# Targeting the Microbiome in the MPTP-Lesioned Mouse Model of Parkinson's Disease: Live Biotherapeutic Products (LBPs) Demonstrate Disease Modifying Effects

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**4D pharma plc** is a pharmaceutical company focussed on developing Live Biotherapeutic products (LBPs) from the human gut microbiome. Live Biotherapeutics are a regulated, emerging and disruptive new class of medicines, which have the potential to transform the way in which we treat many diseases. 4D pharma currently has clinical stage programmes in cancer, asthma, irritable bowel syndrome and Crohn's disease, and a strong pipeline of pre-clinical programmes in diverse therapeutic areas including immuno-oncology, CNS, gastrointestinal, respiratory and autoimmune diseases.

## Aim

To assess the disease-modifying potential of microbiome-derived novel Live Biotherapeutic products **MRx0005** and **MRx0029** in an MPTP-lesioned mouse model of Parkinson's disease (PD).

## Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder predominately affecting dopamine-producing (dopaminergic) neurons in the *substantia nigra*. PD affects more than 10 million people worldwide, making it the most common movement disorder and the second most common neurodegenerative disorder.

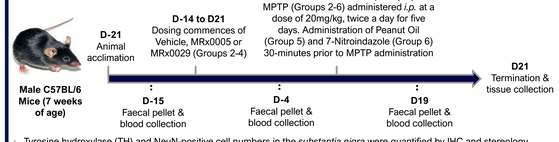
The cause of PD is unknown, with both genetic and environmental factors speculated to play a role. Characteristic of PD is progressive loss of muscle control, leading to tremors, bradykinesia, rigidity, dystonia, vocal symptoms, postural instability and walking/gait difficulties. In addition, 75% of people with PD have gastrointestinal (GI) abnormalities, primarily constipation.

Accumulating evidence suggests an interplay between the GI tract and brain in PD, supported by findings such as an alteration in the gut microbiome composition and presence of α-synuclein deposits in the enteric nervous system (ENS). Research has suggested that the gut microbiome has a potential role in the pathogenesis and treatment of PD. Identification of bacterial strains with disease-modifying actions, be they neuroprotective, neuroregenerative or compensatory to slow, halt or even reverse disease and/or symptom progression would be of great value in the treatment of PD.

## Experimental Design

| 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-Nitro-indazole (7-NI) | 50 mg/kg; *i.p.* | *b.i.d.*; Day 1-5 |

CFU: colony forming units; *q.d.*: once a day; *b.i.d.*: twice a day




- Tyrosine hydroxylase (TH) and NeuN-positive cell numbers in the *substantia nigra* 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

## Results

### MRx0029 protected against MPTP-induced losses in TH+ cell numbers and MRx0005 protected against MPTP-induced losses in striatal DOPAC.

Commencing 14 days prior to start of MPTP (Day -14) and for 35 days following (Day 21), male C57BL/6 mice were administered PBS or bacterial strains MRx0005 or MRx0029 (once daily; *p.o.*). Animals were administered Saline or MPTP (Days 1-5; twice daily; *i.p.*).

**Body weights in the MPTP-lesioned mouse model of PD**

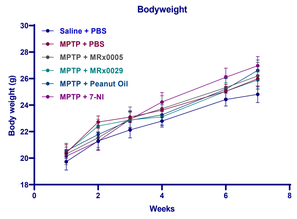



**Figure 1:** Animals were weighed on the first day of acclimatisation and then on a weekly basis thereafter. Data were analysed using a one-way ANOVA and Tukey's multiple comparison *post hoc* test (p > 0.05 *cf.* MPTP + PBS; p > 0.05 *cf.* MPTP + Peanut Oil; N = 10)

**TH+ nigral cell numbers in the MPTP-lesioned mouse model of PD**

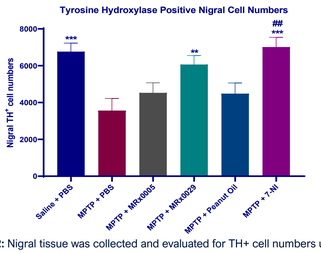



**Figure 2:** Nigral tissue was collected and evaluated for TH+ cell numbers using IHC and stereology. Data were analysed using a one-way ANOVA and Holm-Sidak's multiple comparisons *post hoc* test (*** p ≤ 0.001 *cf.* MPTP + PBS; ** p ≤ 0.01 *cf.* MPTP + PBS; ## p ≤ 0.01 *cf.* MPTP + Peanut Oil; N = 10)

**NeuN+ nigral cell numbers in the MPTP-lesioned mouse model of PD**

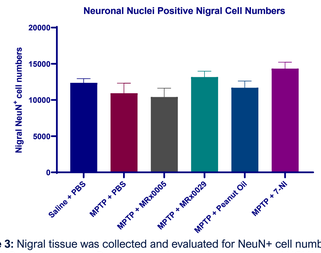



**Figure 3:** Nigral tissue was collected and evaluated for NeuN+ cell numbers using IHC and stereology. Data were analysed using a one-way ANOVA and Holm-Sidak's multiple comparisons *post hoc* test (p > 0.05 *cf.* MPTP + PBS; p > 0.05 *cf.* MPTP + Peanut Oil; N = 10)

**Striatal DA in the MPTP-lesioned mouse model of PD**

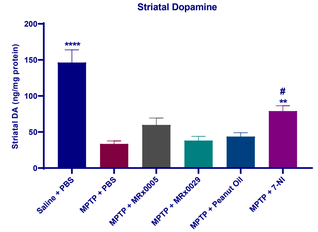



**Figure 4:** Striatal tissue was collected, and DA levels evaluated using LC/MS-MS. Data were analysed using a one-way ANOVA and Fisher's LSD multiple comparisons *post hoc* test (**** p ≤ 0.0001 *cf.* MPTP + PBS; ** p ≤ 0.01 *cf.* MPTP + PBS; # p ≤ 0.05 *cf.* MPTP + Peanut Oil; N = 10)

**Striatal DOPAC in the MPTP-lesioned mouse model of PD**

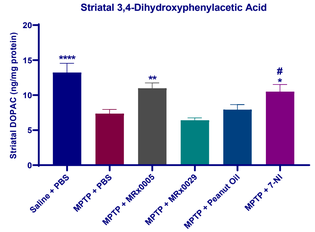



**Figure 5:** Striatal tissue was collected, and DOPAC levels evaluated using LC/MS-MS. Data were analysed using a one-way ANOVA and Fisher's LSD multiple comparisons *post hoc* test (**** p ≤ 0.0001 *cf.* MPTP + PBS; ** p ≤ 0.01 *cf.* MPTP + PBS; * p ≤ 0.05 *cf.* MPTP + PBS; # p ≤ 0.05 *cf.* MPTP + Peanut Oil; N = 10)

**Striatal HVA in the MPTP-lesioned mouse model of PD**

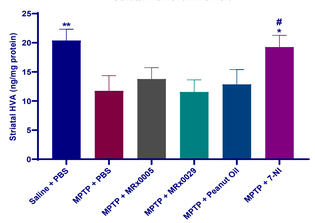



**Figure 6:** Striatal tissue was collected, and HVA levels evaluated using LC/MS-MS. Data were analysed using a one-way ANOVA and Fisher's LSD multiple comparisons *post hoc* test (** p ≤ 0.01 *cf.* MPTP + PBS; * p ≤ 0.05 *cf.* MPTP + PBS; # p ≤ 0.05 *cf.* MPTP + Peanut Oil; N = 10)

**Striatal DA turnover ((DOPAC + HVA)/DA) in the MPTP-lesioned mouse model of PD**

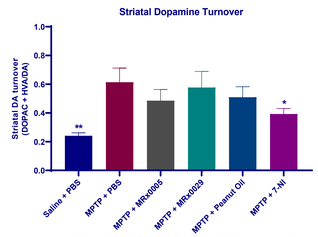



**Figure 7:** Striatal tissue was collected, and DA, DOPAC and HVA levels evaluated using LC/MS-MS. Data were analysed using a one-way ANOVA and Fisher's LSD multiple comparisons *post hoc* test (** p ≤ 0.01 *cf.* MPTP + PBS; * p ≤ 0.05 *cf.* MPTP + PBS; N = 10)

**Striatal DAT binding in the MPTP-lesioned mouse model of PD**

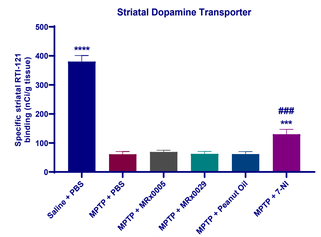



**Figure 8:** Striatal tissue was collected, and DAT levels evaluated using specific [$^{125}$I]-RTI-121 binding. Data were analysed using a one-way ANOVA and Fisher's LSD multiple comparisons *post hoc* test (**** p ≤ 0.0001 *cf.* MPTP + PBS; *** p ≤ 0.001 *cf.* MPTP + PBS; ### p ≤ 0.001 *cf.* MPTP + Peanut Oil; N = 10)

**Representative images of whole brain sections (scale bar is 250 µm). TH and NeuN immunoreactive cells in the *substantia nigra* (SN) of the MPTP-lesioned mouse model of PD**

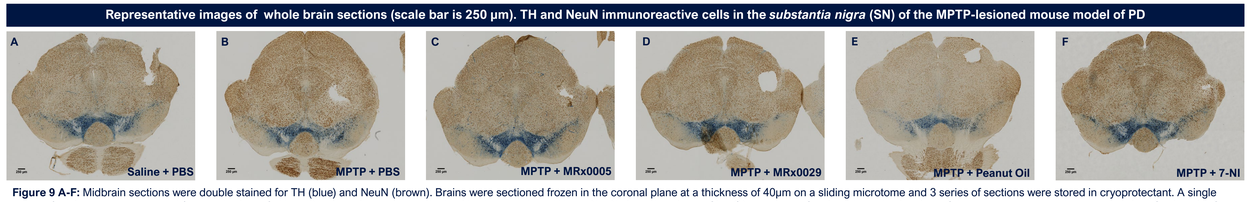



**Figure 9 A-F:** Midbrain sections were double stained for TH (blue) and NeuN (brown). Brains were sectioned frozen in the coronal plane at a thickness of 40µm on a sliding microtome and 3 series of sections were stored in cryoprotectant. A single series of sections were processed for visualisation of TH and NeuN via the biotin-labelled antibody procedure. TH and NeuN stained sections of the SN were used for stereological estimation of dopamine neuron numbers using optical fractionator from the Stereo Investigator software package. Six to eight sections spanning the entire anterior/posterior extent of the SN, separated by 120 µm (1/3 series), were used for counting.

## Bacterial Strains

**MRx0005**: *Parabacteroides distasonis*
**MRx0029**: *Megasphaera massiliensis*

Single strain Live Biotherapeutic products (LBPs) isolated from the gut microbiome of healthy human donors.

## Summary

- The study evaluated the ability of two LBPs, MRx0005 and MRx0029, to reduce dopaminergic deficits in the MPTP-lesioned mouse model of PD.
- Treatment with MRx0029 reduced MPTP-induced deficits in TH+ dopaminergic neurons, in the *substantia nigra* from 46% to 11%.
- Treatment with MRx0005 reduced MPTP-induced deficits in striatal DOPAC from 44% to 17% and dopamine from 77% to 59% (the latter did not reach significance).

## Conclusion

- MPTP administration in the male mouse produced a model of PD with anticipated deficits on dopaminergic function. A Reference Item (7-Nitroindazole; 7-NI) reversed these deficits.
- MRx0029 protected against MPTP-induced loss in nigral TH+ cell numbers. The magnitude of these effects was similar to those of 7-NI.
- Administration of MRx0005 protected against the loss of striatal dopamine and DOPAC to a similar degree as 7-NI.
- This study was conducted in male mice only. It would be interesting to see the effects in female mice in order to investigate the potential influence of sex.
- In conclusion, the LBPs, MRx0029 and MRx0005, had a potential disease-modifying effect in the MPTP model. Further investigation is needed to fully elucidate their potential in the treatment of PD.

## References

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