Live Biotherapeutics to Target the Gut-Brain Axis
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AN INTEGRATED, END-TO-END MICROBIOME PLAYER

4D pharma is a leader in the development of single strain Live Biotherapeutics, a novel class of drug derived from the human gut microbiome.

- **Platform & Research**
  - MicroRx® platform - focus on functionality
  - Research collaboration with MSD in vaccines
  - World-leading IP estate

- **Development & Manufacturing**
  - Unique end-to-end capability and expertise
  - cGMP certified
  - Production for 4 clinical trials in parallel

- **Clinical Development**
  - 4 clinical-stage candidates across multiple TAs
  - Clinical collaboration with MSD in I-O
  - Positive early signals for MRx0518 + Keytruda
WHAT ARE LIVE BIOOTHERAPEUTICS?

Our LBPs are:

• Single-strains of commensal bacteria
• Isolated from healthy human donors
• Encapsulated for oral delivery; formulated for targeted delivery to the intestines
• Highly favourable toxicity/side-effect profile
• Accelerated preclinical development and early-in patient data
Evidence for the importance of the gut-brain axis has been shown across numerous studies, highlighting the crucial role of the gut and in particular the microbiome, on central nervous system processes:
THERAPEUTIC TARGETS WITHIN THE GUT-BRAIN AXIS

• High unmet need for new therapeutic strategies for brain-related conditions

• Current therapies:
  o Target symptoms, not disease-modifying
  o Side effects, low response rate

• Newer therapies:
  o Target mechanism, disease-modifying
  o High clinical failure rate
  o Often limited to one brain mechanism

• MicroRx® screening platform targets:
  o Multiple mechanisms of central nervous system (CNS) pathology
  o Uses overlap, comorbidities, overarching mechanisms
  o Informs on appropriate preclinical models and biomarkers

Neurons, microglia, astrocytes & reporter cell lines

Neuroinflammation & neurodegeneration

MicroRx® bacterial strains

Primary & secondary metabolite analysis

Intestinal epithelial cell models

Inflammation, gut permeability, & disease-specific markers
Screening using MicroRx®
CENTRAL NERVOUS SYSTEM SCREENING PLATFORM

Neuronal
- Hypothalamus
-CRF
- Pituitary
- Brainstem
- Sympathetic nerves
- Baroreceptor

Peripheral
- Adrenal cortex
- HPA axis
- Neurotransmitters (NT), SCFAs and cytokines
- SCFA levels change
- Bacterial metabolite levels change
- LPS

Gastrointestinal
- Immune cell recruitment
- Bacterial translocation
- Enterocytes
- Goblet cell
- Paneth cell
- M cell
- Gut microbiota

Commensal effector molecules
- Neuroprotection
- Neuroinflammation
- Neurodifferentiation
- Protein misfolding and aggregation

Commensal signalling molecules
- Neurotransmitters
- Anti-inflammatory mediators
- Antioxidants
- Polyphenols

Commensal bacterial strains
- Gut barrier function
- Gut dysbiosis
- Short-chain fatty acids (SCFAs)
- Protein misfolding and aggregation

Increased intestinal permeability and gut barrier dysfunction ("leaky gut")
Gut microbiota dysbiosis
Inflammation of the intestinal mucosa
Systemic inflammation due to bloodstream metabolites and molecules
Disruption in the blood-brain barrier
Inflammation in the brain, meninges or spinal cord
Protein aggregation, mitochondrial dysfunction, oxidative stress and neurodegeneration
Stress activates the hypothalamic-pituitary-adrenal (HPA) axis and enhances the secretion of glucocorticoids
Microglia activate an inflammatory response, leading to progressive damage of neurons
**SCREENING OVERVIEW**

**Neuronal**
- Neuroinflammation is a key player in both neuroprotective and neuropathological processes
- Neurodegeneration is associated with genetic mutations and exposure to certain stimuli

**Peripheral**
- Circulating pro-inflammatory cytokines are linked to neuronal injury
- Imbalances to neurotransmitter systems have been associated with many CNS disorders

**Gastrointestinal**
- Increased epithelial permeability is linked to systemic circulation of pro-inflammatory molecules
- Short chain fatty acids (SCFAs) are important in neuro-modulation

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**MicroRx® identifies LBPs:**
- Which have neuroprotective and neuronal anti-inflammatory effects
- With anti-inflammatory effects which are linked to specific diseases such as Parkinson’s disease

**MicroRx® identifies LBPs:**
- With broad anti-inflammatory effects relevant to attenuation of systemic inflammation
- Which produce specific neurotransmitters relevant to target neurodegenerative disorders

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**MicroRx® identifies LBPs:**
- Which increase gut barrier function and reduce translocation of microbiome-derived pro-inflammatory mediators
- With unique SCFA profiles; including the production of SCFAs known to affect disease-specific cellular processes
Neurodegeneration:
Parkinson’s Disease –
MRx0005 & MRx0029
PARKINSON’S DISEASE (PD)

• Most common movement disorder
  o Second most common neurodegenerative disorder
  o ~ 10M people worldwide affected
  o 1.5 times men > women
  o Cause unknown: genetic and environmental factors implicated

• Two forms of PD:
  o Idiopathic:
    ▪ Most common (85 – 90% of PD cases)
    ▪ No known cause
    ▪ Age of onset: ~ 65 years
  o Familial:
    ▪ 10 – 15% of PD cases
    ▪ Genetic mutation in various genes (SNCA, LRRK2, etc.)
    ▪ “Young onset”: before 50 years

• Deterioration of motor function due to loss of dopamine-producing brain cells in the motor region of the brain

• Linked to accumulation or dysfunction of key protein:
  o Misfolded α-synuclein is part of abnormal protein aggregate found in Lewy bodies
NEURODEGENERATION: MRx0005 & MRx0029 IN VITRO DATA

Gut barrier function

Oxidative stress and neuroprotection

Neuroinflammation associated with α-synuclein

Neuronal differentiation

**α-syn induced inflammation in U373 cells**

**MPP+ induced cytotoxicity in SH-SY5Y cells**

**DAT/SLC6A3**
ASSESSMENT OF DISEASE MODIFYING POTENTIAL IN THE MPTP MOUSE MODEL OF PARKINSON’S DISEASE

Model summary

| Model | 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)-Induced Parkinsonian Syndrome |
| Disease Induction | MPTP administered i.p. at a dose of 20mg/kg, twice a day for five days |
| Animal Strain | Male C57BL/6 mice (7 weeks of age) |
| Positive Control | 7-nitroindazole (50 mg/kg; i.p.; b.i.d.) |
| Length of Model | 35 days (14 days pre-treatment + 21 days in vivo) |

Treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th># of Animals</th>
<th>Disease Induction</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Saline</td>
<td>PBS</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>MPTP</td>
<td>PBS</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>MPTP</td>
<td>MRx0005</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>MPTP</td>
<td>MRx0029</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>MPTP</td>
<td>Peanut Oil</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>MPTP</td>
<td>7-Nitroindazole</td>
</tr>
</tbody>
</table>

Key readouts

- DA transporter levels
- Dopamine and metabolites
- Quantification of dopaminergic neurons
- 16S microbiome analysis
- Cytokine profiling
- Metabolomics
- Inflammatory mediators
- Histopathology
- Permeability
- Short chain fatty acid analysis

Study design

- D-21: Animal acclimation
- D-14: Dosing commences
- D1 to D5: MPTP administered i.p. at a dose of 20mg/kg, twice a day for five days
- D21: Termination & tissue collection
- D-15: Faecal pellet & blood collection
- D-4: Faecal pellet & blood collection
- D19: Faecal pellet & blood collection

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ASSESSMENT OF THE DISEASE MODIFYING POTENTIAL IN THE MPTP MOUSE MODEL OF PARKINSON’S DISEASE

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

Dopamine & DOPAC quantification
- MRx0005 protected from loss of striatal dopamine and DOPAC in MPTP-treated mice. The effect is similar to that of the positive control 7-NI.
AUTISM SPECTRUM DISORDER (ASD)

- Neurological development disorder
  - 1 in 160 children affected
  - 4 times boys > girls
  - Cause is unknown: genetic, environmental, psychological, and neurological factors implicated

- ASD categorised in two ways:
  - Primary/Idiopathic:
    - Most common (90% of ASD cases)
    - No underlying medical condition
  - Secondary:
    - 10% of ASD cases
    - Underlying medical condition

- Wide range of symptoms:
  - Social interaction
  - Language and communication
  - Patterns of thoughts and physical behaviour
  - Gastrointestinal complications
ASSESSMENT OF LIVE BIOOTHERAPEUTICS IN A GENETIC MODEL OF AUTISM SPECTRUM DISORDER – THE BTBR MOUSE

Model summary

- **Model**: BTBR genetic mouse model of ASD
- **Animal Strain**: Male BTBR mice (8 weeks of age)
- **Length of Model**: 11 weeks

Key readouts

- **Social**
  - Three chamber social test
  - Social transmission of food preference
  - Marble burying test
  - Grooming behaviour
- **Stereotyped**
  - Elevated plus maze
  - Open field test
- **Anxiety**
  - Forced swim test
  - Female urine sniffing test
- **Depression**
  - Novel object recognition
- **Cognition**
  - Social transmission of food preference
- **Blood**
  - Carmine red
  - Ileum and Colon
- **Ussing chambers**
- **Cytokine profiling**

Study design

- **Week 0**: Animal acclimation
- **Week 1-3**: Dosing commences
- **Week 5**: Three chamber social test and elevated plus maze
- **Week 6**: Female urine sniffing test
- **Week 7**: Forced swim test
- **Week 8**: Forced swim test
- **Week 9**: Termination & tissue collection
ASSESSMENT OF LIVE BIOThERAPEUTICS IN AN ENVIRONMENTAL MODEL OF AUTISM SPECTRUM DISORDER – MIA

Model summary

<table>
<thead>
<tr>
<th>Model</th>
<th>MIA environmental mouse model of ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Strain</td>
<td>Male C57BL/6N mice (8 weeks of age)</td>
</tr>
<tr>
<td>Length of Model</td>
<td>10 weeks</td>
</tr>
</tbody>
</table>

C57BL/6J pregnant mice received a single intraperitoneal injection of the synthetic analogue of viral double-stranded RNA poly(I:C)

Key readouts

- Three chamber social test
- Social transmission of food preference
- Marble burying test
- Grooming behaviour
- Elevated plus maze
- Open field test
- Forced swim test
- Female urine sniffing test
- Novel object recognition
- Three chamber social test
- Social transmission of food preference
- Marble burying test
- Grooming behaviour
- Elevated plus maze
- Open field test
- Forced swim test
- Female urine sniffing test
- Novel object recognition
- Three chamber social test
- Social transmission of food preference
- Marble burying test
- Grooming behaviour
- Elevated plus maze
- Open field test
- Forced swim test
- Female urine sniffing test
- Novel object recognition

Study design

- Week 0: Animal acclimation
- Week 1-3: Dosing commences
- Week 4: Open field, novel object recognition and elevated plus maze
- Week 5: Three chamber social test and marble burying
- Week 6: Grooming and social transmission of food preference
- Week 7: Female urine sniffing test and carmine red
- Week 8: Forced swim test
- Week 9: Termination & tissue collection

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ATTENUATION OF AUTISTIC-LIKE BEHAVIOUR IN GENETIC AND ENVIRONMENTAL MOUSE MODELS OF ASD

**Sociability**

- MRx0006 increased the amount of time that animals spent interacting with a mouse over an object compared to vehicle.

**Reward-seeking behaviour**

- MRx0006 increased the time spent sniffing urine vs. water.
ATTENUATION OF AUTISTIC-LIKE BEHAVIOUR IN GENETIC AND ENVIRONMENTAL MOUSE MODELS OF ASD

Anxiety-like behaviour
- MRx0006 increased the time spent in the inner zone of the open field in BTBR-animals compared to vehicle

Stereotyped behaviour
- MRx0006 decreased the time spent grooming in BTBR-animals compared to vehicle
- MRx0006 decreased the number of marbles buried in MIA-animals compared to vehicle

Depressive-like behaviour
- MRx0006 decreased the immobility time in MIA-animals compared to vehicle
Neuroinflammatory Disorders:
Multiple Sclerosis:
MRx0002 & MRx0005
MULTIPLE SCLEROSIS (MS)

• Progressive, immune-mediated disorder
  o Demyelinating disease
  o ~ 2.1M people worldwide affected
  o 2-3 times women > men
  o Cause is unknown: genetic and environmental factors implicated

• Four main types of MS:
  o Relapsing-remitting MS (RRMS):
    ▪ Most common (85% of MS cases)
    ▪ Temporary periods of relapses or flare-ups
  o Secondary-progressive MS (SPMS):
    ▪ Most people with RRMS transition to SPMS
    ▪ Symptoms worsen steadily over time with/without relapses/remissions
  o Primary-progressive MS (PPMS):
    ▪ 10% of MS cases
    ▪ Slow worsening symptoms with no relapses/remissions
  o Progressive-relapsing MS:
    ▪ Rare (5% of MS cases)
    ▪ Steadily worsening, acute relapses but no remissions
ASSESSMENT OF THE EFFICACY OF LIVE BIOOTHERAPEUTIC PRODUCTS IN AN EAE MODEL OF MS

Model summary

<table>
<thead>
<tr>
<th>Model</th>
<th>Experimental autoimmune encephalomyelitis (EAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease Induction</td>
<td>Administration of MOG peptide in CFA followed by pertussis toxin</td>
</tr>
<tr>
<td>Animal Strain</td>
<td>Female C57BL/6NTac mice (8 weeks of age)</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Dexamethasone (3 mg/kg; i.p.; t.i.d.)</td>
</tr>
<tr>
<td>Length of Model</td>
<td>42 days (14 days pre-treatment + 28 days in vivo)</td>
</tr>
</tbody>
</table>

Disease Induction Administration of MOG peptide in CFA followed by pertussis toxin
Animal Strain Female C57BL/6NTac mice (8 weeks of age)
Positive Control Dexamethasone (3 mg/kg; i.p.; t.i.d.)
Length of Model 42 days (14 days pre-treatment + 28 days in vivo)

Key readouts

- Clinical scores
- Disease incidence
- Inflammatory foci
- Immune cell profiling
- 16S microbiome analysis
- Cytokine analysis
- Inflammatory mediators
- Histopathology
- Permeability
- Short chain fatty acid analysis

Scores Spinal Cord Spleen Faecal Pellets Blood Gut Caecum

Study design

D-21 Animal acclimation
D-14 Dosing commences
D0 Administration of MOG<sub>35-55</sub> peptide in CFA via s.c. injection followed by i.p. injection of pertussis toxin
D1 Administration of pertussis toxin via i.p. injection
D28 Termination & tissue collection
D28 Faecal pellet & blood collection

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ASSESSMENT OF THE EFFICACY OF LIVE BIOThERAPEUTIC PRODUCTS IN AN EAE MODEL OF MS

Clinical score

- MRx0002 and MRx0005 reduced clinical score vs untreated or vehicle
- Reduction in clinical score was comparable to positive control Dexamethasone

Disease incidence

- MRx0002 and MRx0005 reduced disease incidence
- Reduction in disease incidence was comparable to positive control Dexamethasone
ASSESSMENT OF THE EFFICACY OF LIVE BIOTHERAPEUTIC PRODUCTS IN AN EAE MODEL OF MS

**Inflammatory foci**

- MRx0002 and MRx0005 significantly reduced spinal cord inflammation when compared to the untreated control group.

**Immune cell profiling**

- MRx0002 showed a slight expansion of regulatory T-cells in splenocytes.
LBPs for Neurological Diseases

Summary
### Neurodegeneration (PD)
- Decreased IL-6 secretion in glioblastoma cells; and co-culture of glioblastoma and differentiated neuroblastoma cells
- Protected several brain-derived cell types from oxidative stress
- Partial protection from MPP+ induced cytotoxicity in differentiated neuroblastoma cells
- Protected against MPTP-induced losses in striatal DOPAC in vivo

### Autism Spectrum Disorder
- Modulates gene expression of oxytocin and its receptors in hypothalamic cell lines
- Decreased repetitive behaviours in vivo
- Increased social behaviour / reduced anhedonia
- Decreased anxiety-like behaviour

### Multiple Sclerosis
- Strong reduction in clinical scores in vivo
- Complete prevention of disease incidence in vivo
- Significantly reduced inflammation in the spinal cord
- Expansion of Tregs in splenocytes
- Slight reduction in dendritic cell sub-populations
PROGRESS FROM CNS PLATFORM

- Functional screening platform MicroRx® designed to target different aspects of neurological disorders
- Discovered four human gut commensal bacteria that can modulate relevant cell types and pathways via the gut-brain axis
- Demonstrated efficacy in a variety of preclinical models

Rational selection of bacterial strains from proprietary culture collection affecting CNS disorders

Targeted *in vitro* screening for mechanisms involved in gut-brain axis communication can identify novel live biotherapeutic candidates

The strains have complementary characteristics and mechanisms of action

All strains have shown efficacy in an industry-standard animal models

4D is preparing plans to quickly generate clinically-relevant in-patient data
developing science
delivering therapies