Evaluation of a new co-cultured microbiome ecosystem therapy candidate (MaaT034) for clinical testing in combination with immune checkpoint inhibitors in solid tumors

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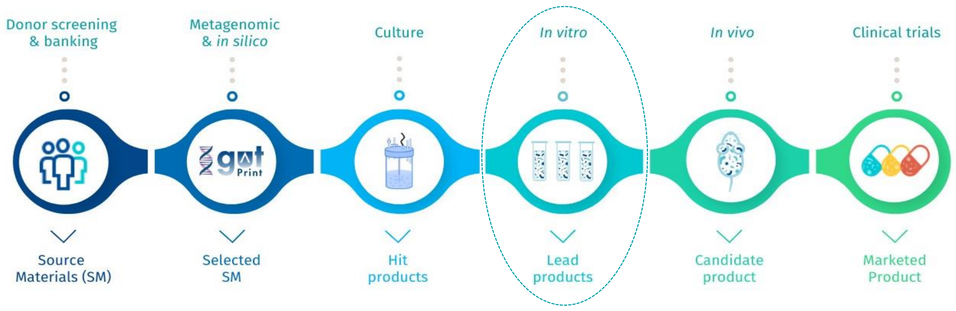
INTRODUCTION

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 [1-4].

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.

METHODS



We assessed the impact of a MET-C hit product (MaaT034) on gut homeostasis and immune cell activation using a combination of methods:

- Metagenomic analysis
- Metabolite quantification
- Caco-2/THP-1 leaky gut model
- Mixed Lymphocyte Reaction (MLR)
- PBMC killing
- MLR-killing

The objective of this study is to assess the impact of a MET-C candidate (MaaT034) on gut homeostasis and immune activation.

RESULTS

Metagenomic analysis reveals the richness and diversity of MaaT034 ecosystem

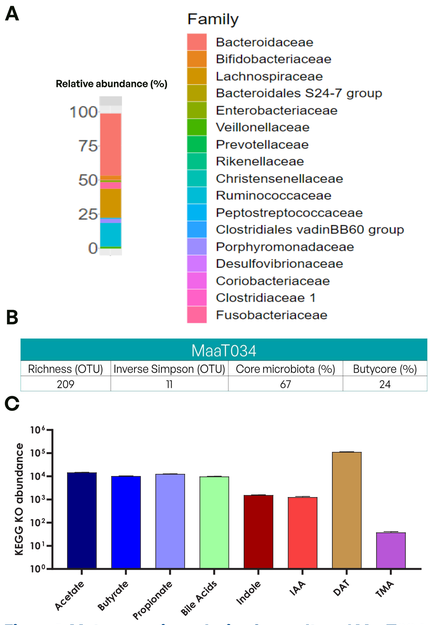


Figure 1. Metagenomic analysis of co-cultured MaaT034 ecosystem. 16S rDNA sequencing was performed to assess taxonomic classification and microbial diversity. **A)** Stacked bar plot of family relative abundances (top 95% abundant families). **B)** Richness (OTU), Inverse Simpson (OTU), % core microbiota and % Butycore® [5] of MaaT034. **C)** Boxplot of the abundance of all KEGG KOs for selected metabolites.

MaaT034 produces key metabolites involved in gut homeostasis and response to immunotherapies

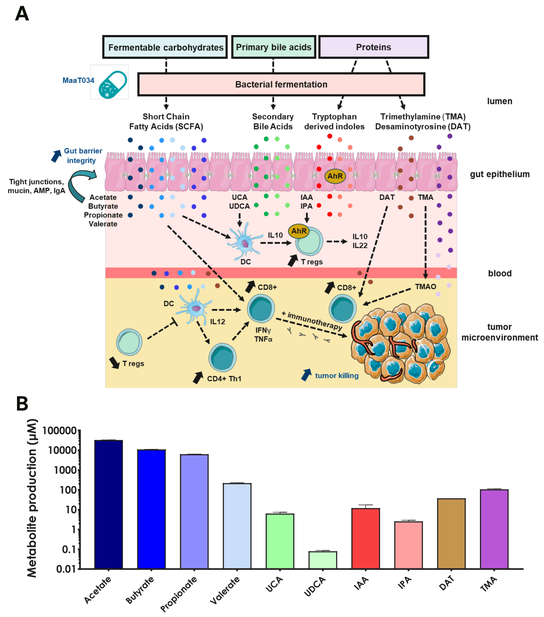


Figure 2. Metabolite quantification. A) Role of microbiota-derived metabolites in gut homeostasis and cancer response to immunotherapies [6]**. B)** The production of key metabolites released by MaaT034 in its conditioned medium was determined by LC-MRM/MS. DAT: desaminotyrosine; TMA: trimethylamine; IPA: indole-3-propionic acid; IAA: indole-3-acetic acid; UCA: ursocholic acid; UDCA: ursodeoxycholic acid.

MaaT034 reveals immunomodulatory potential allowing to restore gut barrier integrity and to promote DC-mediated T cell activation

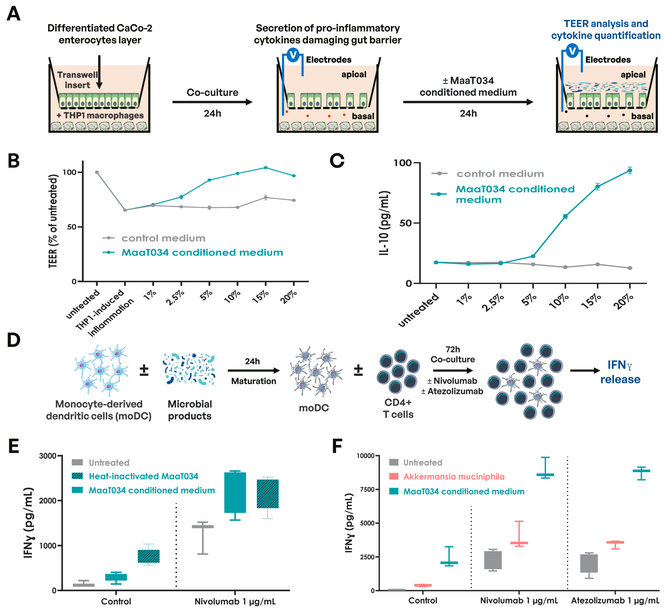


Figure 3. Immunomodulatory potential of MaaT034. A) Transepithelial electrical resistance (TEER) assay in the Caco-2/THP-1 leaky gut model. **B)** Gut barrier integrity in response to MaaT034 conditioned medium. **C)** Anti-inflammatory IL-10 cytokine release. **D)** Allogenic mDC/CD4 Mixed Leukocyte Reaction (MLR). **E)** Quantification of IFNγ release as a marker of T cell activation in response to MaaT034 (heat-inactivated or conditioned medium) and **F)** Akkermansia muciniphila conditioned medium in absence or presence of Nivolumab and Atezolizumab. Example of one out of two HLA-mismatched donor pairs.

MaaT034 promotes DC-mediated T cell activation and tumor cell killing

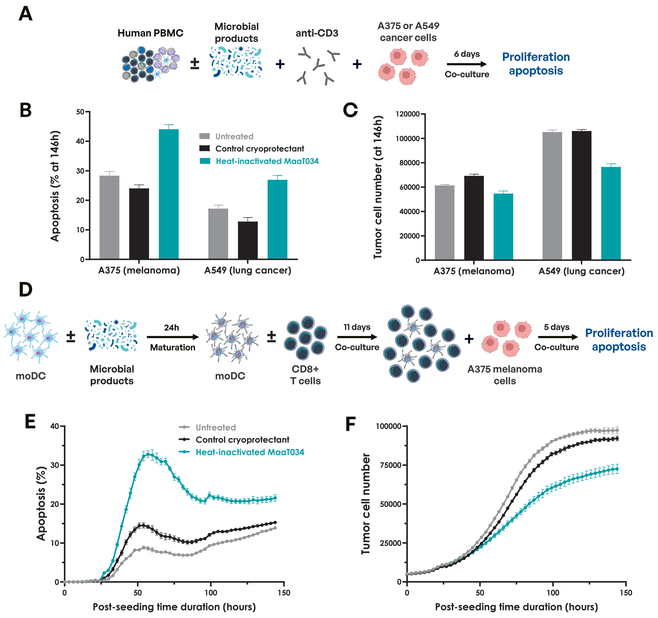
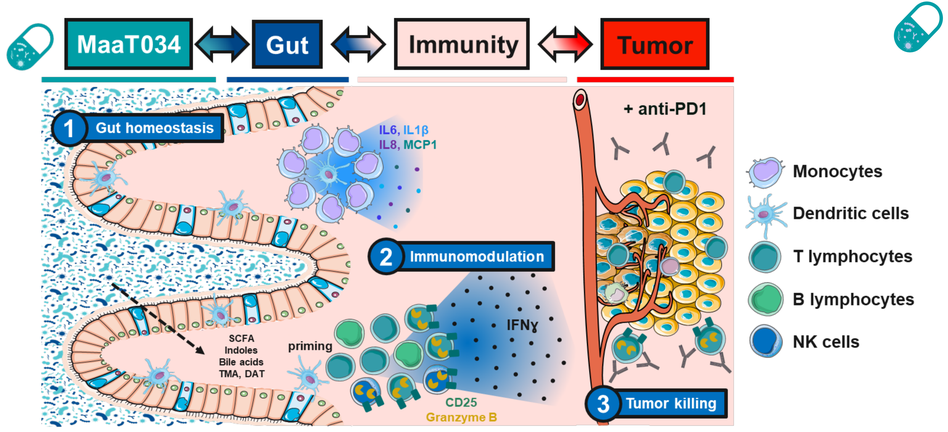


Figure 4. Impact of MaaT034 on immune cell activation and tumor killing. A) PBMC killing assay. Quantification of A375 melanoma and A549 lung cancer cell **B)** apoptosis and **C)** proliferation in response to anti-CD3 activated PBMCs in presence or absence of heat-inactivated MaaT034 via IncuCyte S3™ live cell imaging. **D)** Autologous mDC/CD8 MLR killing. Quantification of A375 melanoma cell **E)** apoptosis and **F)** proliferation in response to CD8+ T cells primed with dendritic cells loaded with or without heat-inactivated MaaT034 via IncuCyte S3™ live cell imaging.

CONCLUSIONS



MaaT034:

- **replicates, at large industrial scale, the richness and diversity** of healthy native-based microbiome ecosystems
- **produces key metabolites** associated with ICI response
- **restores the integrity** of a damaged gut barrier
- **improves immune cell response to ICI therapy**
- **New highlight: MaaT034 activates CD8+ T cell-mediated tumor cell killing**

Altogether, these results reveal the potential of MaaT034 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

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