P059

GB004 Drives Protective Effects on Immune Cells and Epithelial Cells Using Human *Ex vivo* Monolayer and Co-Culture Systems

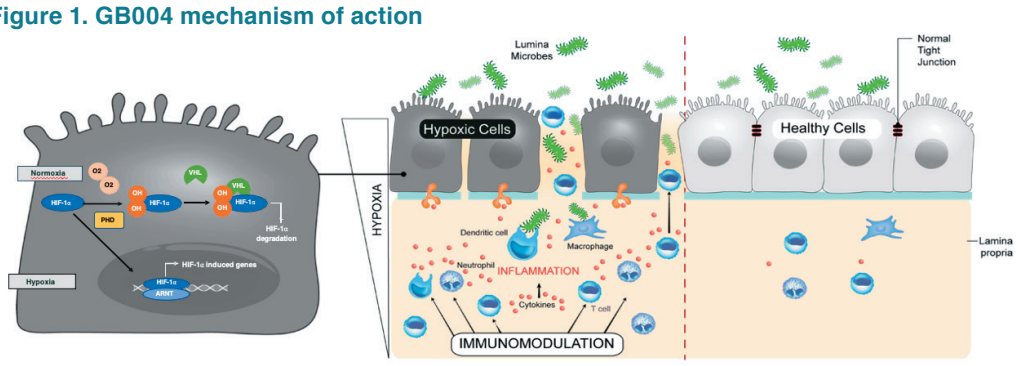
Kristen R. Taylor Meadows, Susan Murphy, Barrett G. Levesque, Laura Carter, Luisa Salter-Cid

Gossamer Bio, Inc.

BACKGROUND

- Inflammatory bowel disease (IBD) is characterized by a breach in intestinal barrier integrity, allowing influx of luminal antigens and setting up a vicious cycle of inflammation and epithelial injury[1]
- GB004 is an oral, gut-targeted, small molecule that stabilizes hypoxia inducible factor (HIF-1α), a key transcription factor involved in the adaptive and protective cellular responses at the intersection of hypoxia and inflammation[2] (**Figure 1**)
- Preclinical efficacy of GB004 has been demonstrated in mouse models of colitis and correlated with HIF-1α stabilization in colonic epithelial cells, induction of HIF-1α target genes, downmodulation of inflammatory cytokines, and improvement in histologic parameters of barrier function[3,4]

Figure 1. GB004 mechanism of action



OBJECTIVE

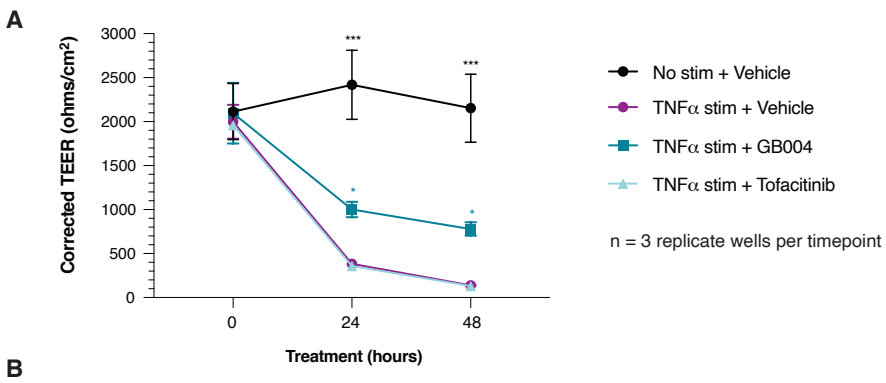
- HIF-1α stabilization by GB004 drives expression of genes that promote barrier restitution and downmodulate inflammation in animal models. To investigate further, we assessed the direct effects of GB004 on barrier integrity using a human differentiated monolayer assay. We also assessed GB004 treatment on *in vitro* immune cell co-culture systems to model effects on local, GI-resident immune cells to further explore its protective mechanism

METHODS

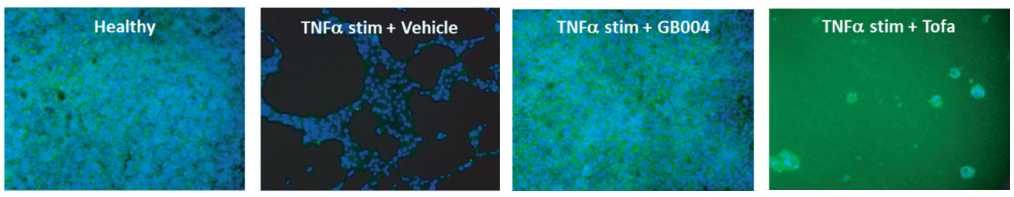
- Repligut human stem-cell-derived monolayer platforms (Altis Biosystems, Chapel Hill, NC) were either unstimulated or stimulated with TNFα and treated with GB004. Barrier integrity was assessed by Transepithelial Electric Resistance (TEER) and tight junction formation and epithelial monolayer viability was investigated by immunofluorescence staining
- In the BioMAP Diversity PLUS panel (Eurofins), human co-culture cell systems were treated with GB004 one hour prior to stimulation and incubated for 24-168 hours. Biomarkers in supernatants, cell viability and proliferation were assessed. BioMAP co-cultures shown in **Figure 4** include venular endothelial cells + PBMC stimulated with TLR4 ligand; venular endothelial cells + PBMC stimulated with TCR ligands; PBMC + B cells simulated with anti-IgM/TCR ligands; bronchial epithelial cells + dermal fibroblasts stimulated with IL-4/TNFα and bronchial epithelial cells stimulated with IL-1β/IFNγ/TNFα, and in **Figure 5** venular endothelial cells stimulated with TNFα/IL-1β/IFNγ; venular endothelial cells stimulated with IL-4/histamine; bronchial epithelial cells stimulated with IL-1β/IFNγ/TNFα; coronary artery smooth muscle cells stimulated with IL-1β/TNFα, IFNγ and lung fibroblasts stimulated with TGFβ/TNFα.
- Unless otherwise noted, data are presented as mean ± SD. Statistical analysis was carried out using GraphPad Prism and one-way ANOVA (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)

RESULTS

Figure 2. GB004 preserves barrier integrity and prevents TNFα-induced cell death in differentiated human monolayer assay system



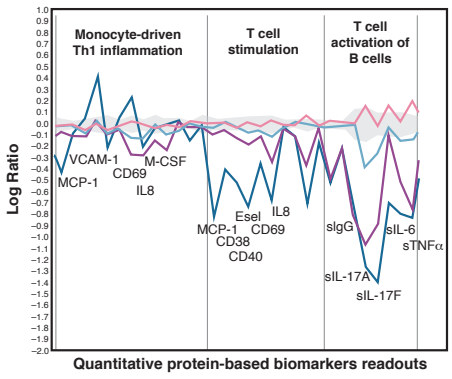
B



(**A**) TNFα-stimulated monolayers were treated with GB004 and TEER measured at 0, 24 and 48 hours to investigate barrier integrity. Data presented as mean ± SD; statistical analysis carried out using one-way ANOVA (*p<0.05, ***p<0.001) (**B**) After 48 hours of treatment with TNFα and compounds (GB004 or tofacitinib), monolayers were stained with ZO-1 (green) and DAPI (blue) and analyzed by immunofluorescence microscopy

- This data provides functional support of previous preclinical studies that demonstrate an upregulation of genes involved in barrier function and integrity following GB004 treatment[4,5]
- Furthermore, this upregulation in genes that restore the integrity of the gut mucosal barrier and increase barrier restitution was also observed in a Phase 1b study[6]

Figure 3. In addition to direct protective epithelial barrier effects, GB004 modulates immune cell function by reducing key mediators of inflammation and immunomodulation

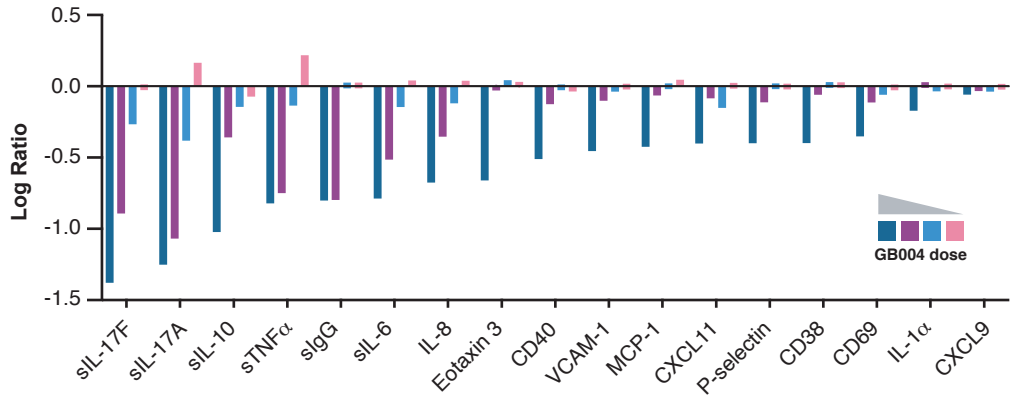


Eurofins BioMAP platform: Dark blue lines denote high GB004 treatment titrated down to the pink lines which depict low GB004 treatment. The X-axis lists the quantitative protein-based biomarker readouts measured in each system. The Y-axis represents a log-transformed ratio of the biomarker readouts for the drug-treated sample (n = 1) over vehicle controls (n ≥ 6)

95% significance envelope generated from historical vehicle controls

- Based on the distribution of GB004 *in vivo*, these results are expected to model effects on immune cells in the gut

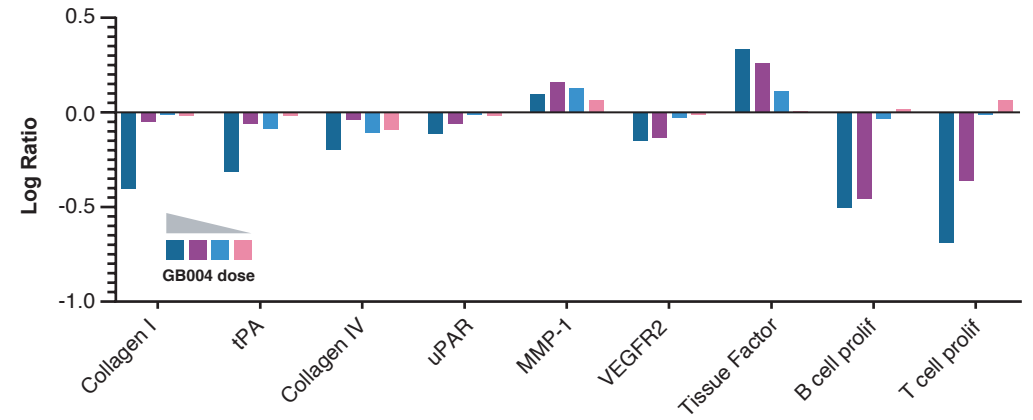
Figure 4. In BioMAP co-cultures, GB004 reduces multiple inflammatory and immunomodulatory mediators associated with chronic inflammation in IBD



Each co-culture assay in the BioMAP platform was dosed with a dose-response of GB004 ranging from high (dark blue), mid-high (purple), mid-low (light blue) and low (pink), as indicated by the grey triangle. The Y-axis represents a log-transformed ratio of biomarker key activities for the drug-treated sample over vehicle controls

- The gut-restricted action of GB004 supports local modulation of immune mediators rather than systemic immune suppression
  - Previous preclinical studies demonstrated a reduction in proinflammatory genes in the gut epithelium of colitis mice[3]
  - In addition, data generated in a Phase 1b study with GB004 downmodulated biomarkers associated with mucosal inflammation locally in the tissue/gut[6]

Figure 5. In BioMAP co-cultures, GB004 modulates tissue remodeling analytes and lymphocyte proliferation



Each system in the BioMAP platform was dosed with a dose-response of GB004 ranging from high (dark blue), mid-high (purple), mid-low (light blue) and low (pink), as indicated by the grey triangle. The Y-axis represents a log-transformed ratio of biomarker key activities for the drug-treated sample over vehicle controls

- Some biomarkers associated with remodeling were reduced, such as Collagen I, but others were elevated, such as Tissue Factor, with GB004 treatment
- In addition, lymphocyte proliferation of B and T cells was reduced in a dose-dependent manner with GB004 treatment
- The direct effect of GB004 on T cell proliferation and its GI-restricted profile could potentially limit the expansion of T cells infiltrating into inflamed tissue in IBD without systemic immune suppression

CONCLUSIONS

- Targeting both barrier function and local colonic inflammation represents a multi-faceted approach to treatment of inflammatory bowel disease
- GB004 demonstrates protective effects on human derived intestinal monolayer cultures by reducing cell death, promoting tight junction formation and improving barrier integrity
- GB004 modulates key anti-inflammatory and immunomodulatory activities in human co-culture assays, supporting a direct mechanism of GB004 to locally modulate colonic inflammation
- A Phase 2 clinical study of GB004 is ongoing in patients with ulcerative colitis (NCT04556383)

REFERENCES

1. Danese S, Fiocchi C. *N Eng J Med.* 2011; 365:1713-1725
2. Robinson S et al. *Gastroenterology.* 2008; 134:145-155
3. Keeley S et al. *Mucosal Immunology.* 2014; 7:114-123
4. Marks E et al. *Inflamm Bowel Dis.* 2015; 21:267-275
5. Taylor Meadows K et al. *UEG Journal* 2020; 8(8S):446
6. Sandborn W et al. *UEG Journal* 2020: 8(8S);462

Presented at the 16th Congress of the European Crohn's and Colitis Organization, July 2-3 & 8-10

Disclosures: KRTM, SM, BGL, and LC are employed by Gossamer Bio, Inc. LSC was formerly employed by Gossamer Bio, Inc. and is a current employee of Pioneering Medicines.