Harnessing the Neuromodulatory Effects of Live Biotherapeutic Products (LBPs) Derived from the Gut Microbiota to Treat Parkinson's Disease (PD)

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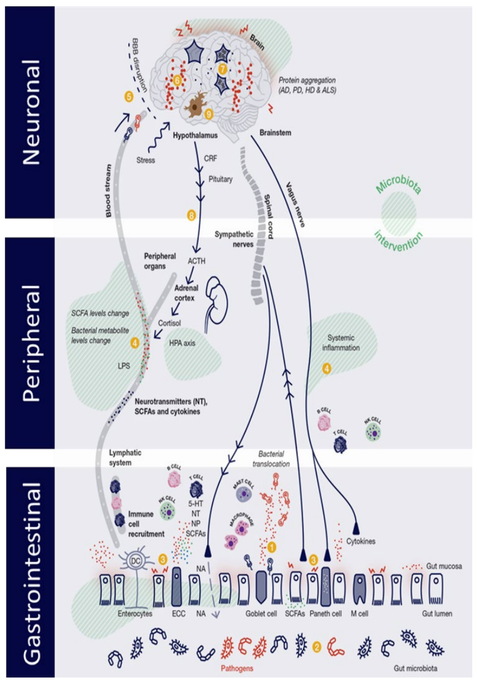


BACKGROUND AND RATIONALE

- Parkinson's disease (PD) is a heterogeneous neurodegenerative disease characterized by the accumulation of misfolded α-synuclein protein and degeneration of dopaminergic neurons in the substantia nigra and other related circuitry, which contribute to the development of both motor and non-motor symptoms.
- The bi-directional communication pathway between the gastrointestinal (GI) tract and the central nervous system (CNS), known as the microbiota-gut-brain axis, has been explored in several preclinical and clinical studies, implicating its role in the pathogenesis of neurodegenerative disorders, such as PD.
- Accumulating evidence suggests that the onset of non-motor symptoms, such as gastrointestinal manifestations, often precedes the onset of motor symptoms and disease diagnosis, lending support to the potential role that the microbiota-gut-brain axis might play in the underlying pathological mechanisms of PD.
- Intestinal hyperpermeability, inflammation, GI dysmotility, microbiome dysbiosis, intestinal α-synuclein inclusions and impaired activity of enteric neurons are early manifestations of PD.
- Novel therapeutics that can restore a functional microbiota-gut-brain axis could thus potentially have disease-modifying properties by delaying or even preventing neurodegenerative processes.

IN VITRO DATA SUMMARY

We identified two single-strain live biotherapeutic products (LBPs), *Parabacteroides distasonis* MRx0005 and *Megasphaera massiliensis* MRx0029, for the treatment of PD.

Neuroprotection

MRx0005 and MRx0029 showed statistically significant rescue of cell viability induced by MPP^+, MRx0029 completely rescuing viability comparable to untreated controls, while MRx0005 was comparable to the positive control (Quercetin).

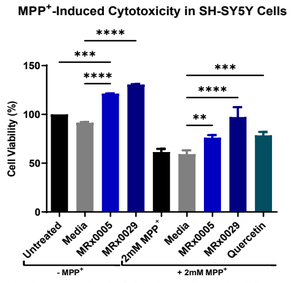


Figure 1: Effect on MPP^+-induced cytotoxicity. After 24 h incubation, 10% bacterial cell-free supernatant of MRx0005, MRx0029 or media were added to the wells and the plates were returned to the incubator. After another 24 h incubation, cell viability was assessed. The plates were incubated with Cell Counting Kit-8 solution for 3 h, allowing the WST-8 to produce a water-soluble formazan dye. Absorbance was measured at 450 nm with a reference wavelength of 655 nm. Data is represented as a percentage of viable cells compared to untreated and/or media controls and 2mM MPP^+ and/or 2mM MPP^+ + media. Statistical analysis of differences between the experimental groups was determined by using one-way ANOVA followed by a Tukey's *posthoc* test. P-values ≤ 0.05 were considered statistically significant. Data are expressed as mean ± SEM (n = 6).

Intestinal Barrier Function

MRx0029 showed an increase in gene expression of intestinal tight junction proteins in an epithelial cell line (HT-29 cells).

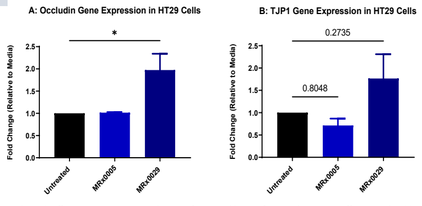


Figure 3: Effect on gene expression changes of intestinal barrier functional proteins. Differentiated HT29-MTX cells were co-incubated with MRx0005 or MRx0029 for 2 h and RNA was isolated for qRT-PCR for analysis of the following proteins: **(A)** Occludin and **(B)** TJP-1/*ZO-1*. Statistical analysis of differences between the experimental groups was determined by using one-way ANOVA followed by a Šidák *posthoc* test. P-values ≤ 0.05 were considered statistically significant. Data are expressed as mean ± SEM (n = 3).

Neuroinflammation

MRx0005 and MRx0029 reduced IL-6 secretion following treatment with wild-type and clinically relevant mutant forms of α-synuclein in U373 cells co-cultured with SH-SY5Y cells.

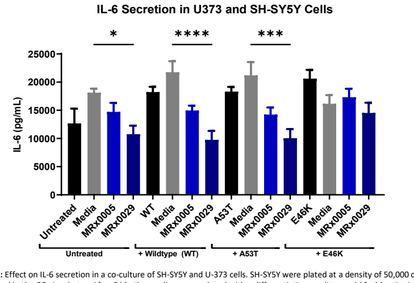


Figure 2: Effect on IL-6 secretion in a co-culture of SH-SY5Y and U-373 cells. SH-SY5Y were plated at a density of 50,000 cells/well and placed in the CO_2 incubator. After 24 h, the media were replaced with a differentiation medium and 10 μM retinoic acid. On Day 10, the differentiation medium was removed and replaced with a growth medium. Cells were co-incubated with U-373 cells grown on 12 transwell plates (50,000/well in 12 well plates, plated 72 h before the experiment) for 24 h. Thereafter SH-SY5Y cells were treated with WT, A53T or E46K alpha-synuclein proteins followed by 10% bacterial cell-free supernatant of MRx0005, MRx0029 or media as control. Cell-free supernatants from U-373 were collected 48 h after for IL-6 secretion measured by ELISA. Statistical analysis of differences between the experimental groups was determined by using one-way ANOVA followed by a Šidák *posthoc* test. P-values ≤ 0.05 were considered statistically significant. Data are expressed as mean ± SEM (n = 3).

Oxidative Stress

MRx0005 and MRx0029 showed an intrinsic antioxidant and ROS-scavenging capacity in several biochemical assays. MRx0005 protects astrocytoma, microglia and differentiated neuroblastoma cells from tert-butyl hydroperoxide (TBPH)-induced ROS (data not shown), while MRx0029 resulted in significant protection from TBHP-induced ROS in neuroblastoma cells.

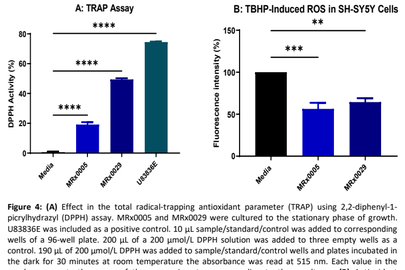
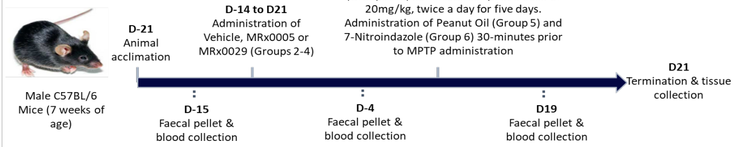


Figure 4: (A) Effect in the total radical-trapping antioxidant parameter (TRAP) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. MRx0005 and MRx0029 were cultured to the stationary phase of growth. U8383E was included as a positive control. 10 μL sample/standard/control was added to corresponding wells of a 96-well plate. 200 μL of a 200 μmol/L DPPH solution was added to three empty wells as a control. 190 μL of 200 μmol/L DPPH was added to sample/standard/control wells and plates incubated in the dark for 30 minutes at room temperature the absorbance was read at 515 nm. Each value in the graph represents the mean of three experiments corresponding to three cultures. **(B)** Antioxidant capacity in retinoic acid-differentiated SH-SY5Y cells stained with 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) probe, then treated with 10% bacterial cell-free supernatant of MRx0005, MRx0029 or media as a control in the presence of TBPH for 2 h, followed by measurement of fluorescence intensity ($F_{excitation}/F_{emission}$ = 485/530nm). Statistical analysis of differences between the experimental groups was determined by using one-way ANOVA followed by a Šidák *posthoc* test. P-values ≤ 0.05 were considered statistically significant. Data are expressed as mean ± SEM (n = 3).

IN VIVO STUDY DESIGN

The disease-modifying potential of MRx0005 and MRx0029 were demonstrated in an MPTP-lesioned mouse model of PD.



Group	# of Animals	Disease Induction	Treatment	Dose & Route	Dosing Schedule
1	10	0.9% Sterile Saline	PBS	N/A; *p.o.*	*q.d.*; Day -14-21
2	10	MPTP	PBS	N/A; *p.o.*	*q.d.*; Day -14-21
3	10	MPTP	MRx0005	1.0 x 10^8 CFU/200μL; *p.o.*	*q.d.*; Day -14-21
4	10	MPTP	MRx0029	1.0 x 10^8 CFU/200μL; *p.o.*	*q.d.*; Day -14-21
5	10	MPTP	Peanut Oil	N/A; *i.p.*	*b.i.d.*; Day 1-5
6	10	MPTP	7-Nitroindazole (7-NI)	50 mg/kg; *i.p.*	*b.i.d.*; Day 1-5

- Tyrosine hydroxylase (TH) and NeuN-positive cell numbers in the SN were quantified by IHC and stereology.
- Striatal dopamine and its metabolites (DOPAC and HVA) levels were quantified by LC-MS/MS.
- Striatal dopamine transporter (DAT) levels were quantified by autoradiography.

IN VIVO DATA SUMMARY

Neuroprotection

MRx0029 protected from loss of tyrosine hydrolase $(TH)^+$ neurons in MPTP-induced brain lesions. Neuroprotection is comparable to positive control 7-Nitroindazole (7-NI).

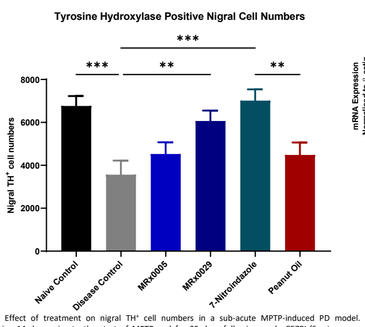


Figure 5: Effect of treatment on nigral TH^+ cell numbers in a sub-acute MPTP-induced PD model. Commencing 14 days prior to the start of MPTP and for 35 days following, male C57BL/6 mice were treated with MRx0005 or MRx0029 via oral gavage (Day -14 to Day 21). Animals were administered saline or MPTP, twice daily from Day 1 to Day 5 via *i.p.* injection. Mice were killed on Day 22. Nigral tissue was collected and evaluated for TH^+ cell numbers using immunohistochemistry and stereology. Statistical analysis of differences between the experimental groups was determined by using one-way ANOVA followed by a Holm-Šidák *posthoc* test. P-values ≤ 0.05 were considered statistically significant. Data are expressed as mean ± SEM (n = 10 per group).

Intestinal Barrier Function

MRx0029 showed an increase in gene expression of intestinal tight junction proteins in the colon and reduced colonic permeability.

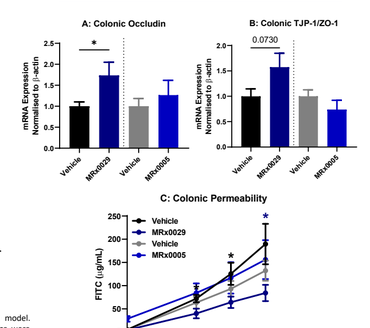
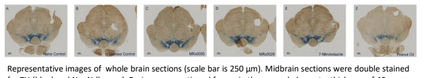


Figure 7: Effect on intestinal barrier function. Mice were treated with MRx0005 or MRx0029 via oral gavage for 6 days (Day 0 to Day 6) and were euthanized at Day 6 by cervical dislocation. The colon was harvested, and RNA was isolated for qRT-PCR analysis of the following proteins: **(A)** Occludin and **(B)** TJP-1/*ZO-1*. **(C)** The colon were harvested and placed in chilled Krebs solution opened along the mesenteric line and carefully rinsed. Preparations were then placed in an Ussing chamber with oxygenated Krebs buffer maintained at 37°C. 4kDa FITC-dextran was added to the mucosal chamber at a final concentration of 2.5 mg/mL. Samples were collected from the serosal chamber every 30 minutes. Statistical analysis of differences between the experimental groups was determined by using an unpaired t-test. P-values ≤ 0.05 were considered statistically significant. Data are expressed as mean ± SEM (n = 6 per group).



Representative images of whole brain sections (scale bar is 250 μm). Midbrain sections were double stained for TH (blue) and NeuN (brown). Brains were sectioned frozen in the coronal plane at a thickness of 40μm on a sliding microtome and 3 series of sections were stored in cryoprotectant. A single series of sections were processed for visualisation of TH and NeuN via the biotin-labelled antibody procedure.

Neurotransmitters

MRx0005 protected from loss of striatal dopamine (DA) and its metabolites (DOPAC) in MPTP-treated mice. The effect is similar to that of the positive control 7-NI. No changes in HVA were observed in this model between treatment groups.

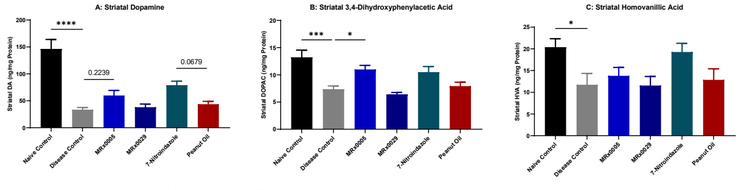
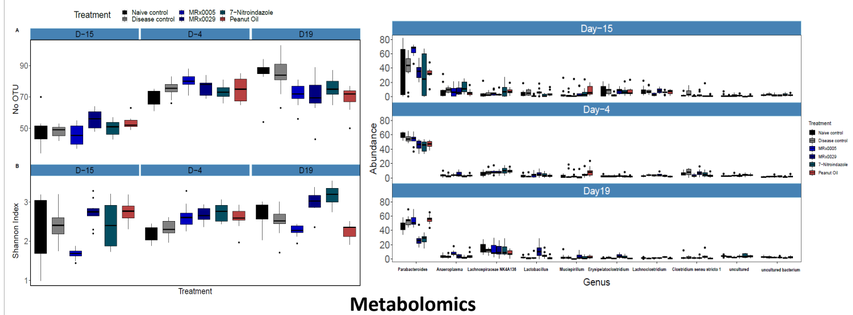


Figure 6: Effect of treatment on striatal **(A)** DA, **(B)** DOPAC and **(C)** HVA levels in a sub-acute MPTP-induced PD model. Commencing 14 days prior to the start of MPTP and for 35 days following, male C57BL/6 mice were treated with MRx0005 or MRx0029 via oral gavage (Day -14 to Day 21). Animals were administered saline or MPTP, twice daily from Day 1 to Day 5 via *i.p.* injection. Mice were killed on Day 22. Striatal tissue was collected and evaluated for DA, DOPAC and HVA levels using LC-MS/MS. Statistical analysis of differences between the experimental groups was determined by using one-way ANOVA followed by a Holm-Šidák *posthoc* test. P-values ≤ 0.05 were considered statistically significant. Data are expressed as mean ± SEM (n = 10 per group).

Microbiome

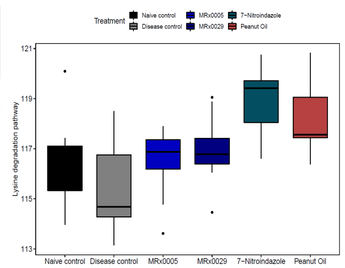
MRx0029 associated with an increase in Shannon index and in the abundance of *Firmicutes, Tenericutes*, *Lactobacillus*, *Anaerotruncus* and *Ruminococcaceae* UCG-013 and a decrease in opportunistic pathogens.

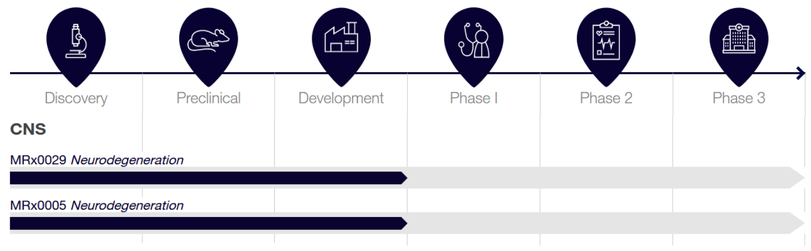


Metabolomics

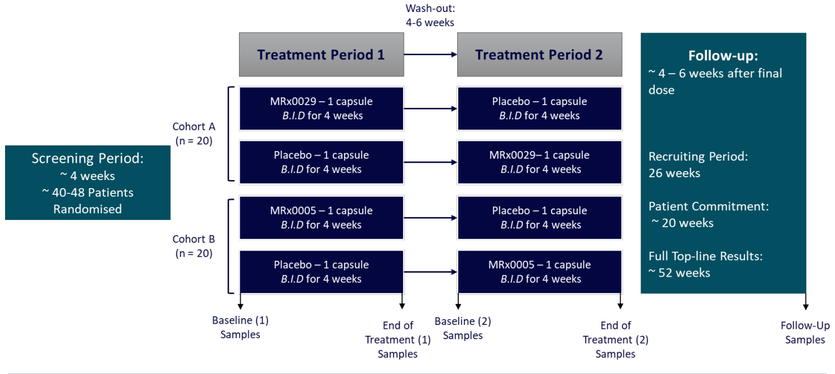
L-lysine degradation found to be enriched in MRx0005, MRx0029 and 7-NI compared to MPTP control. Multiple pathways from this superpathway are enriched in MRx0029.

Pathway	Enriched compounds
L-lysine degradation I	5-aminopentanoate, succinate
L-lysine degradation III	*N*6-acetyl-L-lysine, 2-keto-6-acetamidocaproate, 5-aminopentanoate
L-lysine degradation IV	5-aminopentanoate, succinate
L-lysine degradation V	L-2-aminoadipate, succinate





CLINICAL STUDY DESIGN AND BIOMARKERS



- Safety & Tolerability
- Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) and Movement Disorder Society-Non Motor Symptom Rating Scale (MDS-NMS)
- Pharmacokinetic Profile of Levodopa (alone or in combination with decarboxylase inhibitor or Carbidopa)
- Participants will report dietary habits by filling out a Food Frequency Questionnaire

Plasma	• Neurotransmitters • Metabolomics • Zonulin (Intestinal Barrier Function)	• Lipopolysaccharide (LPS), LPS-Binding Protein, Circulating Cytokines (Systemic Inflammation) • Neurofilament Light Chain (Neuroinflammation)
Urine	• Metabolomics	• Sugar Test (Intestinal Barrier Function)
Stool	• Microbiome • Metabolomics	• Calprotectin (Intestinal Inflammation) • Bristol Stool Chart (Constipation)

The study is sponsored by **4D pharma plc**. For more information, contact clinicaltrials@4dpharmaplc.com.