MaaT013 Pooled Fecal Microbiotherapy Improves Gastrointestinal Physiology and Controls Inflammation which Delays GvHD in a Humanized Mouse Study

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INTRODUCTION

Acute graft-versus-host disease (aGvHD) is a life-threatening complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT), where allogeneic donor T cells recognize recipient tissues as foreign and damage them. Gut microbiota disruption following allo-HSCT has been associated with increased aGvHD incidence, severity, and mortality [1-2]. The gut microbiota plays a key role in the modulation of the immune system, particularly in maintaining immune tolerance. These results prompted the development of fecal microbiotherapies to restore a healthy and diverse gut microbiota and to treat or prevent aGvHD. Growing clinical evidence supports the efficacy of fecal microbiotherapy in decreasing aGvHD mortality [3]. MaaT013, a standardized allogeneic fecal microbiotherapy derived from pooled healthy human fecal microbiota, has demonstrated unprecedented efficacy as third-line treatment of aGvHD with gastro-intestinal (GI) involvement in the pivotal ARES trial.

The objective of this study is to elucidate the mechanisms through which MaaT013 mitigates aGvHD by restoring gut homeostasis and regulating immune activation.

METHODS

We assessed the impact of MaaT013 on gut homeostasis and immune cell activation using a combination of methods:

- Metagenomic analysis (16S and shotgun) of MaaT013 products
- Application of MaaT013 metabolites to Caco-2/THP-1 leaky gut model
- Dendritic Cell (DC) activation assay with heat-inactivated MaaT013
- DC-mediated regulatory T cells (Tregs) modulation *in vitro*
- Engraftment of MaaT013 and quantification of metabolites produced by MaaT013 in germ-free mice
- Humanized mouse model of GvHD

RESULTS

MaaT013 displays high microbial richness and standardized taxonomic composition

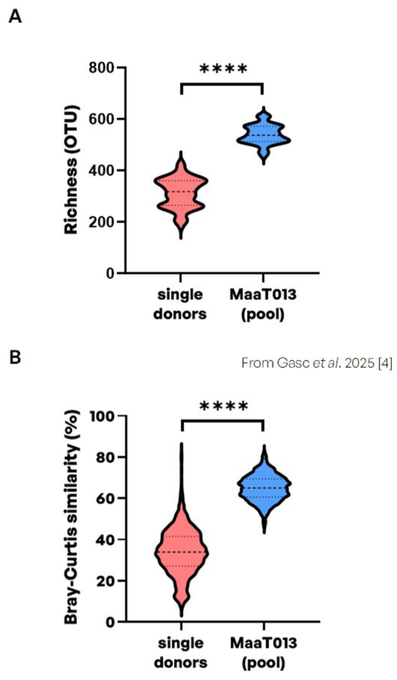


Figure 1. Metagenomic analysis of MaaT013 microbiotherapy products. 16S rDNA sequencing was performed to evaluate A) microbial richness at operational taxonomic unit (OTU) level and B) intra-group Bray-Curtis similarities at OTU level for single-donors (n=78 fecal samples from 22 donors) and corresponding pooled MaaT013 batches (n=38). Median and range are presented. Wilcoxon test was used for statistical analysis (**** p≤0.0001).

MaaT013-derived metabolites control inflammation and restore gut barrier integrity in an *in vitro* leaky gut model

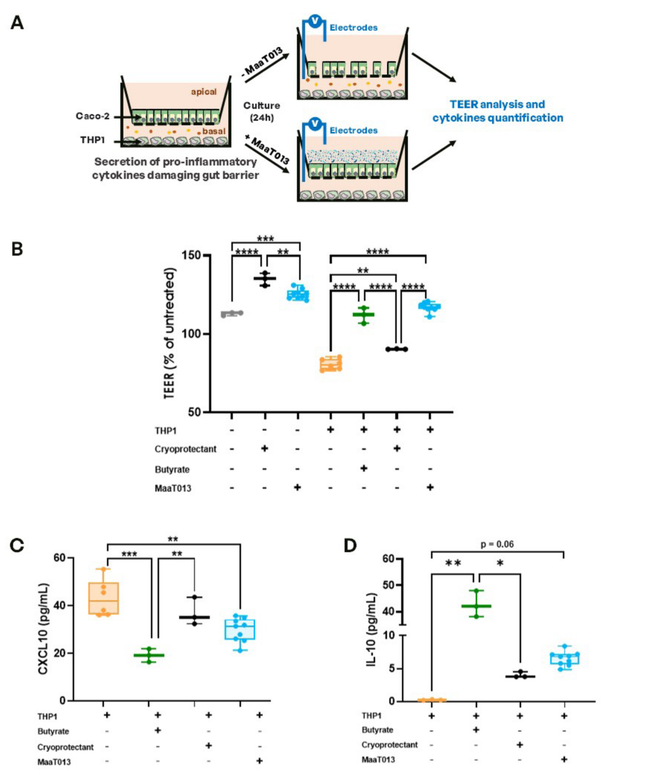


Figure 2. *In vitro* leaky gut assay. A) Experimental design. **B)** Leaky gut (Caco-2/THP1-Blue™ co-culture) assay was performed in presence of MaaT013 supernatants (n=3 batches), or cryoprotectant, sodium butyrate and Caco-2 complete medium as controls. **C)** Quantification of pro-inflammatory chemokine CXCL10 and **D)** anti-inflammatory cytokine IL-10. Data are presented as mean ± SEM. A one-way ANOVA was performed followed by Tukey's multiple comparisons for statistical analysis.

MaaT013 promotes dendritic cell maturation, driving Tregs polarization and activation *in vitro*

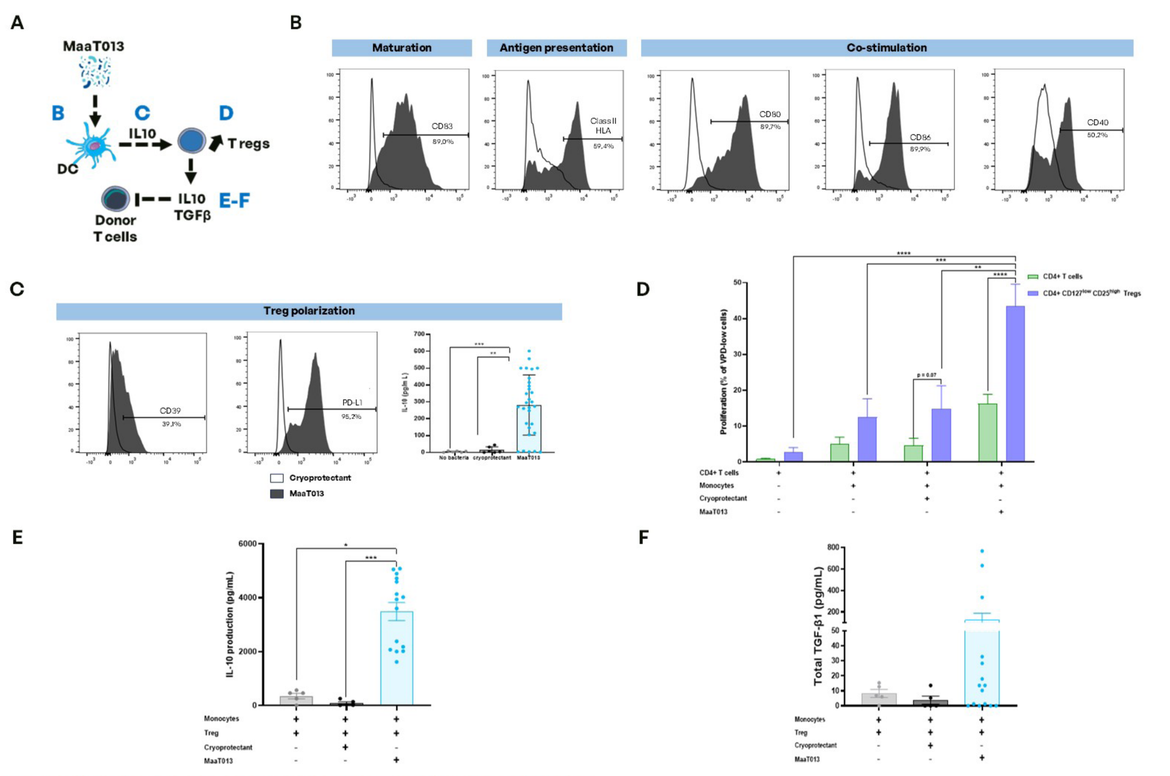


Figure 3. *In vitro* impact of MaaT013 on DC-mediated Treg activation. A) Experimental design. **B)** Naïve monocytes-derived DC primed with heat-inactivated MaaT013 were analyzed by flow cytometry (CD80, CD83, CD86, CD40, class II HLA). **C)** Treg polarization was analyzed by flow cytometry (CD39, PD-L1) and secretion of anti-inflammatory cytokine IL-10 by MaaT013-primed moDC was measured by ELISA. **D)** MaaT013-primed moDC cultured in presence of autologous CD4+ cells. VPD analysis was performed to quantify CD4+ T cells and CD3+ CD4+ CD25+ CD127low Tregs proliferation by flow cytometry. **E)** Secretion of anti-inflammatory cytokines IL-10 and **F)** TGF-β by Tregs activated by MaaT013-primed moDC. All data are presented as mean ± SEM. Kruskal-Wallis test was performed followed by Dunn's multiple comparison test for all experiments except for Treg proliferation, a two-way ANOVA was performed followed by a Tukey's multiple comparison test.

MaaT013 safely engrafts in germ-free mice, restoring healthy gut physiology and immune homeostasis

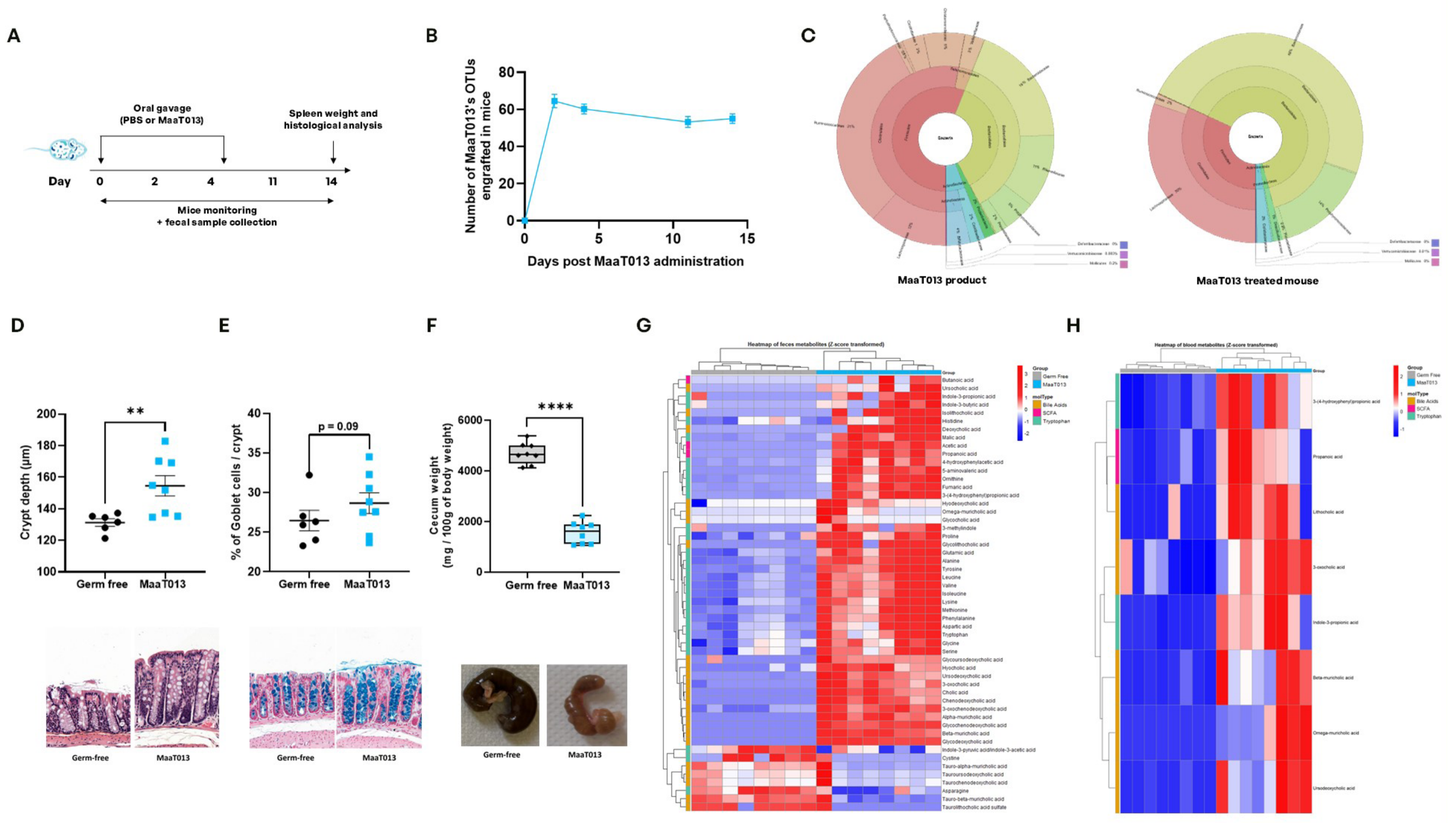


Figure 4. MaaT013 engraftment in a germ-free mouse model. A) Experimental design. 6-8 weeks old male and female C3H/HeN germ-free mice were used. Mice were dosed twice via oral gavage with 100 µL of MaaT013 (10^8 viable bacteria) (8 mice) or sterile water (6 mice) as control at day 0 and 5. **B)** MaaT013 engraftment in mice defined as the number of MaaT013's OTUs detected in mice after treatment. **C)** Krona analysis comparing the taxonomic profile of MaaT013 product to that of a MaaT013-treated mouse (representative example). **D)** Colonic crypt depth in MaaT013-treated and germ-free mice. Representative examples of hematoxylin-eosin stained colon tissues from germ-free and MaaT013-treated mice are provided. Scale bar indicates 20 µm. **E)** Percentage of Goblet cells per colonic crypt in MaaT013-treated and germ-free mice. Representative examples of alcian blue stained colon tissues of germ-free and MaaT013-treated mice are provided. Scale bar indicates 20 µm. **F)** Cecum weight at the time of necropsy expressed as mg/100 mg of body weight. **G)** Hierarchical bi-clustering analysis revealing differential metabolite levels in the feces of germ-free and MaaT013-treated mice. **H)** Hierarchical bi-clustering analysis revealing differential metabolite levels in the plasma of germ-free and MaaT013-treated mice. Data are presented as mean ± SEM. Mann-Whitney test was performed.

MaaT013 delays the appearance of GvHD symptoms in a highly immunodeficient humanized mouse model

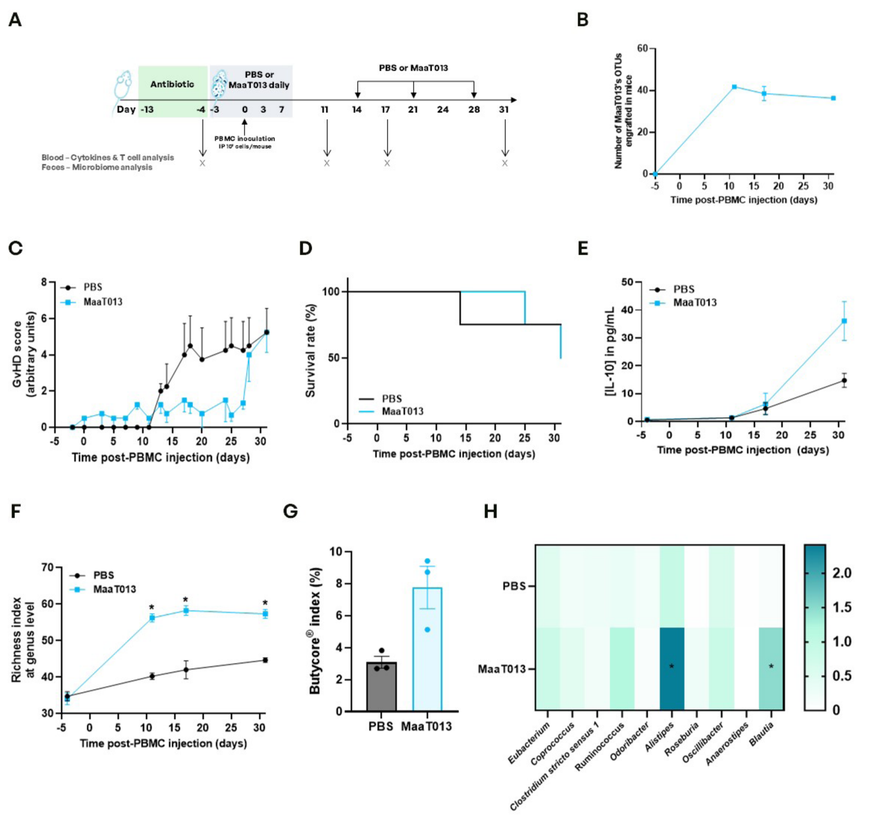
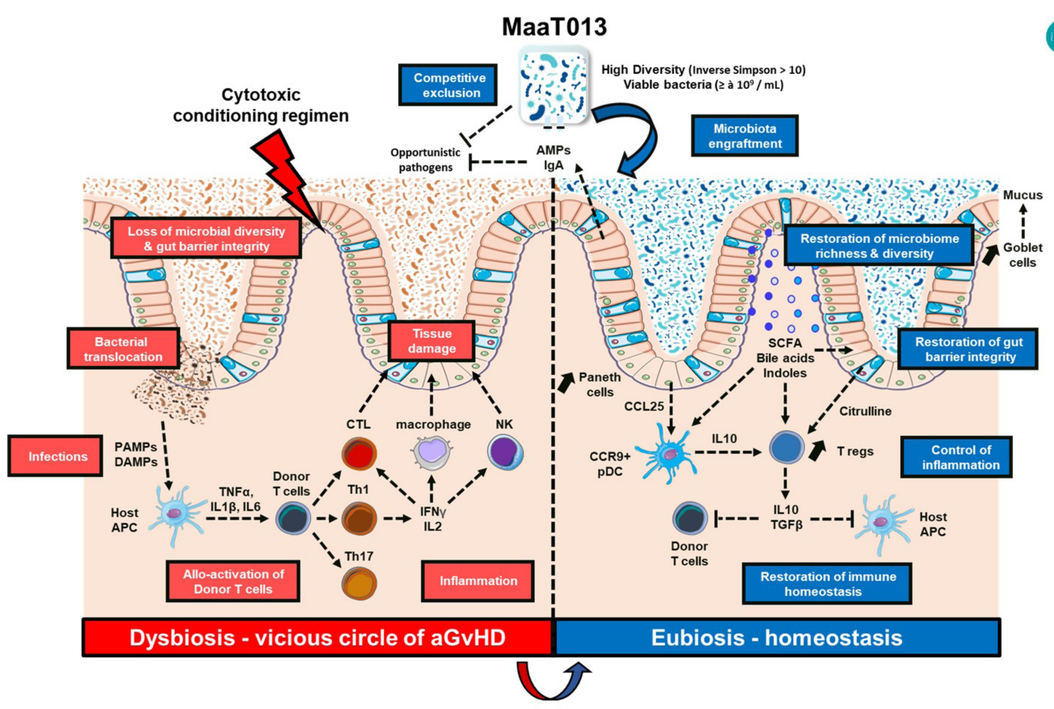


Figure 5. Impact of MaaT013 in a humanized mouse model of GvHD. A) Experimental design. 8 weeks old severely immunodeficient female NOD/Shi-scid/IL-2Rynull (NCG) mice humanized with human PBMCs were used as a model of GvHD (4 mice per group). **B)** MaaT013 engraftment in mice defined as the number of MaaT013's OTUs detected in mice after treatment. **C)** The sum of six individual parameters (i.e. body weight loss, posture, skin integrity, fur texture, activity and paleness) was used to score GvHD progression. **D)** Survival rate. **E)** IL-10 quantification in the plasma of mice. **F)** Microbial richness index at genus level. **G)** Butycore® index at D31. **H)** Heatmap depicting relative abundance of beneficial bacteria at D31. Data are presented as mean ± SEM. Mann-Whitney test was performed.

CONCLUSIONS



MaaT013:

- Displays **high microbial richness** and **standardized taxonomic composition**
- Produces key metabolites **preventing inflammation-induced gut barrier alteration**
- Promotes DC-mediated **Tregs amplification** and **secretion of anti-inflammatory mediators**
- Safely engrafts in germ-free mice, **restoring healthy gut physiology and immune homeostasis**
- **Delays the appearance of GvHD** symptoms in a humanized NCG mouse model

Altogether, these results support the potential of MaaT013 to mitigate aGvHD through restoration of microbial diversity, gut barrier integrity and immune homeostasis leading to control of inflammation.

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