Evaluation of a new co-cultured microbiome ecosystem therapy candidate (MaaT03X) for clinical testing as adjuvant/neoadjuvant to immune checkpoint inhibitors in solid tumors

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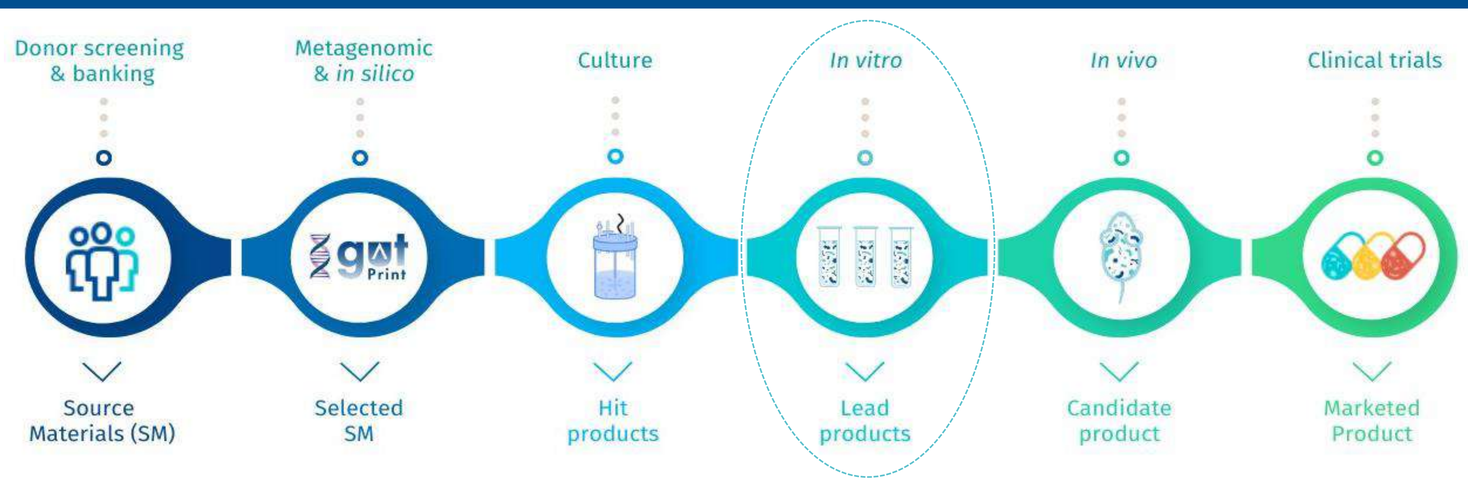
INTRODUCTION

Increasing evidence suggests that **gut microbiome composition modulates tumor response to therapies**, including immune checkpoint inhibitors (ICI). Clinical proofs of concept were obtained using ICI-responder fecal microbiota transplants to modulate the gut microbiome of non-responding cancer patients and improve their response to ICI [1,2]. These results support the development of microbiotherapies replicating the effects of ICI-responders as adjunctive therapies. MaaT Pharma, a clinical-stage biotech pioneer in the development of **Microbiome Ecosystem Therapies (MET) in oncology**, has developed a unique, ground-breaking, patented co-culture process (MET-C). This technology allows to replicate and leverage, at large industrial scale, the richness and diversity of native-based microbiome ecosystems while tuning the resulting product according to indication-specific compositions.

➡ The **objective** of this study is to assess the impact of a MET-C candidate (MaaT03X) on gut homeostasis and immune activation.

METHODS

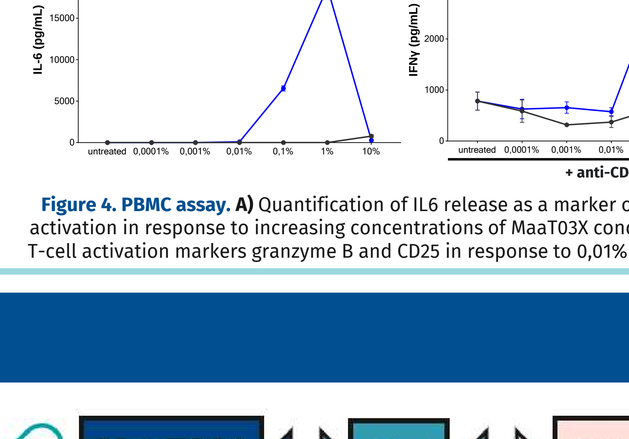


We assessed the impact of a MET-C hit product (MaaT03X) on gut homeostasis and immune cell activation using a combination of methods:

- Metagenomic analysis
- Caco-2/THP-1 leaky gut model
- AhR activation
- PBMC assay
- Mixed Lymphocyte Reaction (MLR)

RESULTS

Metagenomic analysis reveals the richness and diversity of MaaT03X ecosystem



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MaaT03X	
Richness (OTU)	**238**
Inverse Simpson (OTU)	**13**
Core microbiota (%)	**64**
Butycore® (%)	**25**

Figure 1. Metagenomic analysis of co-cultured MaaT03X ecosystem. 16S rDNA sequencing was performed to assess taxonomic classification and microbial diversity. **A)** Stacked bar plot of family relative abundances (top 95% abundant families). **B)** Richness (OTU), Inverse Simpson (OTU), % core microbiota and % Butycore® [3] of MaaT03X.

MaaT03X controls inflammation and restores gut barrier integrity in a Caco-2/THP-1 leaky gut model

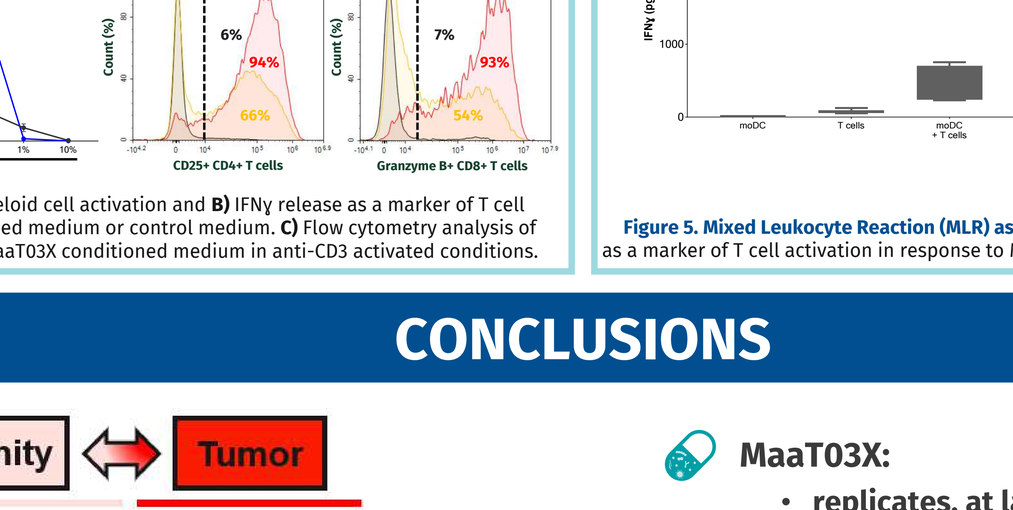

Figure 2. Impact of MaaT03X in a Caco-2/THP-1 leaky gut model. A) Transepithelial electrical resistance (TEER) assay of gut barrier integrity. **B)** Anti-inflammatory IL-10 cytokine release. **C)** Pro-inflammatory TNFα cytokine release.

MaaT03X activates AhR signaling in colon epithelial cells

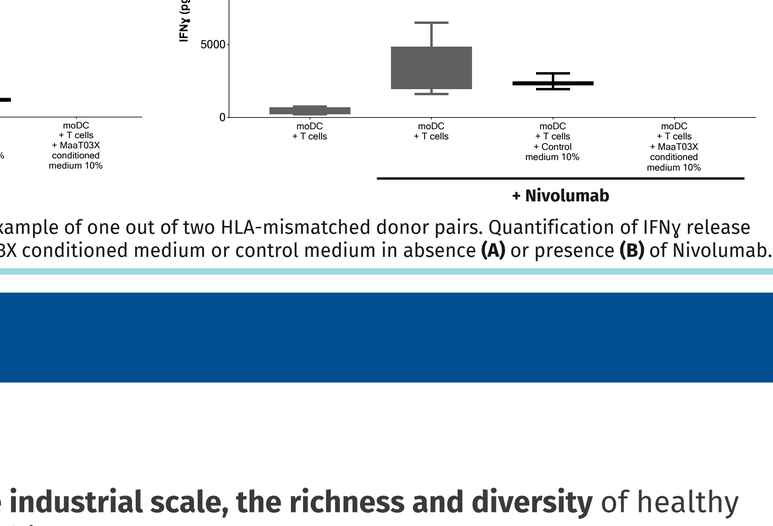

Figure 3. AhR pathway. AhR receptor activation in response to increasing concentrations of MaaT03X conditioned medium in HT29-Lucia™ AhR reporter cells.

MaaT03X stimulates both myeloid and lymphoid immune cells in PBMCs

Figure 4. PBMC assay. A) Quantification of IL6 release as a marker of myeloid cell activation and **B)** IFNγ release as a marker of T cell activation in response to increasing concentrations of MaaT03X conditioned medium or control medium. **C)** Flow cytometry analysis of T-cell activation markers granzyme B and CD25 in response to 0,01% of MaaT03X conditioned medium in anti-CD3 activated conditions.

MaaT03X promotes DC-mediated T cell activation in a Mixed Leukocyte Reaction

Figure 5. Mixed Leukocyte Reaction (MLR) assay. Example of one out of two HLA-mismatched donor pairs. Quantification of IFNγ release as a marker of T cell activation in response to MaaT03X conditioned medium or control medium in absence **(A)** or presence **(B)** of Nivolumab.

CONCLUSIONS

MaaT03X:

- **replicates, at large industrial scale, the richness and diversity** of healthy native-based microbiome ecosystems
- **restores the integrity** of a damaged gut barrier
- **activates AhR pathway** involved in gut homeostasis
- **stimulates both myeloid and lymphoid** immune cells
- **improves immune cell response to ICI therapy**

Altogether, these results highlight the potential of MaaT03X to restore gut barrier integrity and to stimulate immune cell response to ICI treatment.

These outcomes paved the way for the identification of a promising frontrunner, **MaaT034**, slated for further advancements in clinical development.

REFERENCES
1. Davar D, *et al.* Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. Science. 2021
2. Baruch EN, *et al.* Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science. 2021
3. Malard F, *et al.* Pooled allogeneic faecal microbiota MaaT013 for steroid-resistant gastrointestinal acute graft-versus-host disease: a single-arm, multicentre phase 2 trial. eClinicalMedicine. 2023