Detailing Characteristics of Fibrostenotic Crohn's Disease Biology & the Potential of a Local PDE4 Inhibitor Prodrug to Minimize Off-Target Effects & Maximize Efficacy

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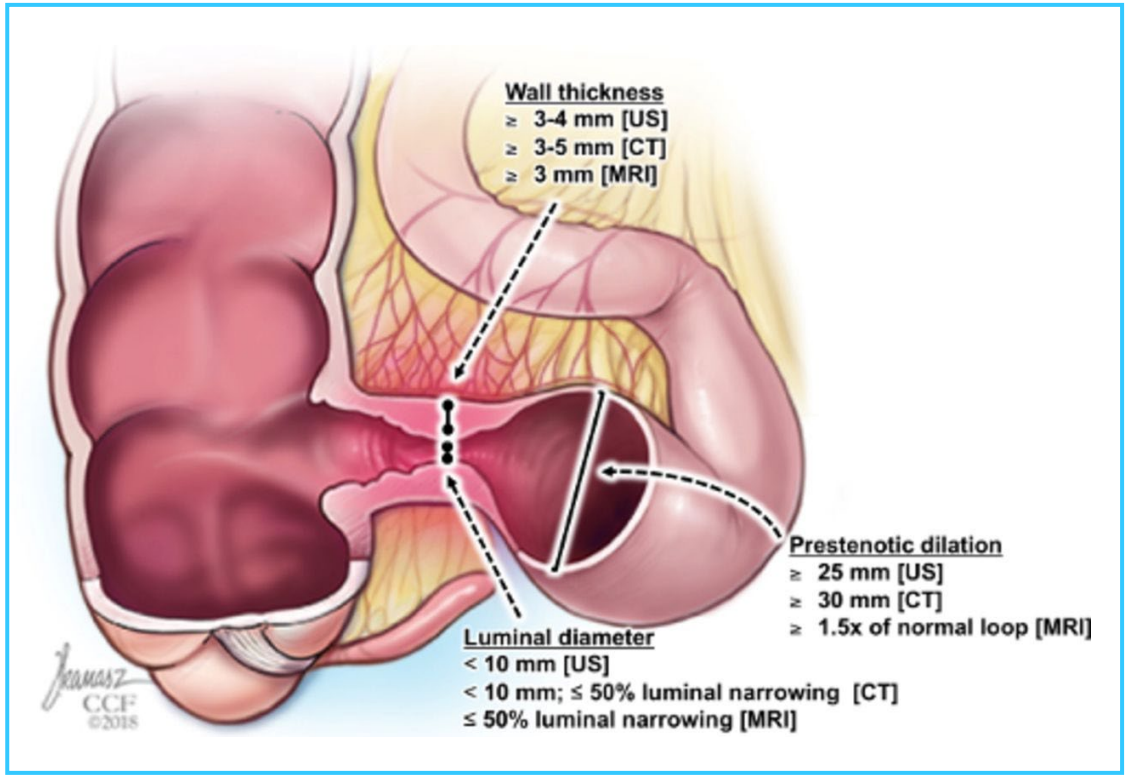
Introduction to IBD and Fibrosis

- IBD Overview:
  - Includes Crohn's disease (CD) and ulcerative colitis (UC).
  - Commonly accompanied by intestinal fibrosis.
  - Currently, there are no specific treatments.
- Symptoms Associated with Fibrosis:
  - CD: Strictures and obstructive symptoms.
  - UC: Urgency and motility abnormalities.
- Significant Unmet Need Particularly in Crohn's Disease
  - In CD, the majority (>50%) of patients experience fibrosis, presenting as strictures and obstructive symptoms, at least once in their lifetime.
  - Often necessitates surgical interventions.



Buttenworth et al. 2019

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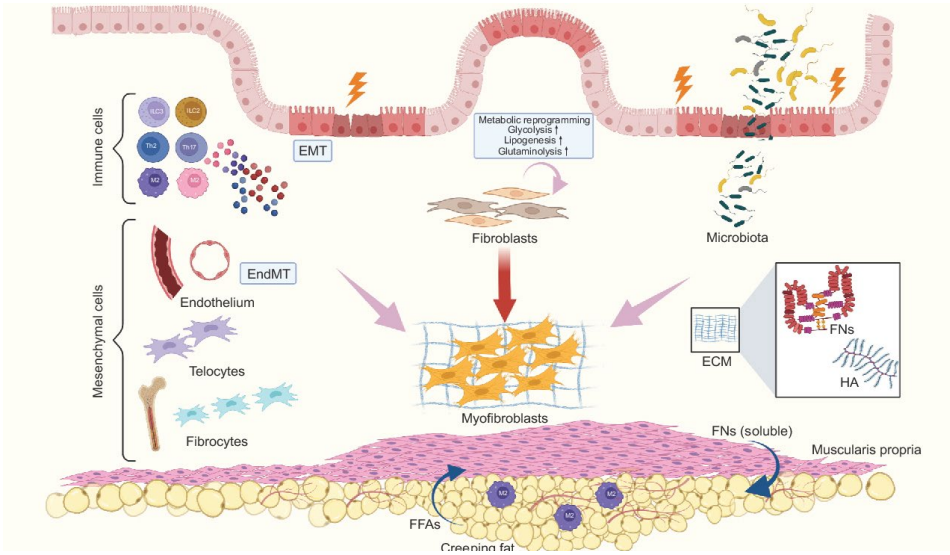
Mechanisms of Intestinal Fibrogenesis

- Extra Cellular Matrix (ECM) Deposition and Scar Formation:
  - One of the hallmark features of IBD.
  - Physiological process for tissue repair.
  - Excessive ECM causes tissue stiffness and functional impairment.
  - Intestinal fibrosis is present even without stricture formation.
- Mesenchymal Cells Important Driver of Fibrosis:
  - Increased MC number in intestinal fibrosis.
  - Heterogeneous population: Fibroblasts, myofibroblasts, smooth muscle cells (SMC).
  - Function: provide the scaffold where cells reside, shaping tissue structure.



Wu et al. 2023

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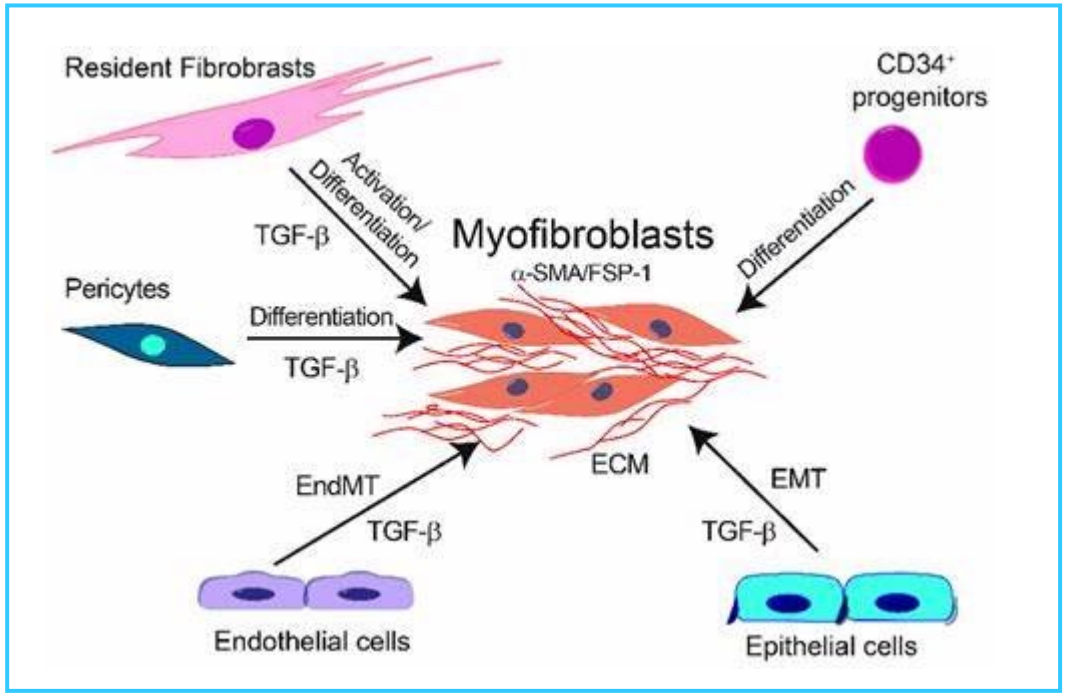
Mesenchymal cells Drive Intestinal Fibrosis

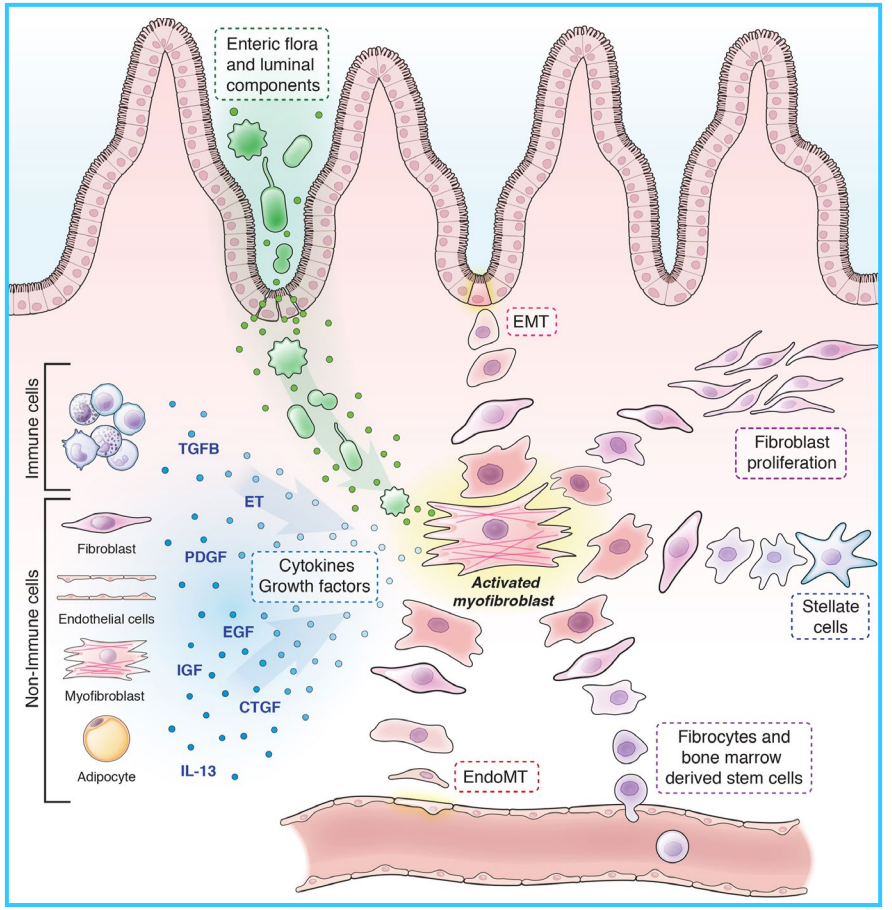
- Intestinal Mesenchymal Cells:
  - Non-epithelial, non-endothelial, non-hematopoietic.
  - Provide scaffold for tissue structure.
- Fibroblasts, Myofibroblasts, and SMCs:
  - Differentiation markers and these transition between phenotypes in inflamed intestine.
  - Example: Exposure to (TGF)-β1 → fibroblasts (α-SMA-poor) differentiate into myofibroblast-like cells (α-SMA-rich)
- Pericytes:
  - Surround blood endothelial cells and exhibit intermediate phenotype between fibroblasts and vascular SMC.
  - Differentiate into fibroblasts.
- Fibrocytes:
  - Bone marrow-derived mesenchymal stem cells.
  - Traffic to inflamed sites and differentiate into fibroblasts/myofibroblasts.



Pardali et al. 2017

Fibroblast-Activating Mediators in Intestinal Fibrosis

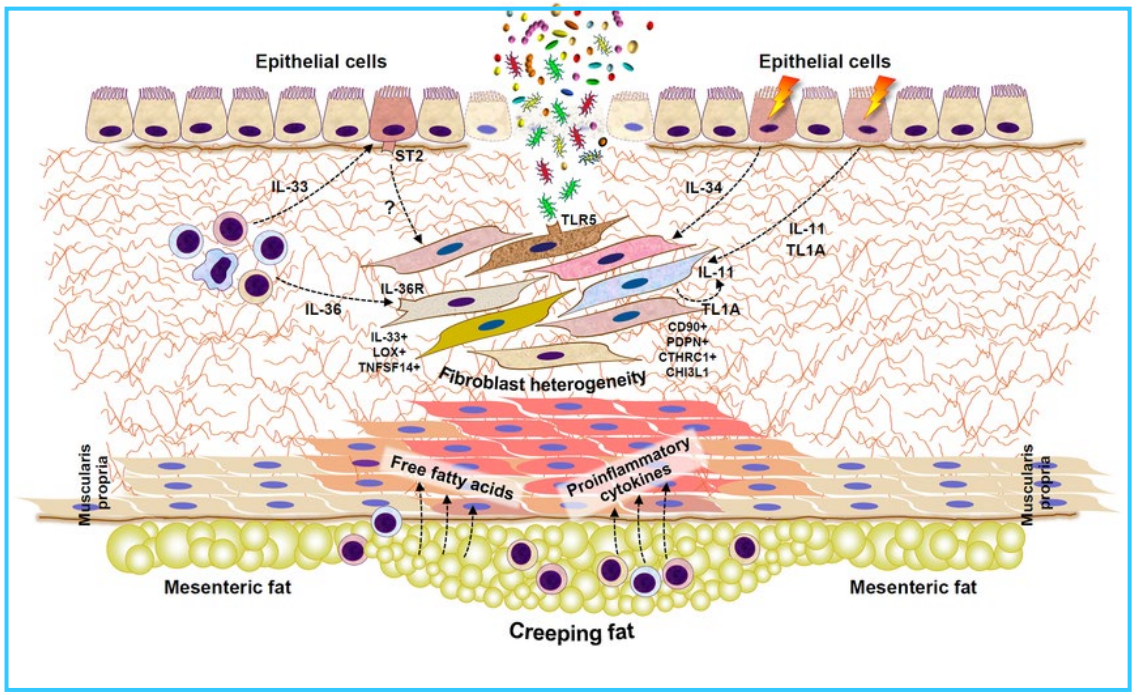
- Complex Microenvironment:
  - Mesenchymal cells exposed to luminal contents and inflammatory mediators.
  - Critical for initiation and perpetuation of IF.
- Observations:
  - Location of strictures follows the location of disease.
  - Degree of fibrosis correlates with the degree of inflammation.
  - No fibrosis in areas not affected by inflammation.



D'Haens et al. 2022

Key Mediators of Intestinal Fibrosis

- TGF-β1:
  - Dominant profibrotic growth factor.
  - Upregulated in fibrotic diseases, including CD.
  - Upregulates α-SMA, collagen I, and fibronectin.
- IL-1α and IL-1β
  - Key mediators of intestinal inflammation and fibrosis.
  - IL-1α activates fibroblasts, while IL-1β promotes collagen production and ECM remodeling.
- IL-6
  - IL-6 is well-established in IBD and acts in a proinflammatory and profibrotic manner.
- TNF and TL1A:
  - Known drivers of intestinal inflammation.
  - Promote fibrosis by increasing collagen production.
- Th1 Response (Il-17):
  - Response involved in development of fibrosis in multiple organs.
- Th2 Response (IL-4 and IL-13):
  - Profibrotic activity of Th2 cytokines.
  - Th1 and Th2 dichotomy of CD4+ T helper cells in fibrostenotic CD controversial.
- IL-34
  - Constitutively expressed in int/colon.
  - Higher expression level in structuring CD.
  - Direct activator of intestinal fibroblasts.



Wang et al. 2021

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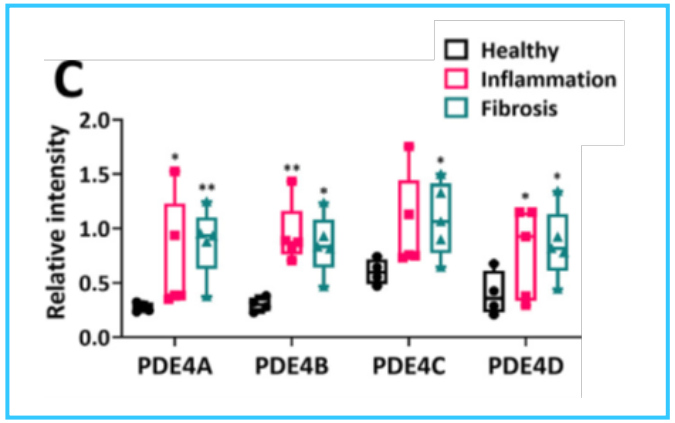
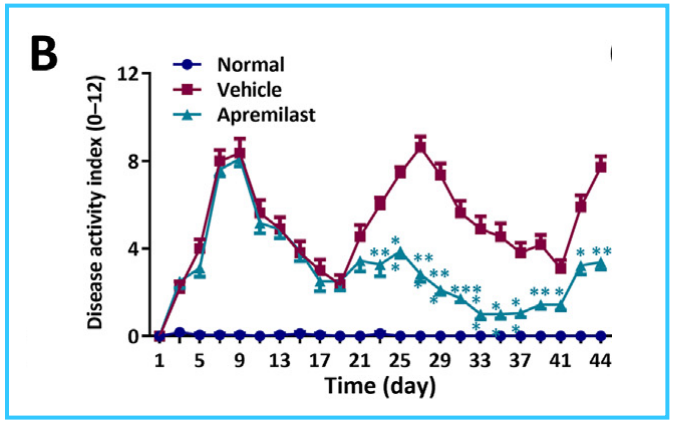
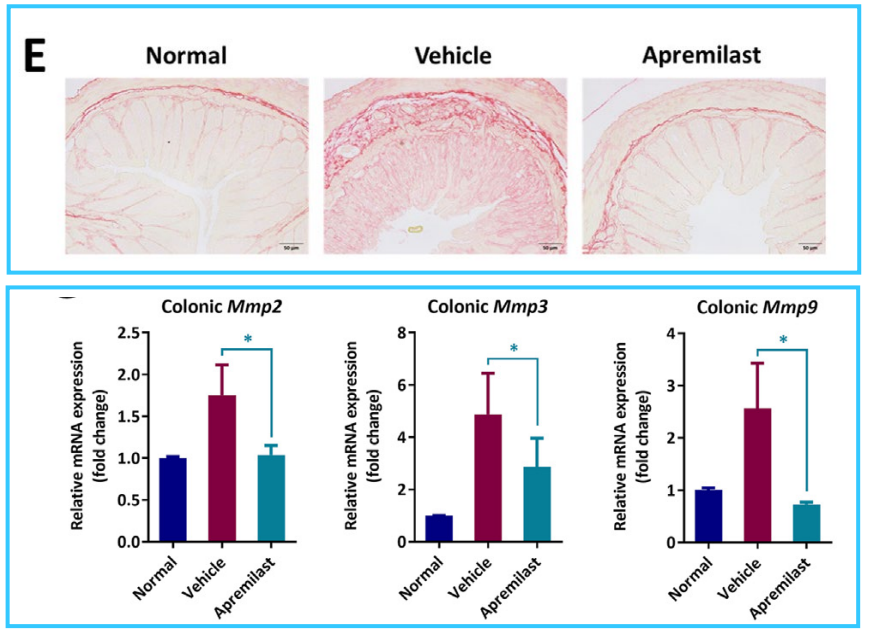
PDE4 is an Important Target which Affects Multiple Mediators of Intestinal Fibrosis

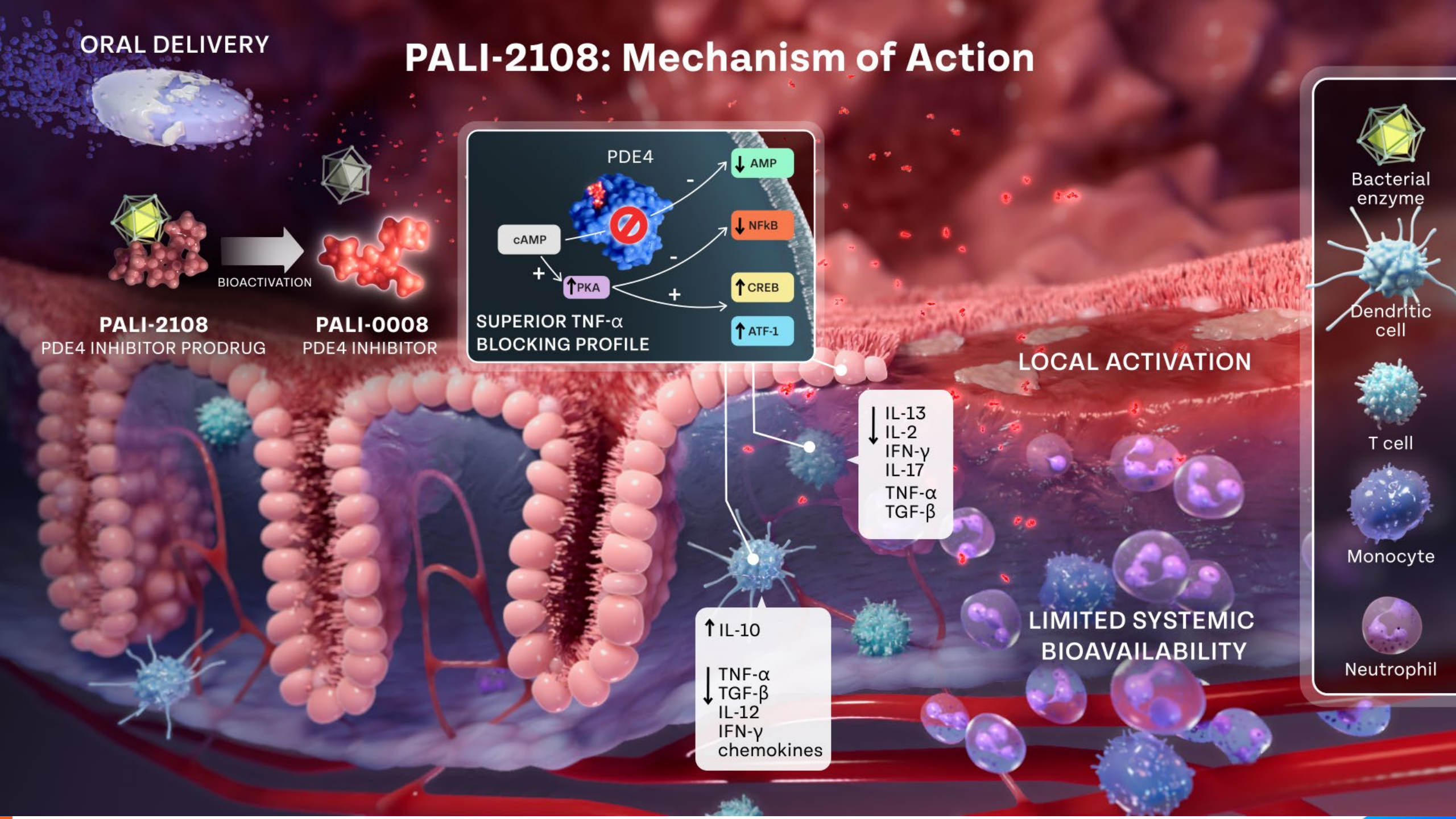
- Patients with Intestinal Fibrosis show increased levels of PDE4 enzymes in intestinal tissues.
- Mouse models of IF have been used to demonstrate PDE4 inhibitors significantly improve disease activity.
- PDE4 inhibitors prevent breakdown of cAMP and inhibit fibroblast functions including mitigating tissue remodeling.
- The important mediator of fibrosis, TGF-beta, can modulate cAMP levels and PDE4 inhibition is particularly effective in the presence of TGF-beta-induced fibroblast stimulation (Togo et al. 2009)
- As seen below, mouse models of IF have been used to demonstrate, intestinal α-SMA and MMPs are normalized with PDE4 inhibitor treatment (Li et al. 2021)





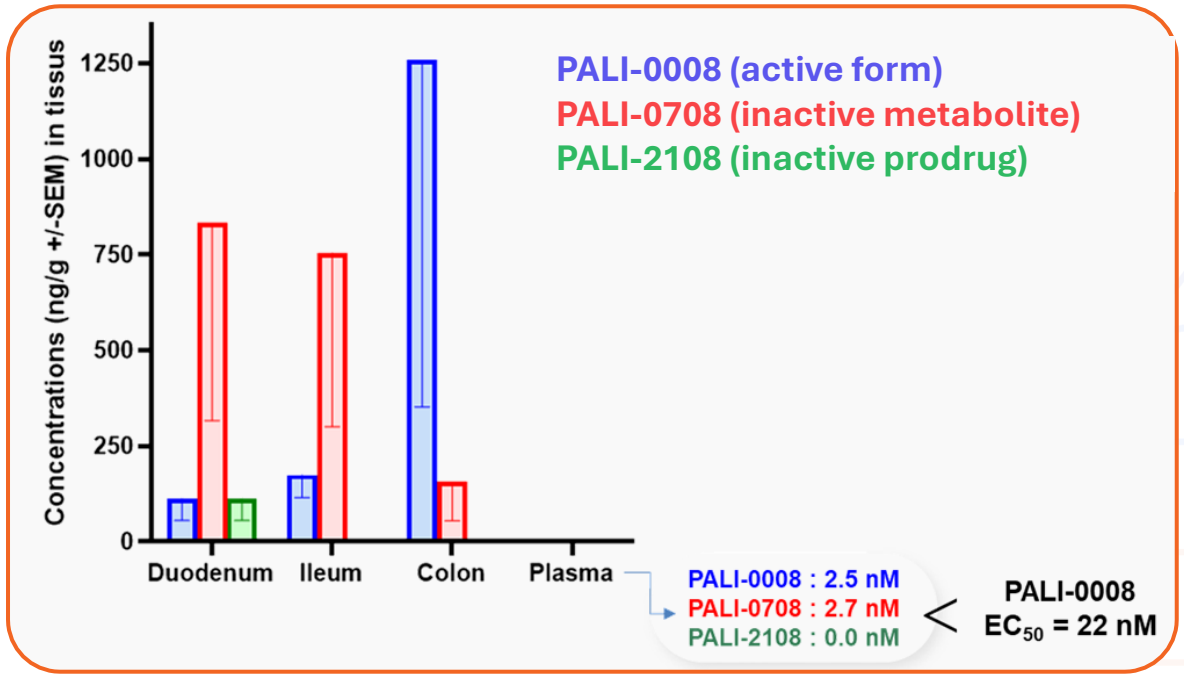
Li et al. 2021




ORAL DELIVERY
PALI-2108: Mechanism of Action
BIOACTIVATION
PALI-2108
PDE4 INHIBITOR PRODRUG
PALI-0008
PDE4 INHIBITOR
PDE4
cAMP
PKA
AMP
NFkB
CREB
ATF-1
SUPERIOR TNF-α
BLOCKING PROFILE
LOCAL ACTIVATION
IL-13
IL-2
IFN-γ
IL-17
TNF-α
TGF-β
IL-10
TNF-α
TGF-β
IL-12
IFN-γ
chemokines
LIMITED SYSTEMIC
BIOAVAILABILITY
Bacterial enzyme
Dendritic cell
T cell
Monocyte
Neutrophil

Limited Systematic Activity in Preclinical Model Provides Potential for Improved Safety Profile

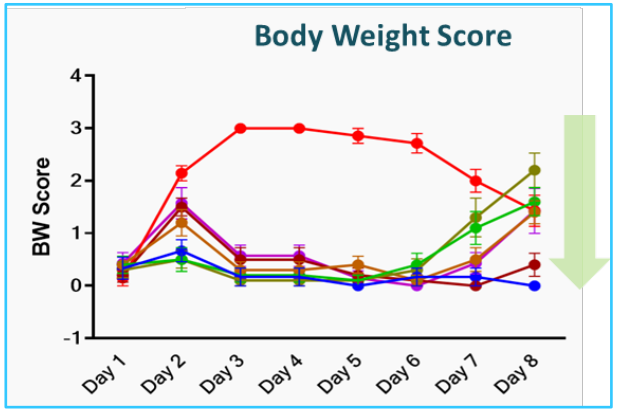
Lack of Plasma Activity is Believed to Mitigate CNS Side Effects, like Nausea, Which is Seen with Systemic PDE4 Inhibitors

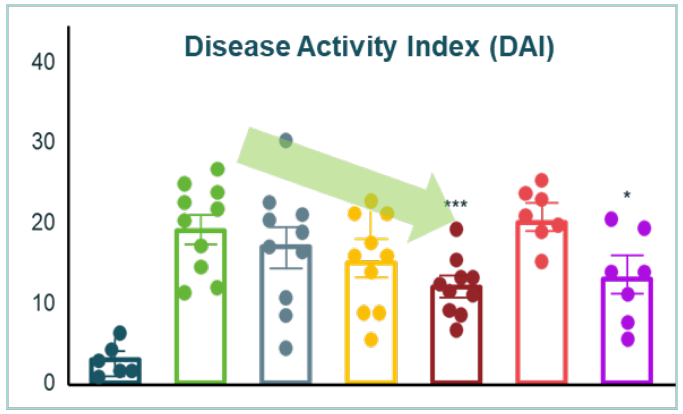


- ✓ Prodrug is released within the ileum and bio-converted by bacterial enzymes in the colon
- ✓ Prodrug is locally absorbed and inhibits PDE4 enzyme intracellularly
- ✓ Improved therapeutic window enables maximal PDE4 inhibition supporting higher cAMP tissue levels (supporting data in next slide)

Demonstrated Improved Efficacy Over Standard of Care and Systematic PDE4 Inhibitors in DSS Colitis Mouse Model

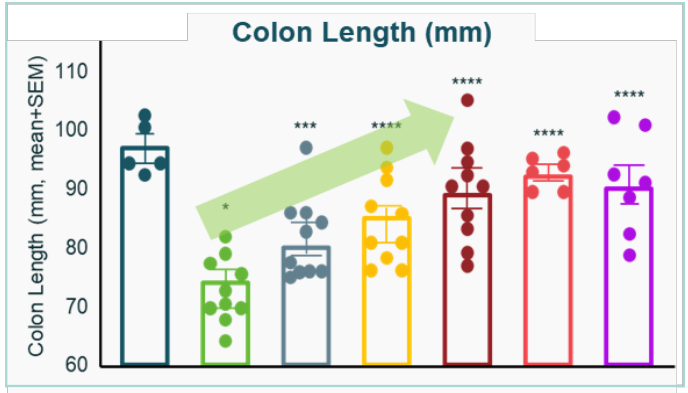
Dose Dependent Efficacy Response in Two DSS Colitis Mouse Models





<0.05,***p<0.001, ****p<0.0001: Dunnett's test compared to 4%DSS+Vehicle

- Non-Treatment naive
- 4% DSS + Vehicle (PO)
- 4% DSS + PALI-2108/ 40mg/kg (PO)
- 4% DSS + PALI-2108/ 80mg/kg (PO)
- 4% DSS + PALI-2108/ 160mg/kg (PO)
- 4% DSS + Cyclosporin A 80mg/kg (PO)
- 4% DSS + Apremilast/ 25mg/kg (PO)



****p<0.0001,***p<0.0001, *p<0.05: Dunnett's test compared to DSS+Vehicle

PALI-2108 demonstrated clear dose dependent improvements in clinical outcome measures and was better than SOC in DSS colitis mouse model

While apremilast showed efficacy, it was at a dose not well tolerated when translated to human, including nausea*

Danse et al. evaluated 60mg and 80mg daily doses in UC patients which is ~1mg/kg; whereas we used the HED of ~2mg/kg in this study

*Danese et al

PALI-2108

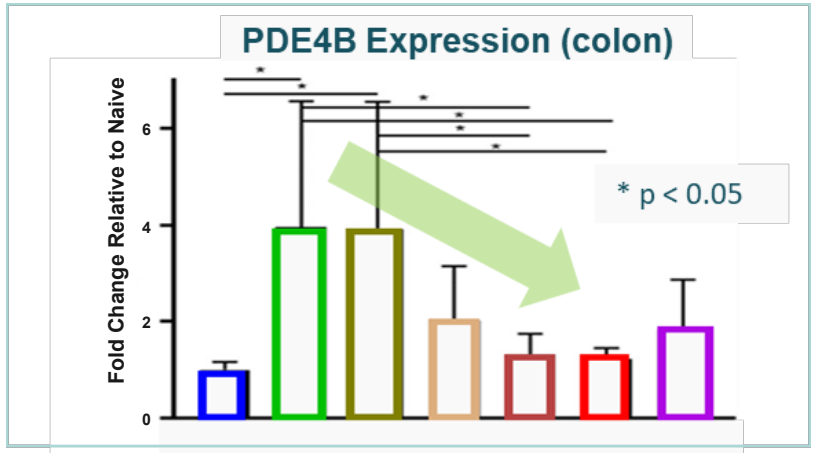
Demonstrated Improved Efficacy Over Standard of Care and Systematic PDE4 Inhibitors in DSS Colitis Mouse Model

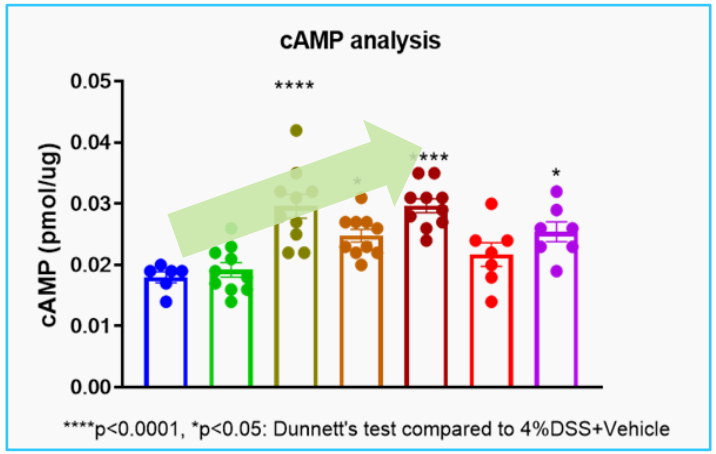
Dose Dependent Efficacy Response in DSS Colitis Mouse Model

Colon tissue PDE4B Expression (mRNA) was reduced in response to PALI-2108 in a dose dependent manner together with local PDE4B inhibition and increased intracellular cAMP

cAMP levels were increased more with PALI-2108 and TNF-alpha levels in colon tissue are normalized on average an in most animals

*Danese et al





- Non-Treatment naive
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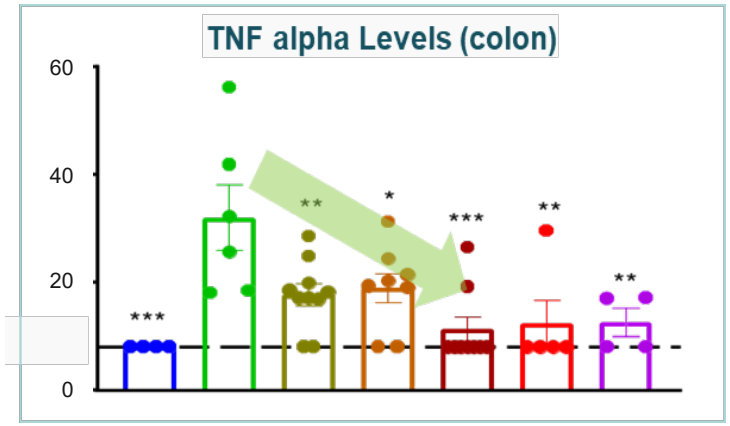

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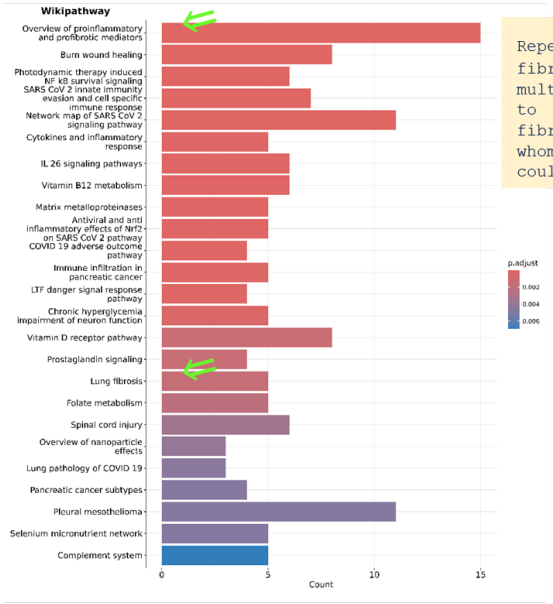
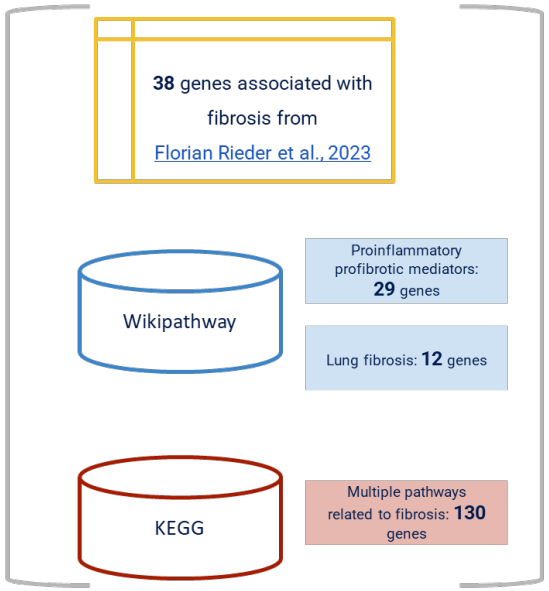
PALI-2108

Prominent markers from meta-analysis are associated with fibrotic pathways

Enrichment



Repeated enrichment of **2** fibrosis related pathways in multiple meta-analyses led us to accumulate a list of fibrotic pathway markers, on whom the effect of *PALI-2108* could be assessed.



Combined list of **187** fibrotic gene expression was used to estimated combined change in variation of fibrotic pathway expression in a dosage dependent manner.

We built a >1,600 UC and CD patient database including patients with ileal and colon tissue RNAseq data and built a genomics workflow to identify markers and enriched pathways of fibrosis in UC and CD

We included genes from fibrostenotic CD review literature and profibrotic mediators from Wikipathway and KEGG

A combined list of 187 fibrotic genes were used to estimate the combined change in variation of the fibrotic pathway gene expression after DSS and with PALI-2108 treatment.

*Strand

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Demonstrated Dose Response for Engagement of Most Important Fibrotic Pathways in UC and Crohn's

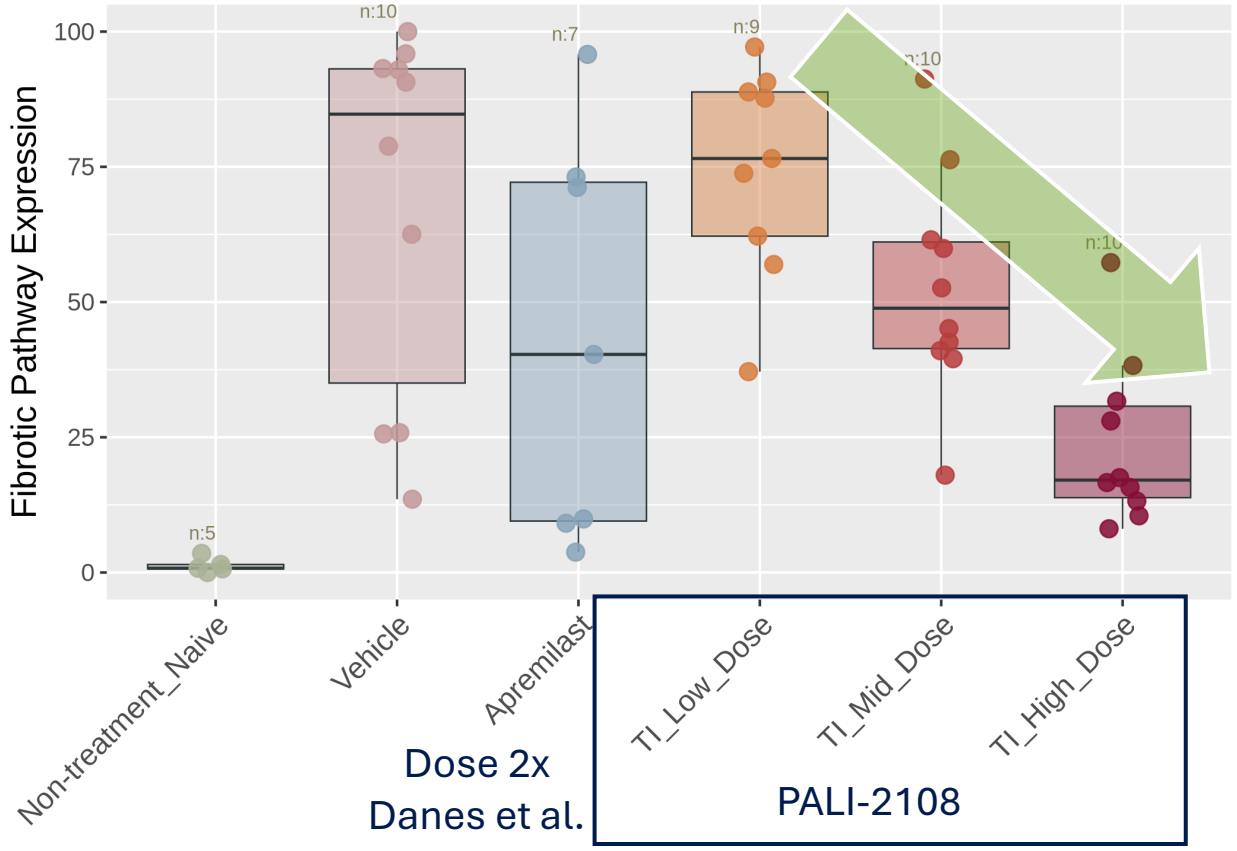
Engagement of genes from the four IBD fibrotic pathways (187 genes)

Consolidated enrichment score for these markers was obtained for each mouse sample

Score represents the overall up- or down-regulation of the fibrotic pathway signature in each sample

PALI-2108 may offer a promising solution for fibro stenotic Crohn's disease by enhancing efficacy, safety, and therapeutic potential compared to traditional treatments

PALI-2108 demonstrated localized bioactivation and potent antifibrotic pathway engagement



Precision Medicine-Based Approach

Machine Learning Technology for Patient Selection

Targeting patients with elevated PDE4 activity (PDE4 related biomarkers) to potentially enhance responder population

Process

Identify Markers of Colitis → **RNAseq DiffExp** → **Top Ranked Genes (U or D)** → **Top Ranked in Multiple Studies** → **Relation with PDE4**

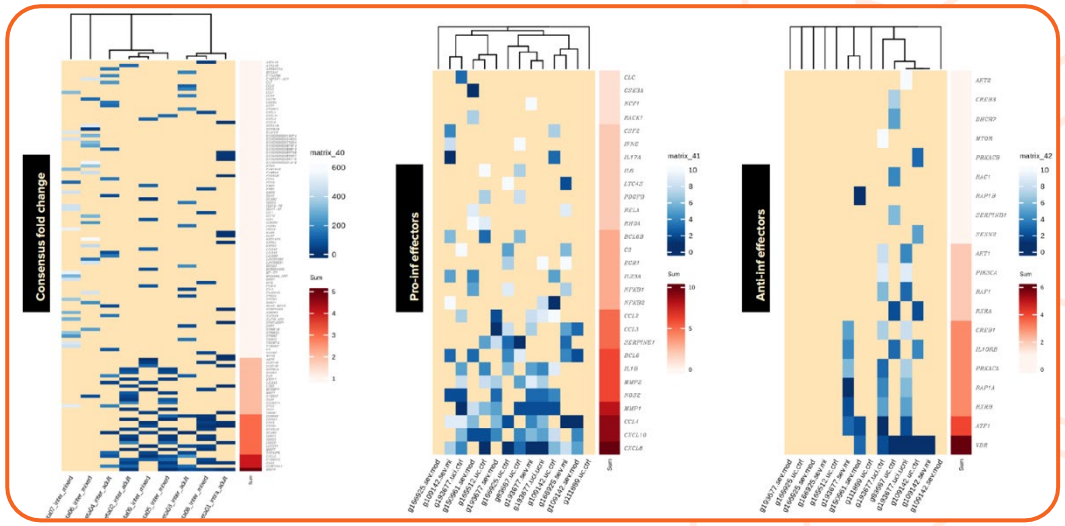
Output

Identified pool of PDE4-effector genes predictive of potential patient response to PDE4 inhibitor

Genes ranked by weights were selected from meta-analysis, pro-inflammatory and anti-inflammatory

Generate a weighted sum DAS score

Meta-Analysis



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First Precision Medicine Test with PDE4B-Based Selection Alone Could be a Breakthrough

Elevated PDE4B Expression:
Consistently observed in adult patients in >1600 patients and ~10 studies

PDE4BX

Dataset	Age Group	Expression Threshold	UC (%)	UC Severe Moderate (%)	UC Inactive Mild (%)	UC Inflammatory (%)	Control (%)
GSE193677	Adult	1.13	57	70	52	70	42
GSE193677	Adult	0.8	72	79	70	81	62
GSE193677	Adult	1.12	58	70	53	70	43
GSE109142	Pediatric	5.67	34	90	73	NA	5
GSE109142	Pediatric	5.01	37	91	86	NA	5
GSE83687	Adult	4.9	69	NA	NA	NA	34
GSE166925	Adult	2.59	38	70	0	55	1
GSE166925	Adult	1.14	75	80	67	80	12
GSE166925	Adult	1.92	58	80	17	70	4
GSE150961	Pediatric	0.52	90	90	NA	NA	NA
GSE111889	Adult	2.81	70	NA	NA	NA	41
GSE165512	Adult	1.31	70	NA	NA	NA	29

Table 2.8.1: *Summary of PDE4B expression cut-off above which 70% (Adult) or 90% (Pediatric) of the disease samples are observed*

Broad Applicability: Average expression of 1.6 TPM (range 0.8-2.81 TPM) covers over 70% of moderate to severe cases.

Potential FDA-Approved test: PDE4B expression provides a reliable marker for patient stratification, essential for optimizing our UC PDE4 prodrug therapeutic.

Development: Integration of PCR-based assays with RNAseq will standardize TPM values and improve qPCR Ct value correlations, ensuring precise and effective patient targeting.

First approach leverages PDE4B expression to enhance treatment efficacy and personalization, solidifying our position as a leader in precision medicine for ulcerative colitis.