S-MER peptide specifically binds to serum amyloid A (SAA), a stress pro-inflammatory protein, which generates aggregated amyloid deposits in the IBD colon (de Villiers at al Cytokine 12:1337-1347,2000). Our studies further provide in vitro evidence, strengthened by in vivo experiments, that SAA is a significant target of this pentamer. To this end, the S-MER peptide (but not the corresponding scrambled peptide) inhibits the release of the pro-inflammatory cytokines IL-6 and IL-1β from SAA-activated fibroblasts. Furthermore, the S-MER peptide was found to retard the early stages of amyloid-type aggregation of SAA in solution (Fig 2). Adopting the β-sheet conformation, MTADV would display opposing hydrophobic and hydrophilic faces that could interact with the β-sheet-forming amyloidogenic sequences in SAA. This suggests that the mechanism of action for the S-MER peptide in vivo may depend on its ability to slow the aggregation of SAA, thus reduce its contribution to chronic inflammation. Finally, using bioinformatics and qRT-PCR, we have found the pentamer up-regulates a set of genes involved in resistance to chronic inflammations. Hence, our study provides both a new potential drug (MTADV) and a new therapeutic target candidate (SAA) for IBD.

**Background:**
T cell costimulation has been strongly implicated in the pathogenesis of IBD, yet CD28 costimulatory pathway inhibitors (e.g. abatacept, CTLA4-Fc) have not proven clinically efficacious, implicating an alternative costimulatory pathway.

**Methods:**
Primary cell assays were performed with PBMC stimulated with K562 (CD80+, CD86+, ICOSL+, anti-CD3 (OKT3) (+) to evaluate suppression of cytokine release and compare to single pathway inhibition. ALPN-101 was assessed in the CD4+/CD45RB+ T cell-induced colitis model either singly dosed on Day 0 or 14 or repeat dosed 2x/week starting at Day 0 or 14 through Day 41, respectively. Serum cytokine and flow analysis of blood was performed throughout the study. Clinical presence of colitis was assessed using a disease activity index based on weight loss and stool consistency. At end of study, colon were measured and assessed histologically. Results: ALPN-101 suppressed cytokine release (IFNy, IL-2) from healthy or IBD patient PBMCs superior to single pathway inhibitors. In vivo, preventively or therapeutically, a single dose of ALPN-101 was efficacious to significantly improve multiple colitis readouts. Repeat dosing completely prevented onset of colitis. ALPN-101-treated mice gained weight and had colon weight-to-length ratios similar to the no-colitis cohort and demonstrated significant suppression of T cells and pro-inflammatory cytokines (e.g. TNFα, IL-12/23, IL-6). Conclusion: Dual pathway inhibitor ALPN-101 is superior to single pathway inhibition in human in vitro and mouse in vivo translational studies and may be a novel therapeutic candidate for the treatment of IBD. Clinical trials for ALPN-101 in multiple inflammatory diseases are planned and underway.

**AZD4205, A SELECTIVE, GI TRACT-ENRICHED SELECTIVE JAK1 INHIBITOR FOR CROHN’S DISEASE: PRECLINICAL EVIDENCE AND PHASE I DATA**
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Background: AZD4205 is an oral, ATP-competitive, JAK1 selective inhibitor. Nonclinical data showed its higher drug concentration within the mucosal layer of the colon, thus demonstrating its potential for the treatment of IBD.

**Results:**
AZD4205 reduced TNBS-induced colitis in a mouse model of ulcerative colitis. In a Phase I clinical trial, AZD4205 was well tolerated and demonstrated clinical responses in up to 60% of patients with moderate to severe left-sided ulcerative colitis.
gastrointestinal (GI) tract relative to plasma in the rodents, suggesting its potential as an effective and safe treatment option for patients with Crohn’s disease (CD). AZD4205 also has been evaluated in a phase I study in healthy volunteers (HV). Here we report AZD4205 preclinical data in cells and animal models, and phase I data from HVs.

Method: Enzymatic activity of AZD4205 was assessed in *in vitro* biochemical assay with human isolated JAK kinase domains. The cellular activity of AZD4205 was evaluated in human peripheral blood mononuclear cells (PBMCs) by assessing cytokine induced phosphorylated-STATs (pSTATs). The in vivo efficacy of AZD4205 was assessed in a murine CD model induced by subcutaneous injection of 10 mg/kg indomethacin, and AZD4205 was orally administered at different doses for 5 consecutive days. The ileum and bone marrow tissues were collected post AZD4205 treatment to measure pSTAT3 expression using immunohistochemistry. The drug concentration of AZD4205 in ileum and plasma were measured by HPLC.

Phase I study of HVs (Clinicaltrial.gov Identifier: NCT03728023) comprised of single ascending dose (SAD) of 5 to 150 mg, food effect (FE) with 50 mg single dosing with or without food, and multiple ascending dose (MAD) of 25 to 100 mg once daily for 14 days. Safety monitoring was conducted, and blood samples were collected to assess PK of AZD4205.

Result: In enzymatic assay, AZD4205 exhibits greater than 200-400-fold selectivity over other JAK family kinases. AZD4205 inhibits pSTAT1, pSTAT3, and pSTAT5 in human PBMCs with IC_{50} of 50, 308, and 90 nM, respectively.

The drug concentration of AZD4205 in murine ileum was around 100-fold higher than that in plasma. In murine CD model, the pSTAT3 was inhibited by 93% in ileum but only 43% in bone marrow at 4 hr post AZD4205 treatment. AZD4205 exhibited a dose-dependent effect on improving body weight loss and decreasing colon density in this model.

In the NCT03728023 study, 66HV’s were dosed with AZD4205 or matched placebo, SAD (n=20 in AZD4205, n=10 in placebo), FE (n=12 in AZD4205 and MAD (n=18 in AZD4205, n=6 in placebo). AZD4205 was well-tolerated, and no ≥ G3 drug related AE was observed. A dose-proportional increase of AZD4205 plasma concentrations was detected in HVs. The T_{1/2} was close to 48 hr and hence accumulation of about 3-fold in C_{max} and AUC was observed after multiple once daily dosing. No relevant food effect was observed to significantly impact AZD4205 PK.

Conclusion: Based on preclinical murine data, AZD4205 has unique GI-tract enriched PK properties, as a promising agent for CD. Clinical data in HVs demonstrated its favorable safety and PK profiles. A phase II study in moderate to severe CD is planned.

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**CREATION OF A CHRONIC PERIANAL CROHN’S FISTULA IN SWINE**

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Introduction: Peri-ianal Crohn’s disease (PCD) is associated with worse outcomes including poor quality of life and higher medical costs. PCD is notoriously difficult to treat despite advancements in biologic therapies for luminal disease. In order to test innovative therapies for PCD, an animal model that closely mimics human perianal disease is warranted. Previous efforts were successful in creating peri-ianal fistulas in swine, however, these closed after removal of the setons. A chronic model that maintains the fistula open and replicates the inflammatory component found in clinic is necessary.

Methods: Under general anesthesia, 4 female pigs underwent creation of surgical peri-ianal fistulas using basic surgical technique with scalpel and hemostat. Size 24 French silicone Foley catheter setons were placed to maintain patency of the fistula tract. Each pig had 3 fistulas created – 1 rectovaginal and 2 perianal. The setons were left in place for 4 weeks. After removal of the setons, trinitrobenzenesulfonic acid (TNBS) was administered into the fistula tract twice a week to create local inflammation. After 2 weeks of TNBS, an MRI was obtained to assess the fistula tracts and the pigs were sacrificed to review histopathology.

Results: MRI showed successful creation of chronic fistula tracts, and maintenance of these tracts 2 weeks after removal of setons. We noted a transphincteric fistula with supravelvator component as well as an associated abscess 3.3 x 1.0 cm containing fluid and gas (Fig 1). Gross specimen revealed patent fistula tracts. Histopathology found significant chronic active inflammation on standard H&E staining (Fig 2).

Conclusions: A chronic peri-ianal fistula model in pigs was successfully created using large bore rubber setons and TNBS stimulation that strongly resembles that seen in humans. Further studies are needed to standardize the conditions required to have a reproducible model with well-defined levels of inflammation, fistula patency, and other parameters.

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**DISRUPTION OF ENDOGENOUS ITACONATE PRODUCTION EXACERBATES EXPERIMENTAL COLITIS**

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Background: Inflammation is a hallmark of inflammatory bowel disease and alterations in tricarboxylic acid cycle (TCA) metabolism have been identified as major regulators of immune cell phenotype during inflammation and hypoxia. The TCA cycle metabolite, itaconate, is produced by the enzyme aconitate decarboxylase 1 (Acod1) and is highly upregulated during classical macrophage activation and during experimental colitis. Itonoxate and cell permeable derivatives have robust anti-inflammatory effects on macrophages, therefore we hypothesized that Acod1-produced itaconate has a protective, anti-inflammatory effect during experimental colitis.

Methods and Results: Wild type (WT) control and Acod1^{-/-} mice were administered 3% Dextran Sulfate Sodium (DSS) in water for 7 days to induce experimental colitis. After DSS was discontinued, Acod1^{-/-} mice had significantly reduced body weight recovery with increased macroscopic disease severity, and upon dissection had decreased colon length and more severe inflammation. To determine if myeloid cells are the critical Acod1-itaconate-producing cell types, we generated myeloid-specific Acod1 deficient mice, however no differences in weight loss, colon length or inflammatory gene expression were detected compared to WT controls. To test