Live Biotherapeutics to Target the Gut-Brain Axis

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INTEGRATED DEVELOPMENT FROM CONCEPT TO CLINIC

Bench to bedside in-house – true end-to-end microbiome company
- Fully developed infrastructure and expertise, from strain isolation through to manufacturing
- Significant experience in navigating the regulatory landscape to deliver clinic-ready candidates

Extensive library of strains
- Well characterised functionality and defined mechanism of action

Multi-functional discovery platform, MicroRx®
- Adaptable platform identifies candidates with functionality applicable to diverse therapeutic areas

3000L, cGMP-certified manufacturing capabilities, unique in the microbiome space
- CMC and ‘manufacturability’ integrated early into candidate development
- De-risks subsequent development stages, accelerating progression into the clinic

Largest IP estate in the space
- Patentability of LBPs now established
- Multi-layered protection facilitated by detailed mechanistic understanding
CORE PIPELINE ADDRESSESS KEY GLOBAL DISEASES

**Programmes**

**Immu-no-oncology**
- MRx0518 *Solid tumours – Combination study with Keytruda®*
- MRx0518 *Solid tumours – Monotherapy study (Tx naïve neoadjuvant)*
- MRx0518 *Pancreatic cancer – Monotherapy study (neoadjuvant)*
- MRx0518 *TNBC*
- MRx0573 *New solid tumour types*
- MRx1299 *HDACi – New solid tumour types*

**Gastro-intestinal**
- Blautix® *Irritable Bowel Syndrome*
- Thetanix® *Crohn’s Disease*

**Respiratory**
- MRx-4DP0004 *Asthma*

**CNS**
- MRx0005 *Neurodegeneration*
- MRx0029 *Neurodegeneration*

**Platform**
- **Vaccines**
- **Autoimmune**
FOCUS ON FUNCTIONALITY: MicroRx® PLATFORM

**Product development**
- Integrated scale-up and optimization
- Strain-specific fermentation and formulation

**Strain engineering**
- Gene disruption/deletion
- Recombinant protein expression

**Isolation**
- Significant culturomics expertise
- Broad coverage and diversity
- Previously unisolated organisms

**Host-response assays**
- Suite of host cells, spheroids/organoids
- Rodent models (disease, SPF, GF)

**Genome mining**
- Whole genome sequencing
- Comparative genomics and bioinformatics

**Proteomics/lipidomics**
- Cell surface shaving
- Characterisation of secreted proteins
- Targeted and driven by host signalling data

**Metabolomics**
- Metabolomic fractionation
- Exhaustive profiling of effector molecules
Gut-Brain Axis:
MicroRx® Screening

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The gut microbiota can affect neurological processes and pathophysiology, from stress response and behaviour to depression and anxiety (Cryan and Dinan, *Nat Rev Neurosci* 2012; Foster et al., *Neurobiol Stress* 2017).

Recent studies have demonstrated significant differences in the gut microbiota of patients with PD and other neurodegenerative disorders when comparing faecal samples to age-matched healthy controls (Scheperjans et al, *Mov Disord* 2015; Bedarf et al, *Genome Med* 2017; Boertien et al., *J Parkinsons Dis.* 2019).

Gut constipation often manifests as a pre-motor or prodromal symptom years before any clinical manifestation of central nervous system (CNS) neurodegeneration is confirmed (Stirpe et al., *Eur J Neurol* 2016).

Deposition of αSyn in the brain is preceeded by accumulation in and degeneration of the enteric nervous system, thought to propagate to the CNS via the vagus nerve. This causes gut motility disorders, increased intestinal permeability, oxidative stress and inflammation (Nair et al., *J Neurogastroenterol Motil*, 2018; Liddle, *Brain Res*, 2018).
THERAPEUTIC TARGETS WITHIN THE GUT-BRAIN AXIS

- High unmet need for new therapeutic strategies for neurological conditions

- Current therapies:
  - Target symptoms, not disease-modifying
  - Side effects
  - Low response rate

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

- MicroRx® screening platform targets:
  - Multiple mechanisms of central nervous system (CNS) pathology
  - Uses overlap, comorbidities, overarching mechanisms
  - Informs on appropriate preclinical models and biomarkers
CENTRAL NERVOUS SYSTEM SCREENING PLATFORM

**Neuronal**
- Neuronal
- Gastrointestinal
- Peripheral

**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

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SCREENING OVERVIEW

**Neuronal**
- Neuroinflammation is a key player in 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

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**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

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Gut-Brain Axis
Neurodegenerative disorders:
Parkinson’s Disease
MRx0005 & MRx0029
PARKINSON’S DISEASE (PD)

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

- Two forms of PD:
  - Idiopathic:
    - Most common (85–90% of PD cases)
    - No known cause
    - Age of onset: ~65 years
  - 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:
  - Misfolded α-synuclein is part of abnormal protein aggregate found in Lewy bodies
MicroRx® platform has identified two gut commensal bacterial strains which show strong potential for the treatment of neurodegenerative disease:

- MRx0029 – *Megasphaera massiliensis*
- MRx0005 – *Parabacteroides distasonis*

**SUMMARY OF NEURODEGENERATION PROGRAM TO DATE**

- Reduction of neuroinflammation
- Induction of dopaminergic neuronal differentiation
- Promotion of gut barrier integrity
- Protection of neuronal cells from oxidative stress
- Protects dopaminergic neurons and increases DOPAC *in vivo*

**LBP candidates progressing through development cycle**

**Rapid progression into first-in-man trial in patients**
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)

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<th>Disease Induction</th>
<th>Treatment</th>
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<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-1 to D5**: MPTP administered i.p. at a dose of 20mg/kg, twice a day for five days
- **D-15**: Faecal pellet & blood collection
- **D-14**: Dosing commences
- **D-21**: Animal acclimation
- **D1** to **D5**: Administration of MPTP
- **D19**: Faecal pellet & blood collection
- **D19**: Faecal pellet & blood collection
- **D21**: Termination & tissue collection
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 the positive control 7-NI
MRx0029 increases tight junction protein in vitro, in vivo and ex vivo

Intestinal hyperpermeability is observed in patients with PD and likely contributes to microbiota-initiated inflammation in PD

- MRx0029 increased gene expression of tight junction proteins associated with gut barrier function (Villin, Occludin, TJP)
- MRx0029 decreased gut permeability (as measured by FITC/Ussing chambers)

**In vitro (HT29-MTX)**

- Villin
- Occludin
- TJP1
- TJP2

**In vivo (colon)**

- TJP1
- Occludin

**Ex vivo**

- Colon Permeability

Parkinson's Disease

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MRx0005 AND MRx0029 DOWN-REGULATE IL-6 SECRETION IN BRAIN CELL LINES

Elevated plasma concentrations of pro-inflammatory cytokines correlate with increased risk of PD

- Investigated the ability of MRx0005 and MRx0029 to attenuate IL-6 secretion in glioblastoma astrocytes (U373) and microglia cells (HMC3)
- MRx0005 and MRx0029 reduce secretion of IL-6 after LPS treatment in U373 cells and TNF-α treatment in HMC3 cells
MRx0005 AND MRx0029 INDUCED CELL-DEPENDENT DOWN-REGULATION OF NF-κB ACTIVATION AND IL-6 SECRETION BY α-SYNUCLEIN PROTEINS

Mutated forms of α-synuclein (A53T, E46K and A30P) are linked to familial PD and early disease onset

- In HEK-TLR4 cells treated with mutated α-syn proteins, MRx0005 significantly decreased NF-κB activation
- In U373 cells co-cultured with SH-SY5Y cells treated with αSyn WT and mutated proteins, both MRx0005 and MRx0029 decreased IL-6 secretion
- In contrast with HEK-TLR4 cells, MRx0029 significantly reduced secretion of IL-6, suggesting a specificity associated with MRx0029
MRx0029 PROMOTED NEURONAL PROTECTION FROM MPP+ INDUCED CYTOTOXICITY ON NEUROBLASTOMA CELLS

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a prodrug to the neurotoxin MPP+, which causes permanent symptoms of PD by destroying dopaminergic neurons in the substantia nigra of the brain.

- MRx0029 completely rescued mitochondrial damage induced by MPP+, while MRx0005 showed a partial protection.
- These findings suggest that MRx0029 might have a specific tropism for neuron-like cells.

**MPP+** is a neurotoxin that interferes with oxidative phosphorylation in mitochondria, leading to the depletion of ATP and eventual cell death.

**MPP+** is the toxic metabolite of MPTP. MPTP is converted in the brain into MPP+, ultimately causing parkinsonism by killing dopamine-producing neurons in the substantia nigra.
MRx0029 INDUCES A DOPAMINERGIC-LIKE NEURON PHENOTYPE IN NEURONAL CELLS

- MRx0029 induced neuronal differentiation in SH-SY5Y cells via upregulation of MAP2 at the gene and protein level.
- MRx0029 induced expression of DAT and LMX1B, which are considered markers of dopaminergic neurons.

Microtubule-associated protein 2 (MAP2) is considered a marker of terminal neuronal differentiation:

- MRx0029 induced neuronal differentiation in SH-SY5Y cells via upregulation of MAP2 at the gene and protein level.
- MRx0029 induced expression of DAT and LMX1B, which are considered markers of dopaminergic neurons.

**MAP2 expression in SH-SY5Y cells**

- Normalised MAP2

**β3-Tubulin expression in SH-SY5Y cells**

- Normalised β3-tubulin

**MAP2 expression changes**

- Field change

**LMX1B expression changes**

- Field change

**DAT/SLC6A3 expression changes**

- Field change

**Phalloidin, MAP2, DAPI images**

- A: Media
- B: MRx0005
- C: MRx0029

- D: Phalloidin
- E: MAP2
- F: DAPI

- G: B3T
- H: DAPI

- J: Media
- K: MRx0005
- L: MRx0029
Neurodevelopmental Disorders:
Autism Spectrum Disorder
MRx0006
AUTISM SPECTRUM DISORDER (ASD)

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

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

• Wide range of symptoms:
  o Social interaction
  o Language and communication
  o Patterns of thoughts and physical behaviour
  o Gastrointestinal complications
MicroRx® platform has identified a gut commensal bacterial strain which shows strong potential for the treatment of autism spectrum disorder:

- MRx0006 – Blautia stercoris

SUMMARY OF NEURODEVELOPMENTAL PROGRAM TO DATE

Modulates neuropeptide gene expression in vitro and ex vivo

Anti-inflammatory effects in vitro and in vivo

Increased social behaviour / reduced anhedonia

Decreased anxiety-like behaviour in open-field test

Decreased stereotyped behaviour, suggesting reversal of repetitive behaviours in vivo

LBP candidate progressing through development cycle
ASSESSMENT OF LIVE BIOTHERAPEUTICS IN A GENETIC MODEL OF ASD - 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
  - Marble burying test
  - Elevated plus maze
  - Forced swim test
  - Female urine sniffing test
- **Stereotyped**
  - Grooming behaviour
  - Open field test
  - Novel object recognition
  - Social transmission of food preference
  - Social transmission of food preference
- **Anxiety**
  - Elevated plus maze
  - Open field test
  - Depression
- **Depression**
  - Elevated plus maze
  - Open field test
  - Social transmission of food preference
- **Cognition**
  - Elevated plus maze
  - Open field test
  - Social transmission of food preference
- **Social**
  - Three chamber social test
  - Marble burying test
  - Elevated plus maze
  - Forced swim test
  - Female urine sniffing test
- **Stereotyped**
  - Grooming behaviour
  - Open field test
  - Novel object recognition
  - Social transmission of food preference
  - Social transmission of food preference
- **Anxiety**
  - Elevated plus maze
  - Open field test
  - Depression
- **Depression**
  - Elevated plus maze
  - Open field test
  - Social transmission of food preference
- **Cognition**
  - Elevated plus maze
  - Open field test
  - Social transmission of food preference

Study design

- **Week 0**: Animal acclimation
- **Week 1-3**: Dosing commences
- **Week 4**: Marble burying and grooming test
- **Week 5**: Three chamber social test and elevated plus maze
- **Week 6**: Open field, novel object recognition and social transmission of food preference
- **Week 7**: Female urine sniffing test
- **Week 8**: Carmine red
- **Week 9**: Forcend swim test
- **Week 10**: Termination & tissue collection

Dodero et al., PLoS ONE 2013
ASSESSMENT OF LIVE BIOThERAPEUTICS IN AN ENVIRONMENTAL MODEL OF ASD - MIA

Model summary

<table>
<thead>
<tr>
<th>Model</th>
<th>MIA environmental mouse model of ASD</th>
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<tbody>
<tr>
<td>Animal Strain</td>
<td>Male C57BL/6N mice (8 weeks of age)</td>
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<tr>
<td>Length of Model</td>
<td>10 weeks</td>
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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
- Ileum and Colon
- Carmine red
- Cytokine profiling

Study design

- Week 0: Animal acclimation
- Week 1-3: Dosing commences
- 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

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

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**Sociability in BTBR model**
- Interaction time (s)

**Sociability in MIA model**
- Interaction time (s)

**Female urine sniffing test in BTBR model**
- Interaction time (s)

**Female urine sniffing test in MIA model**
- Interaction time (s)
• MRx0006 decreased the immobility time in MIA animals

• MRx0006 increased the time spent in the inner zone of the open field in BTBR animals

• MRx0006 decreased the time spent grooming in BTBR animals

• MRx0006 decreased number of marbles buried by MIA animals

ATTENUATION OF AUTISTIC-LIKE BEHAVIOUR IN GENETIC AND ENVIRONMENTAL MOUSE MODELS OF ASD
Pituitary neuropeptides and their receptors

- Oxytocin and arginine vasopressin:
  - Neuropeptides
  - Synthesized in the hypothalamus
  - Secreted from the posterior pituitary gland
  - Implicated in complex behaviours such as:
    - Social behaviours
    - Trust
    - Romantic bonds
    - Aggression

- Increases in oxytocin and arginine vasopressin may be linked to improvement in autistic-like behaviour:
  - MRx0006 increased oxytocin and oxytocin receptor mRNA expression in mHypoA2-28 cells
  - MRx0006 increased arginine vasopressin in the hypothalamus of BTBR mice
LBPs for Neurological Diseases:
Summary
PROGRESS FROM PRE-CLINICAL CNS PLATFORM

• Functional screening platform MicroRx® designed to target different aspects of neurological disorders
• Discovered 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 LBPs

Strains have complementary characteristics and mechanisms relevant to different conditions of the CNS

All strains have shown efficacy in industry-standard animal models of neurological diseases

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