Modulation of gut microbiota by Indoleamine-2,3-dioxygenase 1 (IDO1) inhibitor during antiretroviral suppressed SIV infection in rhesus macaques

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Indoleamine-2,3-dioxygenase 1 (IDO1) and tryptophan metabolism

- IDO1 is an immuno-modulatory enzyme initiates the kynurenine pathway of tryptophan metabolism and likely plays an important role in HIV specific T-cell dysfunction.

- Inhibition of IDO1 balances host mucosal reactivity by activating alternative tryptophan metabolism via gut microbiota in mice, in particular Lactobacillus.

Zelante et al. 2013, Immunity
Longitudinal microbiome profiling of IDOi in SIV infected rhesus macaques

Study design (57 samples):
- 12 rhesus monkeys, 6 receiving IDOi and 6 receiving placebo.

Clinical metadata:
- Immune cell counts: Total Treg/Th17, Monocyte, IL-17, IL-22, IL-21, IL-2, TNFa, IFNr producing CD4+/CD8+, etc.
- Plasma metabolites: Kynurenine, Tryptophan, Indoleacetate, Indolelactate, etc.
- RNA-Seq: Lymph Node, Rectal Biopsy, Peripheral Blood.
Reversal microbiome profiles prior and post SIV infection

- Increased alpha diversity and decreased *Prevotella* at SIV infection, which were reversed at HAART and IDOi/Placebo.
- Increased *Lactobacillus* in both Placebo and IDOi treatments.
Reversal changes of multiple genera prior and post SIV infection

- A gut microbiome dysbiosis during SIV infection which can be potentially ‘corrected’ by the HAART and IDOi treatments.
Non-Lactobacillus genera significantly different between IDOi VS Placebo

- *Slackia, Parabacteroides, Dorea, Succinivibrio* increased more greatly, and *Treponema* decreased more greatly in IDOi than in placebo (ANCOVA).
Dorea, Slackia and Treponema significantly correlate with tryptophan metabolites
**Lactobacillus** not significantly different between IDOi VS Placebo

- Lactobacillus genus and individual species increased during IDOi/Placebo treatment, but not significantly different between IDOi and Placebo.
Potential role of microbiota in differentially regulating host key processes

- Significant opposite correlation of multiple microbial genera with both host **metabolic** processes and **immune/cancer related** pathways.

  - Single-sample gene set enrichment analysis (ssGSEA) on host RNA-Seq data.
  - Data from lymph node transcriptomics which were most strongly correlated with gut microbiota.

**Flexispira**

- TRYPTOPHAN_METABOLISM
- PANCREATIC_CANCER
- SMALL_CELL_LUNG_CANCER

**Prevotella**

- GLYCOSPHINGOLIPID_BIOSYNTHESIS
- NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY
- CYTOKINE_RECEPTOR_INTERACTION

**Methano-brevibacter**

- PRIMARY_BILE_ACID_BIOSYNTHESIS
- JAK_STAT_SIGNALING_PATHWAY
- TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY
Differential immuno-modulatory effects between human and bacterial tryptophan metabolites

- Human tryptophan metabolites (L-Kynurenine) are likely pro-inflammatory while bacterial tryptophan metabolites (indole derivatives) are anti-inflammatory.

Human L-Kynurenine

Bacterial Indole-3-carboxaldehyde (IAld)
Summary

- Reversed microbiome profiles before and after SIV infection, indicating a SIV infection related dysbiosis which can be ‘corrected’ by HAART and IDOi.

- Genera other than *Lactobacillus* (*Dorea, Slackia, Succinivibrio* and *Treponema*) significantly different in IDOi vs Placebo and significantly correlated with bacterial tryptophan metabolites, thus could potential be involved in alternative tryptophan metabolism in IDOi treatment in non-human primates.

- Opposite correlation of multiple microbial genera with host metabolic processes and immune/cancer related pathways, indicating a potential role of microbiota in differentially regulating host key processes.

- Differential immuno-modulatory effects between human and bacterial tryptophan metabolites, in support of the positive role of gut microbiota in maintaining host immune homeostasis.
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All studies were conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.
Backup slides
Tryptophan metabolism related functions inferred by PICRUSt

- Increase of Tryptophan catabolism and decrease of Tryptophan anabolism genes in IDOi/Placebo.

**KEGG Genes:**
- K01695: Tryptophan synthase alpha chain
- K01696: Tryptophan synthase beta chain
- K06001: Tryptophan synthase beta chain 2
- K03835: Tryptophan-specific transport protein
- K02846: N-methyl-L-tryptophan oxidase
- K00453: Tryptophan 2,3-dioxygenase
- K00465: Tryptophan 2-monooxygenase
- K01667: Tryptophanase

Legend:
- Increase
- Decrease
- ▲ 181A_IDOi
- ▲ 181A_Placebo

1. Pre-bleed
2. Baseline
3. SIV Infection
4. HART
5. IDO/Placebo
Changes of tryptophan metabolic functions IDOi VS Placebo

– Greater decrease of tryptophan synthase beta chain (K06001, ANCOVA P=0.029) and greater increase of tryptophanase (K01667) and tryptophan transport protein (K03835) in IDOi compared to placebo.

**K01695**: Tryptophan synthase alpha chain (P=0.648)
**K01696**: Tryptophan synthase beta chain (P=0.681)
**K06001**: Tryptophan synthase beta chain 2 (P=0.029)

**K03835**: Tryptophan-specific transport protein (P=0.344)
**K02846**: N-methyl-L-tryptophan oxidase (P=0.686)
**K00453**: Tryptophan 2,3-dioxygenase (P=0.371)
**K00466**: Tryptophan 2-monoxygenase (P=0.794)
**K01667**: Tryptophanase (P=0.956)

ANCOVA results

Changes compared to HAART
Treponema and Succinivibrio contributed significantly to tryptophan metabolic functions.

K01695 tryptophan synthase alpha chain

K01696 tryptophan synthase beta chain

K06001 tryptophan synthase beta chain 2

K03835 tryptophan-specific transport protein
Dorea, Slackia, Succinivibrio and Treponema

– **Dorea**: a known component in human gut microbiota. Associated with increased bacterial diversity and resistance to pathogenic colonization (Bäumler et al. 2016). In some studies associated with GI disorder (Guinane et al. 2013) and increased intestinal permeability (Leclercq et al. 2014).

– **Slackia**: a gut associated bacteria that have been suggested to play roles in host lipid and xenobiotic metabolism (Cho et al. 2016). *Slackia* equolifaciens are known to convert dietary isoflavones into equol (Jin et al. 2010), a known inflammation suppressant.

– **Succinivibrio**: a characteristic bacterium in primate and traditional human gut microbiota (as part of the rumen ecosystem). A starch fermentator whose level increases from a high fiber to a high starch diet (Petri et al. 2013).

– **Treponema**: a characteristic bacterium in primate and traditional human gut microbiota (Schnorr et al. 2014). A fiber metabolizer whose level decreases from a high fiber to a high starch diet (Obregon-Tito et al. 2015).

– IDOi possibly switched a fiber metabolizing microbiota to a starch metabolizing one?
Single-sample GSEA on RNA-Seq data

– Single-sample GSEA (ssGSEA), an extension of GSEA, calculates separate enrichment scores for each pairing of a sample and gene set.

– Each ssGSEA enrichment score represents the degree to which the genes in a particular gene set are coordinately up- or down-regulated within a sample.

– ssGSEA transforms a single sample's gene expression profile to a *gene set (i.e. pathway)* enrichment profile, essentially reducing the dimensionality of the dataset.