# Validation of hPTM-001, a humanized candidate therapeutic antibody for promoting mucosal wound healing in IBD

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## Abstract:

A hallmark of the clinical course of patients with Inflammatory Bowel Disease (IBD) is poorly healing erosions and ulcers in the intestinal mucosa. Despite the adverse clinical consequences of non-healing mucosal wounds in IBD, current front-line therapies that selectively target mucosal wound healing are not available.

Recent studies revealed an association between colonic inflammation and aberrant glycosylation of epithelial CD44v6. Under conditions of inflammation, epithelial CD44v6 was shown to be decorated with the terminal glycan sialyl Lewis A. Importantly, targeting sialylated Lewis glycans on CD44v6 with a murine antibody GM35 (mPTM-001) was shown to promote mucosal wound healing in cell lines and in biopsy based wounding assays as well as dextran sodium sulfate (DSS) induced murine colitis models.

We sequenced CDRs from GM35 and produced a humanized antibody. Eight candidate human IgG1 clones were produced and screened. hPTM-001 was selected from the eight candidates based on glycan affinity and selectivity compared to GM35. In vitro wound healing studies revealed that PTM-001 was as effective as GM35 in promoting repair of scratch wounds with human intestinal epithelial cells. Furthermore, intraperitoneal injection of mice with hPTM-001 during induction of DSS colitis resulted in reduced weight loss compared to control IgG. These results suggest that hPTM-001 is well-positioned as a unique potential candidate therapeutic for IBD that can be used to selectively promote healing of mucosal wounds and ulcers.

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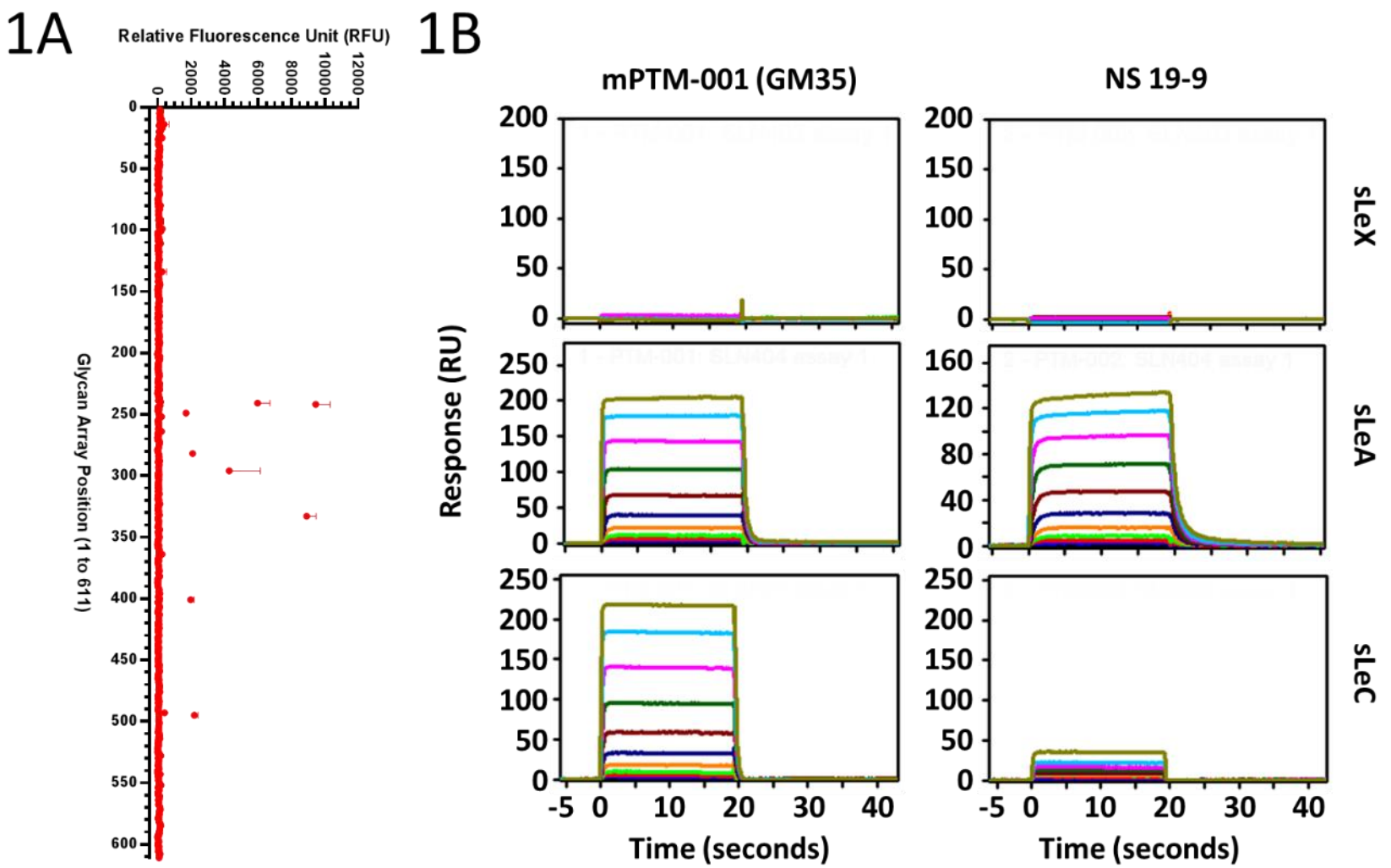
# Figure 1

Previously, GM35 (here termed mPTM-001) was demonstrated to bind to the terminal O-linked glycan sialyl Lewis A (sLeA) found on CD44v6 on colonic epithelial cells [1].

The glycan specificity of mPTM-001 was analyzed on a 611 glycan containing array by the National Consortium for Functional Genomics (NCFG, Boston, MA). mPTM-001 exhibited measurable binding on 8/611 glycans (fig. 1A). The 8 mPTM-001 reactive glycans were variations of sLeA [1].

To confirm the binding data from the CFG glycan array, glycans sialyl Lewis X (sLeX), sialyl Lewis C (sLeC, precursor to sLeA), and sLeA (all from Dextra Biosciences, Reading, UK) were flowed over mPTM-001 coated Biacore sensor chips to measure binding kinetics (Biosensor Tools, Salt Lake City, UT, USA). mPTM-001 bound to sLeA and sLeC but not sLeX. NS 19-9, an antibody to sLeA, had minimal reactivity to sLeC (fig. 1B).




[1] Brazil, J. C., et. al., *J Immunol* 2013; 191:4804-4817

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# Figure 2

mPTM-001 was sequenced (Digital Proteomics, San Diego, CA, USA). 4 light chain variable domains (VL, L1 to L4) and 3 heavy chain variable domains (VH, H1 to H3) were designed. Transiently transfected HEK293 cells expressing the combination of VL and VH sequences (fig. 2A) were generated at Absolute Antibodies (Boston, MA, USA) and purified. Two VH/VL combinations (humanized clones 1 and 4) had low expression levels and were deemed unusable.

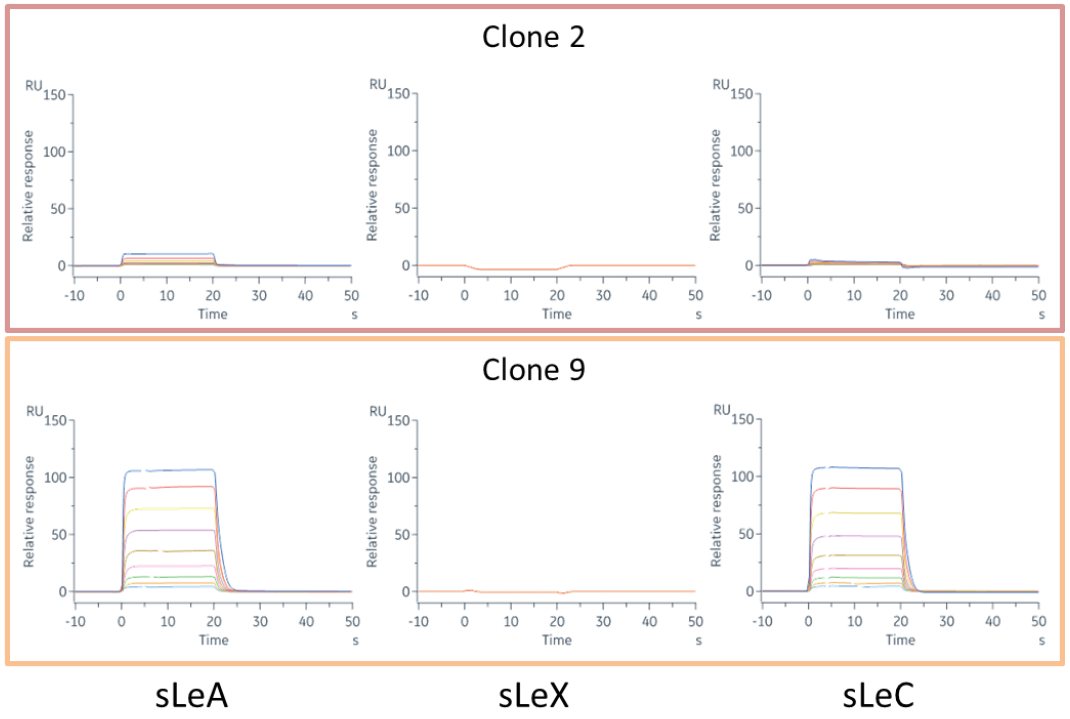
mPTM-001 and purified antibodies from clones 2, 3, 5, 6, 7, 8, and 9, were examined for glycan reactivity by Biacore (Antibody Analytics, Newhouse, UK). Clones 2, 3, and 5 had poor binding to glycans, clones 6 to 9 engaged sLeA and sLeC well (fig. 2B). Clone 9 was most like mPTM-001 in binding kinetics (no binding to sLeX, $K_D$ of 48.3µM and 77.1µM to sLeA and sLeC versus 44.0µM and 73µM to sLeA and sLeC for mPTM-001). Clone 9 was selected and renamed hPTM-001.

## 2A

| Heavy Chain Used | | | Humanized Clone # | Yield (mg/L) | sLeA | sLeC | sLeX |
|---|---|---|---|---|---|---|---|
| H1 | H2 | H3 | | | $K_D$ (µM) | | |
| L1 | | | 1 | 9 | Low Expression, No Data | | |
| | L1 | | 2 | 26 | 731.7 | 306.3 | N.B. |
| | L2 | | 3 | 146 | 620.7 | 538.0 | N.B. |
| | L3 | | 4 | 10 | Low Expression, No Data | | |
| | L4 | | 5 | 132 | 552.0 | 460.0 | N.B. |
| | | L1 | 6 | 134 | 81.3 | 103.7 | N.B. |
| | | L2 | 7 | 136 | 69.1 | 93.2 | N.B. |
| | | L3 | 8 | 135 | 59.2 | 85.4 | N.B. |
| | | L4 | 9 | 128 | 48.3 | 77.1 | N.B. |
| | | | mPTM-001 | | 44.0 | 73.0 | N.B. |

**N.B. = No Binding**

## 2B



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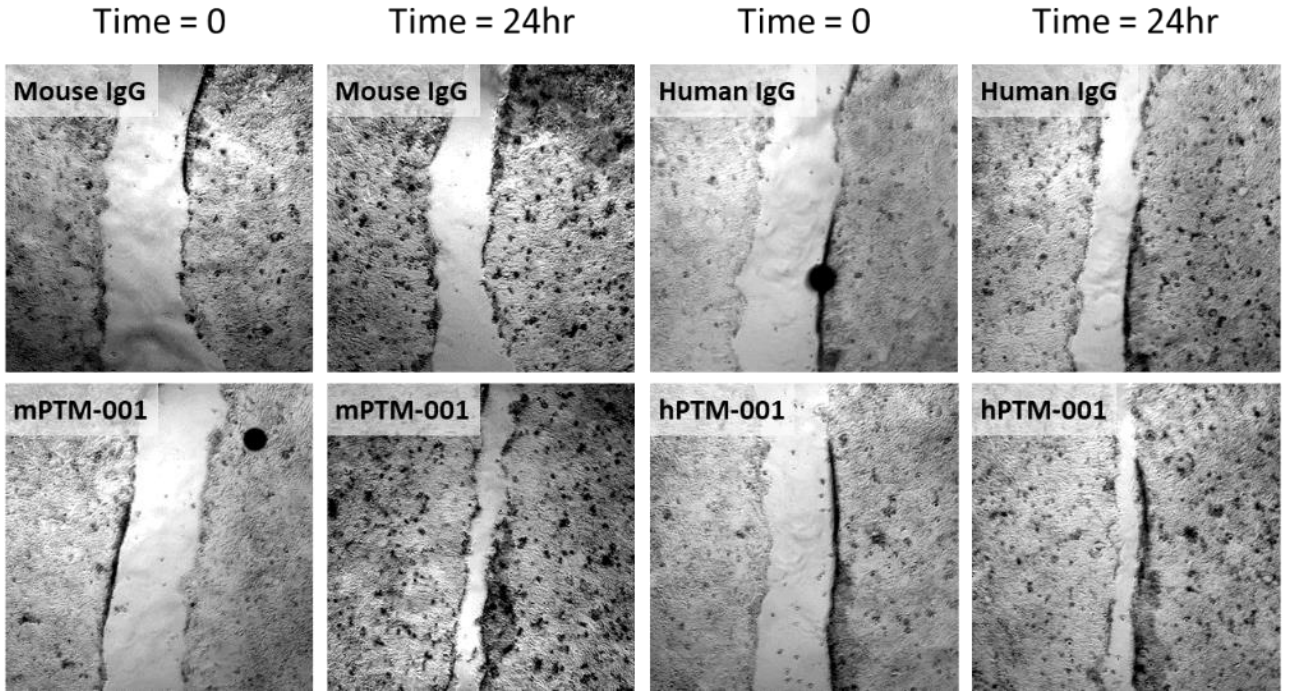
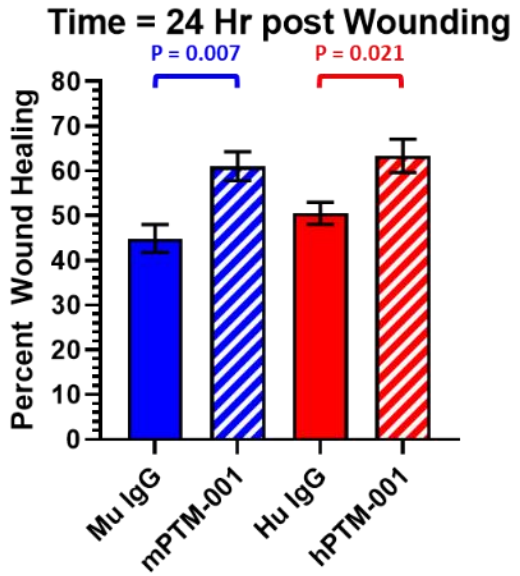
# Figure 3

mPTM-001 was previously shown to accelerate intestinal epithelial wound healing *in vitro* [2]. hPTM-001 was used in a scratch wound assay to examine its ability to enhance intestinal epithelial wound healing. mPTM-001 was used as a comparator. As can be seen in figure 3A, mPTM-001 and hPTM-001 both accelerated intestinal epithelial wound healing in a 24 hour assay when compared to mouse and human IgG controls. Figure 3B shows the quantitated percent wound healing outcome. As can be seen, mPTM-001 (blue hash column) and hPMT-001 (red hash column) demonstrated 10-15% increase in intestinal epithelial wound healing. This outcome demonstrates that hPTM-001 maintained the *in vitro* wound healing properties of mPTM-001.

3A




3B




[2] Kelm, M, et. al., *JCI Insight.* 2020;5(12):e135843

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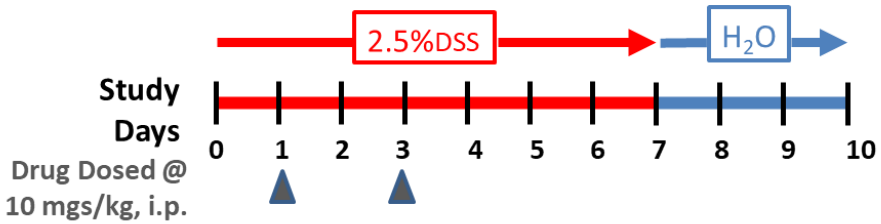
# Figure 4

Previously, mPTM-001 was demonstrated to protect against acute DSS-induced colitis in mice [2]. The study was performed in a prophylactic manner, where the mice were given 10mg/kg mPTM-001 by intraperitoneal (IP) injection on study day -1 (24 hours before administering DSS) and day 2 (24 hours after administering DSS) [2].

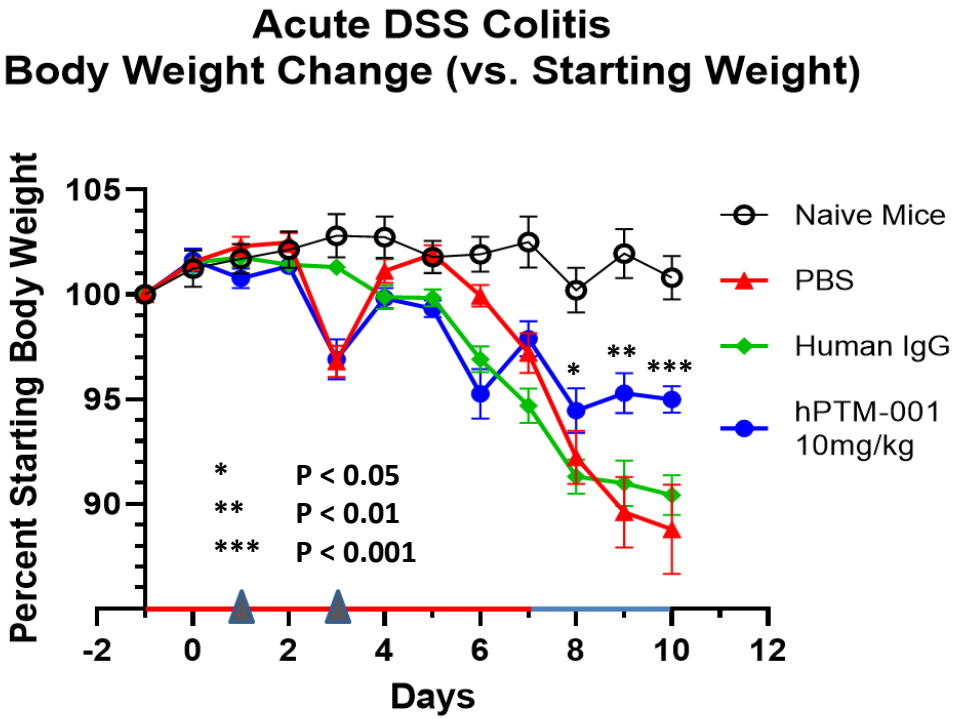
hPTM-001 was validated in an acute DSS colitis model in mice (Crown Biosciences San Diego, San Diego, CA, USA) with 2.5% DSS in drinking water administered to mice starting on day 0, on day 7, the 2.5% DSS in drinking water was replaced by normal drinking water and the study continued until day 10. The study was conducted in a therapeutic manner: 10mg/kg of hPTM-001 was administered by IP injections on days 1 and 3, similarly administered human IgG1 (hIgG, 10mg/kg) and PBS was used as controls (N=10 mice per treatment group, fig. 4A).

Compared to hIgG and PBS treated mice, hPTM-001 treated mice suffered less body weight loss: 5.02% ± 0.73% vs 11.20% ± 2.12% and 9.57% ± 1.00% for IgG and PBS treated mice, respectively (fig. 4B).

4A




4B




[2] Kelm, M, et. al., *JCI Insight.* 2020;5(12):e135843

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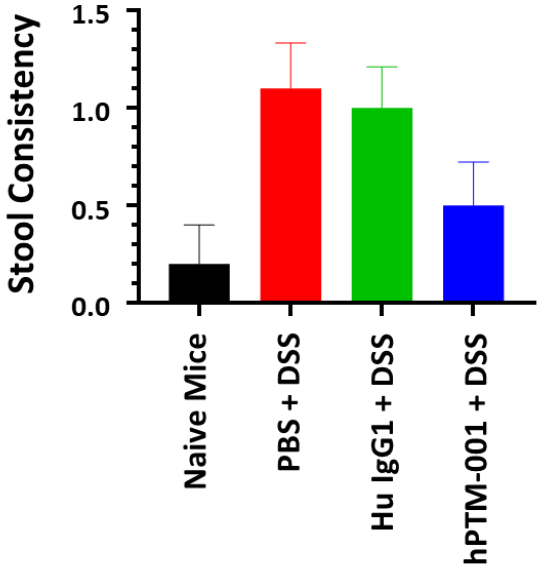
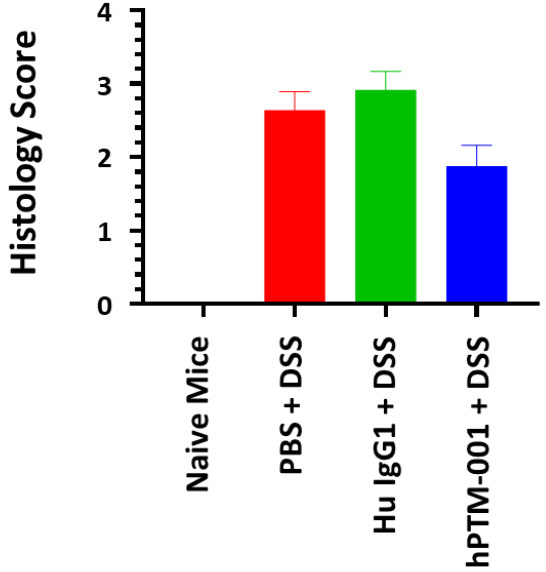
# Figure 5

hPTM-001 treated mice had better stool consistency score (based on the state of stool and presence of blood via hemoccult test, 0 = normal) compared to PBS or hIgG treated mice (fig. 5A). Histology scoring of swiss rolled colon tissue shows better scoring (a summary of inflammation, edema, erosion, hyperplasia, and necrosis observed) in hPTM-001 treated groups versus PBS or hIgG groups (fig. 5B). Figure 5C shows representative images of colon tissue from naïve mice, PBS, hIgG, and hPTM-001 treated groups, illustrating improved colon morphology in hPTM-001 treated groups.

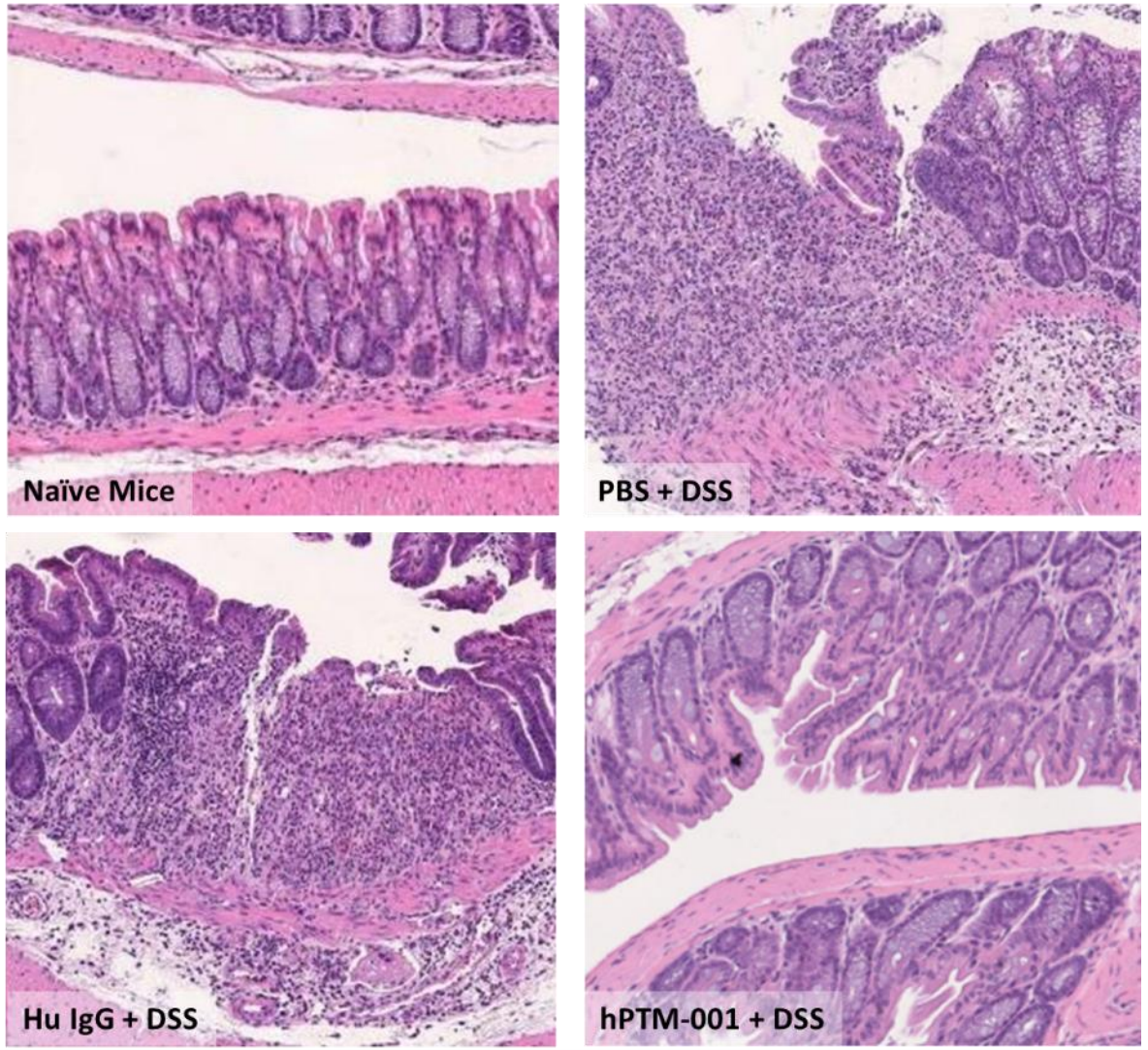
5A




5B




5C



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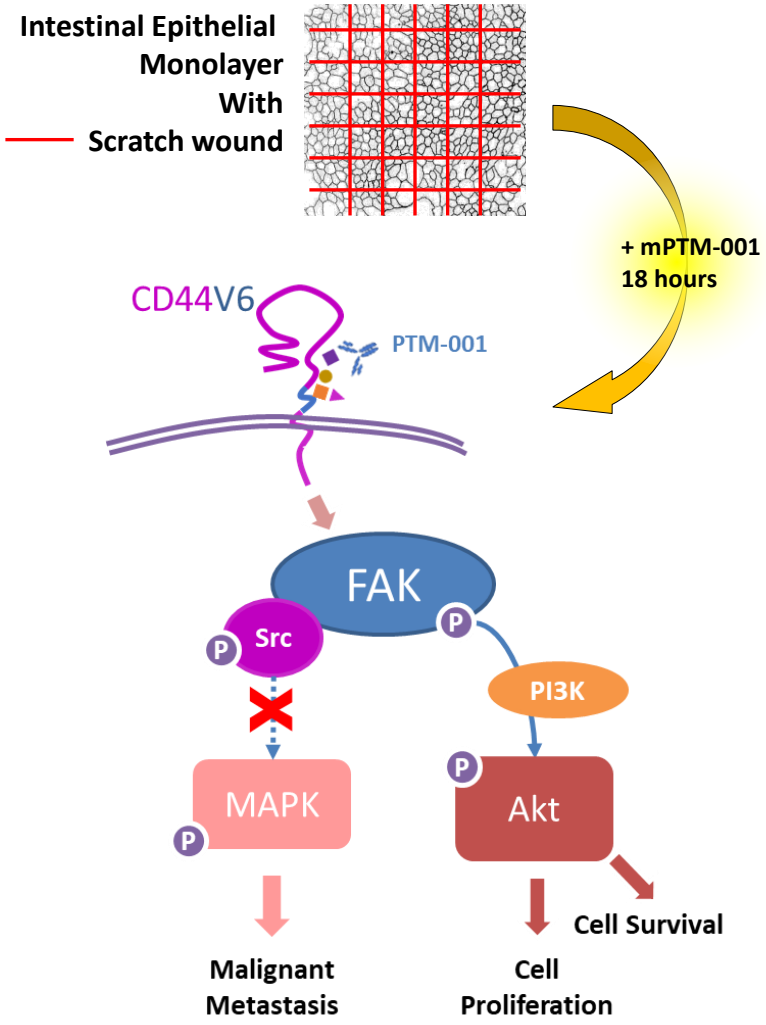
# Conclusion




IBD is characterized by immune dysfunction and ulcerating wounds in the intestinal mucosa. Although mucosal wound healing has been shown to confer better clinical outcomes, current IBD therapeutics have not been able to adequately address mucosal wound healing in IBD.

PTM Therapeutics has humanized and validated hPTM-001, a therapeutic antibody targeting an epithelial expressed carbohydrate epitope, sLeA on CD44v6, to enhance intestinal mucosal wound healing.

The parental antibody of hPTM-001, mPTM-001 (GM35) has been shown to accelerate epithelial wound healing *in vitro* on human intestinal epithelial monolayers and enhance the repair of mechanically wounded murine intestinal mucosa *in vivo*. On intestinal epithelial cells undergoing wound repair (18 hours) *in vitro*, mPTM-001 is shown to trigger a signaling cascade involving FAK, PI3K, Src, Akt, but not MAP Kinase [2]. This signaling cascade was not induced by an isotype control. The signaling cascade, specifically FAK, has been shown to be important for maintaining colon homeostasis [3].

hPTM-001 has similarly been demonstrated to enhance *in vitro* epithelial wound healing on scratch wounded human intestinal epithelial cell monolayers.

In *in vivo* acute DSS induced colitis disease model, mice treated with 2 doses of hPTM-001 (10mg/kg administered by IP injections) experienced less body weight loss, and better stool consistency scores when compared to hIgG and PBS treated animals. Histological assessment of the mouse colons also shows that mice treated with hPTM-001 had better histology scores (scored on the degree of inflammation, edema, erosion, hyperplasia, and necrosis).

Taken together, the data demonstrate that hPTM-001 is a therapeutic antibody that drives mucosal wound repair in the gut and thus may represent a novel therapeutic strategy for promoting restitution of intestinal homeostasis in IBD.


[2] Kelm, M, et. al., *JCI Insight*. 2020;5(12):e135843
[3] Owen, K.A., et. Al., *PLoS ONE,* 2011; 6(8):e23123