared to Control groups. OTUs are organized on the y-axis according to the lowest taxonomic classification possible

Vogt et al. (2017)

Patient-Level Representation shows the Relative Abundance of Phyla, Families and Genera Present in Gut Microbiota

Ring slices correspond to the relative abundance of bacteria in one sample of the ANAVEX2-73® study



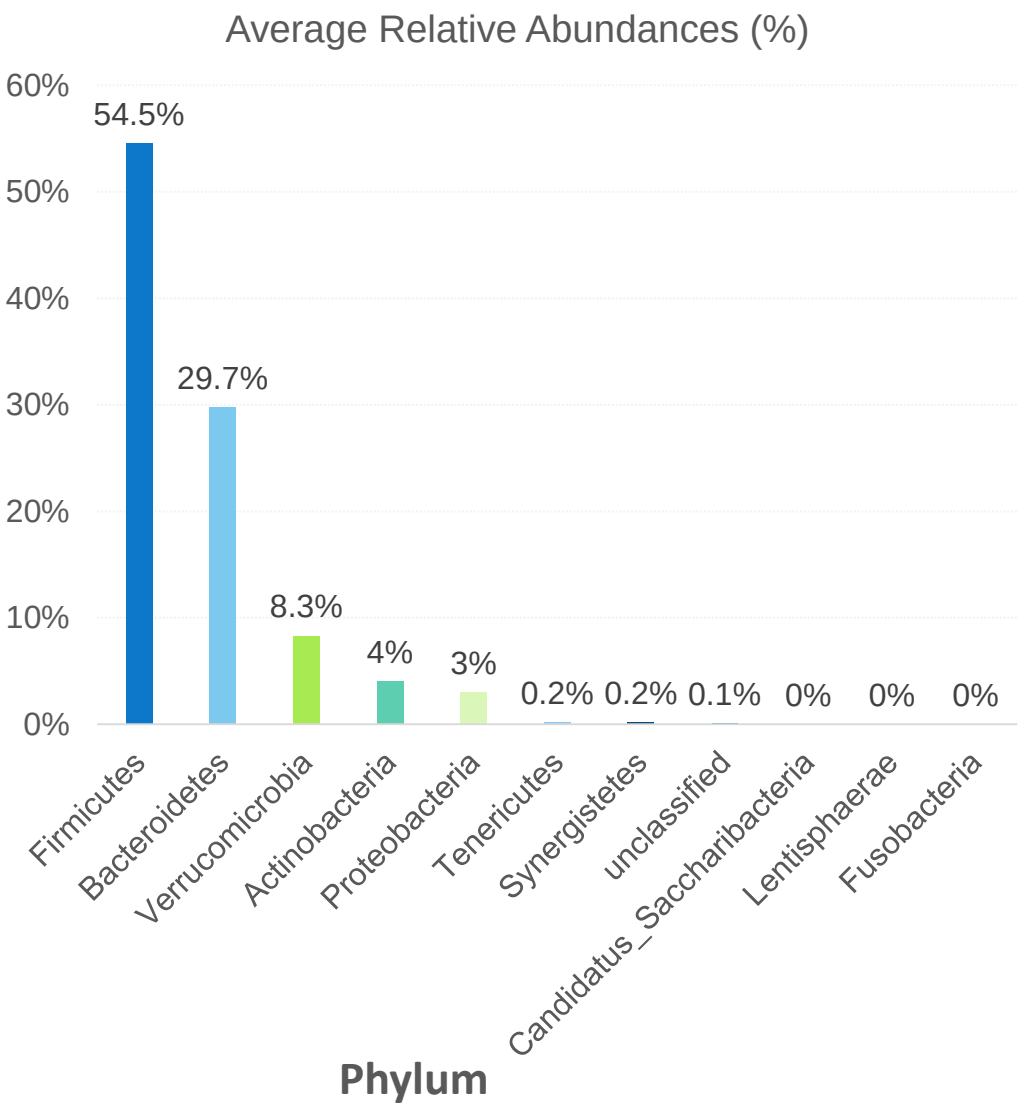


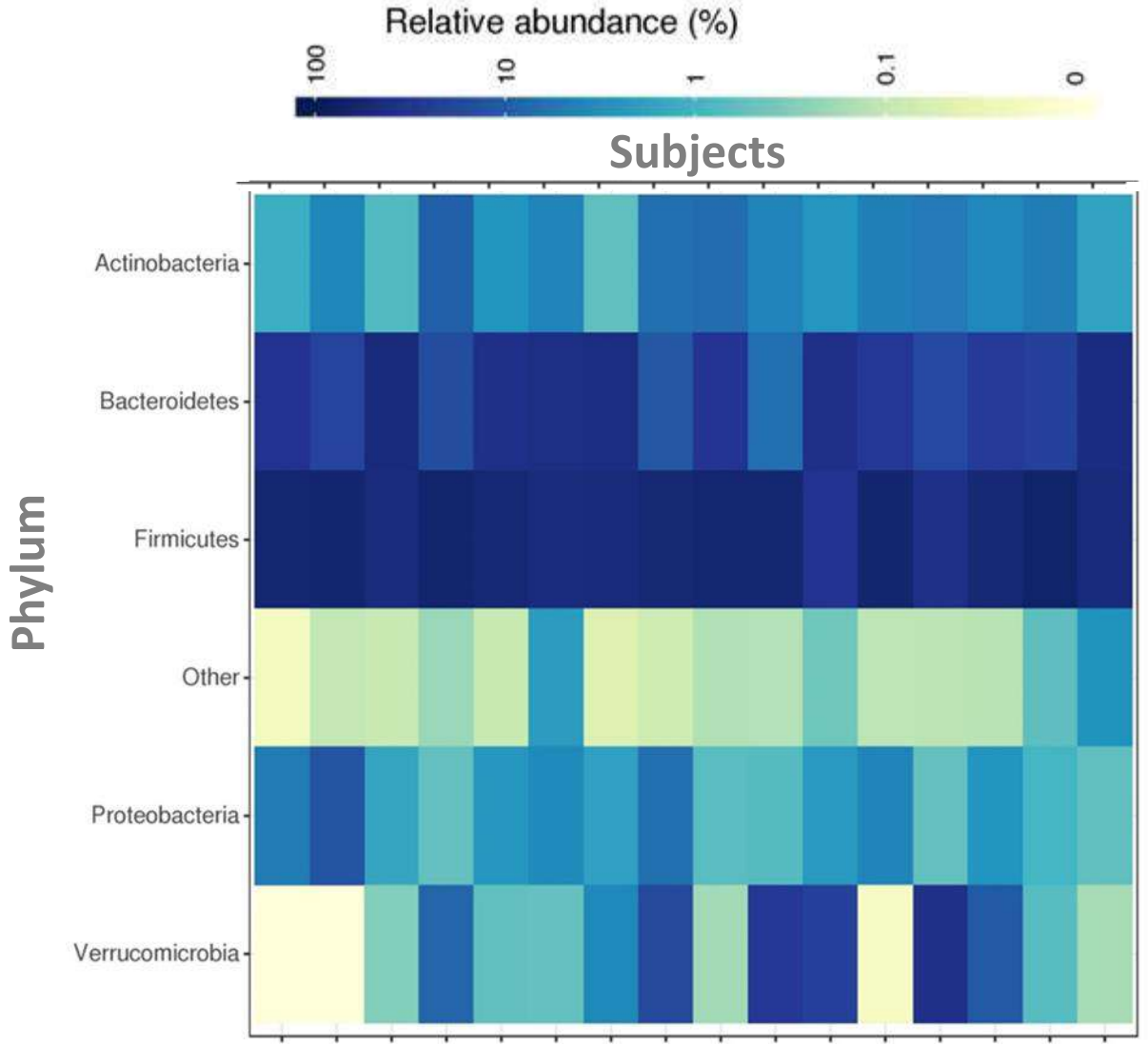
Most common phyla are *Firmicutes* and *Bacteroidetes*, which are known to be the most abundant bacteria in human's gut (Arumugam et al., Nature, 2011).

Most common genus is *Bacteroides*.

Example of a responder patient to ANAVEX®2-73

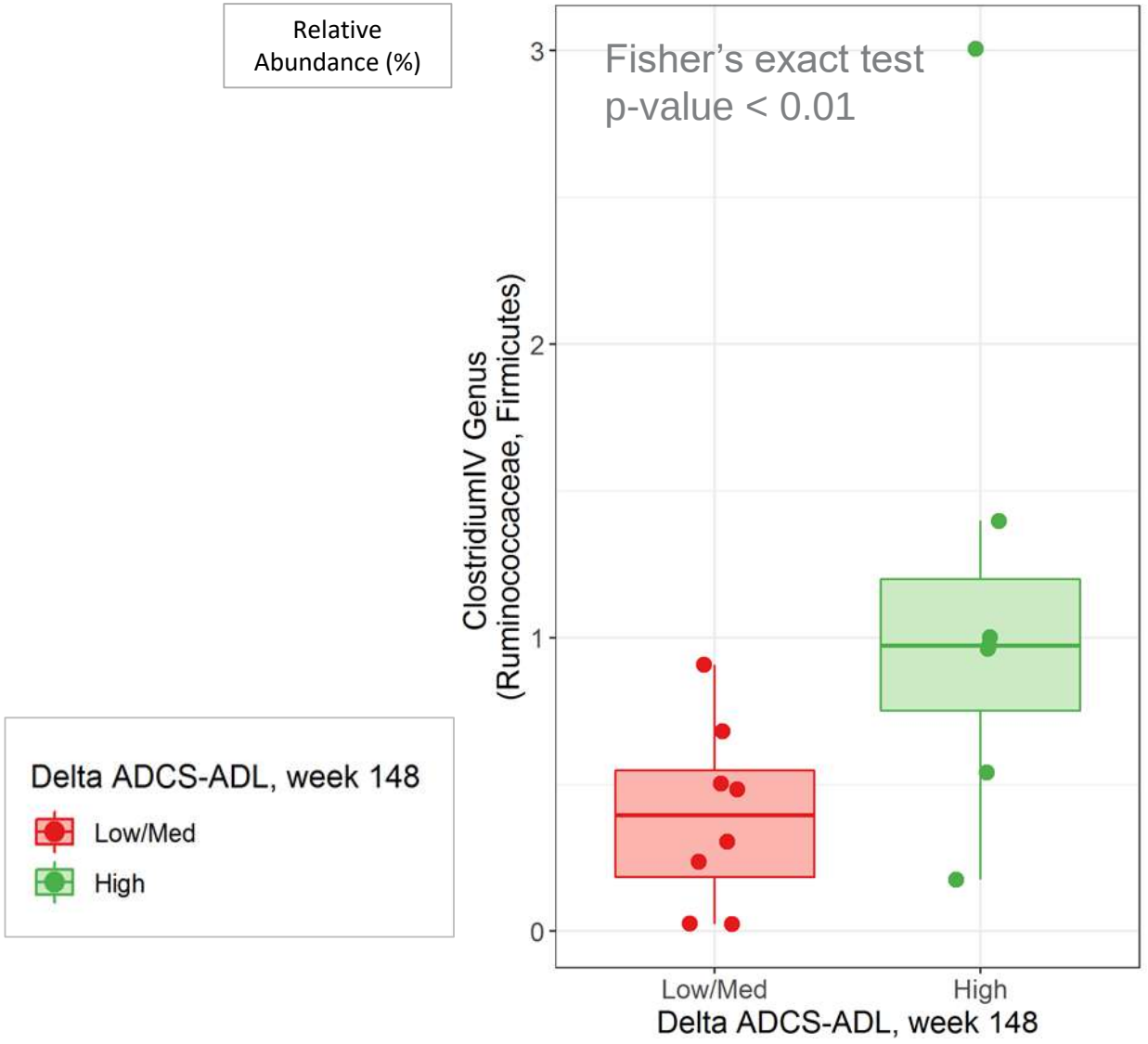
ANAVEX®2-73-Treated Patients have *higher* Abundance of *Bacteroidetes* and *Firmicutes* Phyla in Gut Microbiota

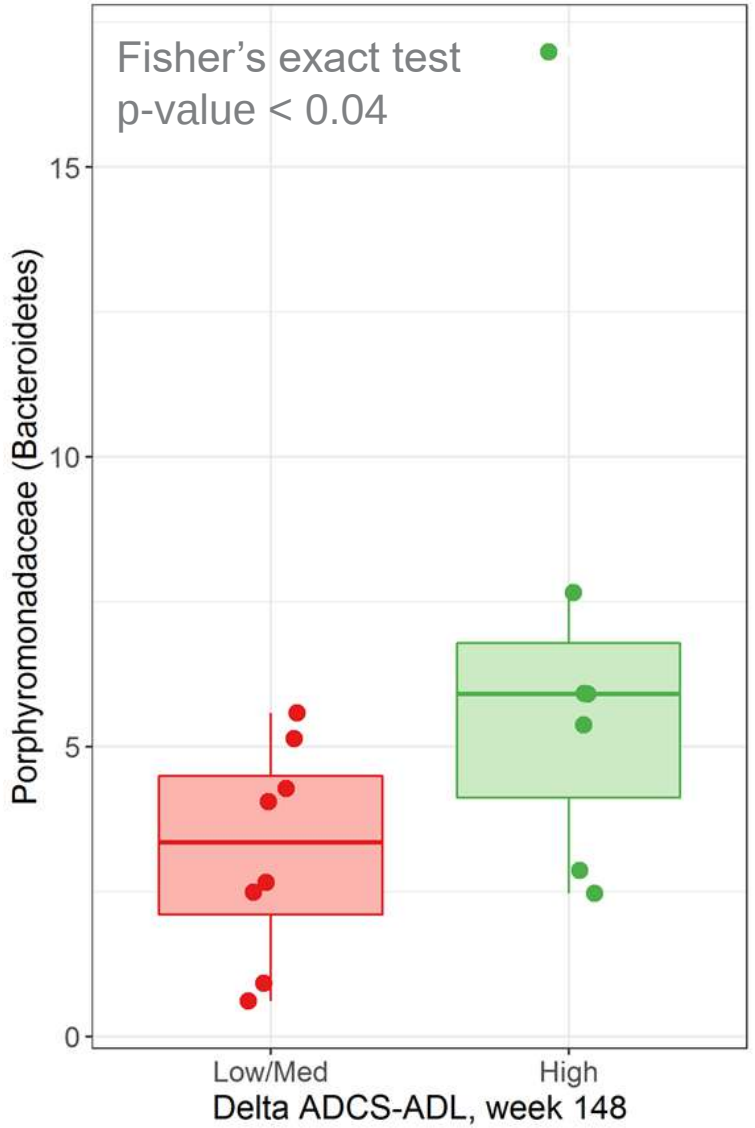




KEM® Identifies Changes in two Gut Microbiome Families - *Ruminococcaceae* and *Porphyromonadaceae* - Associated with Response to ANAVEX®2-73

Relative Abundance (%)





Increase in relative abundance

*High Delta ADCS-ADL correspond to **improvement** (improvement or limited functional change)*
*Low Delta ADCS-ADL correspond to **worsening** (functional decline)*

Conclusions

- **Communication between gut microbiota and the brain is a critical component of a healthy brain function** – identified in studies in the last decade [1,2,3]
- **Less richness and diversity of the microbiome found in AD participants** – compared to healthy control participants
- **E.g. Lower levels of *Ruminococcaceae*** – found in AD patients compared to healthy control subjects [5]
- **Higher levels of microbiota families i.e. *Ruminococcaceae* and *Porphyromonadaceae*** – associated with *improved* ANAVEX®2-73 response at week 148 ($p<0.01$ and $0<0.04$, respectively)
- **Human Data** – ANAVEX®2-73 has undergone a Phase 2a trial in Alzheimer's disease with favorable safety and exploratory efficacy results through 148 weeks [4]
- **Systematic Unbiased Analysis using KEM® AI Framework** – enables initial data-driven analysis of gut microbiota of AD patients in a clinical trial setting without *a priori* hypotheses
- **Analysis shows Target Engagement Data** – Dose-dependent ANAVEX®2-73 target engagement with the Sigma-1 receptor and **beneficial effect on neuro-inflammation**
- **Precision Medicine Using AI Improves Chance of Clinical Success** – KEM platform to integrate clinical and microbiota data and identify potential biomarkers of response for ANAVEX ®2-73 in addition to testing for genomic biomarkers with improved clinical response to ANAVEX®2-73 in Alzheimer's patients carrying wild-type (WT) SIGMAR1 and COMT genes

ANAVEX®2-73 may have beneficial homeostatic effect on brain-gut-microbiota axis

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Acknowledgements

- Principal Investigators & clinical sites' study staff
- Data safety review committee
- Anavex SAB
- Most of all, grateful acknowledgement of the contribution of the participating AD patients and their caregivers

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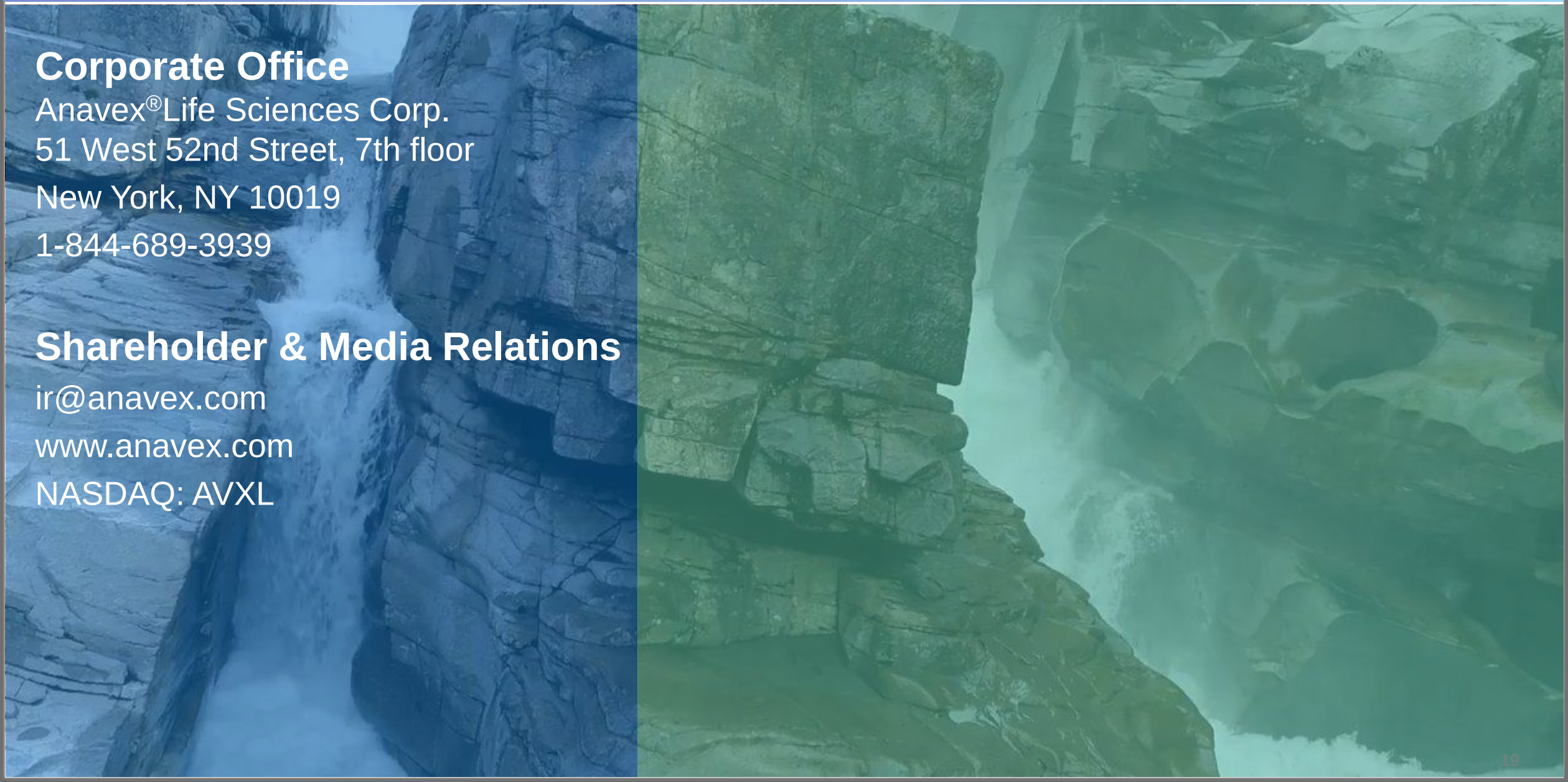

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