Live Biotherapeutics in Immuno-Oncology: From Discovery to the Clinic

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PRESENTATION OVERVIEW

Overview - The role of the gut microbiome in cancer

- Therapeutic approaches for the gut microbiome
- Challenges and opportunities of microbiome-based interventions

4D Pharma PLC

- Microbiome company developing live biotherapeutic products
- Current clinical programmes
- MicroRx® discovery platform

MRx0518 immuno-oncology programme

- Preclinical efficacy
- Mode of action
- Clinical trial designs
The gut microbiome in immuno-oncology
Microbiome impacts efficacy of ICIs in patients

• Anti-PD-1-responsive patients have significantly increased microbiome diversity compared with non-responders (Gopalakrishnan et al., 2018, Routy et al., 2018)

This effect is causative, not just a correlation

• Animals humanised with ‘responder’ microbiota have delayed tumour growth and enhanced response to anti-PD-1 therapy (Gopalakrishnan et al., 2018)

Single-strain LBPs inhibit tumour growth and increase microbiome diversity

• Single-strain live biotherapeutics are effective in reducing tumour growth (Sivan et al., 2015, Vetizou et al., 2015, 4D pharma data) and increase microbiome diversity and stability in animal models (4D pharma data)
Microbial taxa enriched in responders (blue ribbons) or non-responders (red) to cancer immunotherapy agents (anti-CTLA-4, anti-PD-1) increase gut microbes alter systemic immune function via local changes within the gut mucosa and GALT to induce increased antitumor immune function.
MICROBIOME-DERIVED THERAPEUTIC APPROACHES IN IMMUNO-ONCOLOGY

Translation of microbiome-derived therapeutics in cancer

- Microbiota analysis: differences between health and disease
  - Compositional and functional differences

- Strategies to modify the gut microbiome composition
  - FMT (healthy, ICI responders)
  - LBPs, consortia
  - Antibiotics, drugs
  - Dietary intervention

- Therapeutic interventions
  - LBPs, consortia

Biggest challenges to success in the clinic

- Proof in human clinical trials is still missing
- More understanding needed of mechanisms of action
  - More complex than drugs acting on a target molecule
  - Live bacteria – growth, behaviour, activity, manufacturing
Company overview
4D PHARMA – WORLD LEADER IN LIVE BIOOTHERAPEUTICS

Live Biotherapeutics

• Single-strain commensal bacteria encapsulated for oral delivery
• Highly favourable toxicity/side-effect profile
• Accelerated preclinical development and early in-patient data

Industry leader with differentiated approach

• 4D pharma is an integrated platform & product company
• Mechanistic approach focused on function: targeting and addressing known disease pathways via effector molecules
• Sector-leading IP estate; more than 400 granted patents
• Multiple value inflection points; proof-of-concept clinical data on the horizon

Breakthrough class of medicines with potential to change the way we treat disease
### CORE PIPELINE ADDRESSING KEY GLOBAL DISEASES

<table>
<thead>
<tr>
<th>Focus</th>
<th>Discovery</th>
<th>Preclinical</th>
<th>Development</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
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<tbody>
<tr>
<td><strong>Immuno-oncology</strong></td>
<td>MRx0518 Solid tumours – Combination study with Keytruda®</td>
<td>Phase II study enrolling</td>
<td>MRx0518 Solid tumours – Monotherapy study (Tx naïve neoadjuvant)</td>
<td>Phase I study enrolling</td>
<td>MRx0518 Pancreatic cancer – Monotherapy study (neoadjuvant)</td>
<td>Phase I study opening H2 2019</td>
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<td>MRx0573 Immuno-oncology</td>
<td>Phase I/II opening Q2 2019</td>
<td>MRx1299 Immuno-oncology</td>
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**Gastro-intestinal**

- **Blautix® Irritable Bowel Syndrome** - Phase II enrolling
- **Thetanix® Crohn’s Disease** - Phase II in planning

**Respiratory**

- **MRx-4DP0004 Asthma** - Phase II study opening Q2 2019

**CNS**

- **Neurodegeneration**

**Platform**

- **Rosburix® Ulcerative Colitis**
- **MRx0006 Rheumatoid Arthritis**
- **MRx0002 Multiple Sclerosis**
- **Autism**
FOCUS ON FUNCTIONALITY: MICRORX PLATFORM

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

**Host-response assays**
- Targeted immune screening approaches
- Suite of host cells, spheroids/organoids
- Host receptors/MAMPs

**Genome mining**
- WGS and genome mining
- Comparative genomics and bioinformatics

**Metabolomics**
- Metabolomic fractionation
- Exhaustive profiling of effector molecules

**Strain engineering**
- Gene disruption/deletion
- Recombinant protein expression
- Anti-sense RNA gene silencing
- CRISPR-Cas9 gene editing

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

**Proteomics**
- Cell surface shaving (surfaceome)
- Characterisation of secretome
- Targeted and driven by host signalling data
Programme:
MRx0518 in immuno-oncology
MRx0518

• Gram-positive, motile, anaerobic bacterium of the *Enterococcus* genus
• Selected for immunostimulatory host response profile, unique within the 4D strain library

**Efficacy in vivo**

• Monotherapy: reduction of tumour volume in three syngeneic cancer models
• Combination: boosts efficacy of checkpoint inhibition

**Mechanism of action**

• Increases tumour CD8+/Treg ratio
• Immune stimulation through action of bacterial flagellin on TLR5

**Ongoing clinical studies**

• Phase Ib neoadjuvant biomarker study (UK)
• Phase I/II combination study with anti-PD-1 (US)
MRx0518 was tested in an EMT6 breast carcinoma syngeneic mouse model

- MRx0518 significantly inhibited tumour growth (T/C 44%) and increased survival (125%)
- MRx0518 monotherapy outperformed species reported to have anti-tumour effects
- The effect of MRx0518 was not *Enterococcus* genus-wide
MRx0518 was subsequently tested in models of kidney, lung and breast cancer

- MRx0518 inhibited tumour growth in syngeneic RENCA and LLC1 cancer models
- Efficacy was observed in model refractory to monotherapy checkpoint blockade
The effects of MRx0518 on the tumour immune microenvironment were investigated

- MRx0518 increases T cells, CD8+ T cells and NK cells populations in the tumour
- MRx0518 increases the expression of chemokines, cytokines and TLRs
- Correlation between MRx0518-treated individual tumour size and IFNγ expression
- MRx0518 and anti-CTLA-4 increase the ratio CD8+/FoxP3+ cells in the tumour
The MRx0518-induced cytokine signature was investigated in different cell types

- MRx0518 increases the production of a cytokine/chemokine signature that includes IL-8, IFN-γ, IL-6, TNF-α, IL-1β, IL-23, CCL20, CXCL1, CXCL3, CXCL9 and CXCL10
The immune cell signature induced by MRx0518 was investigated

**PBMC co-culture assay**

<table>
<thead>
<tr>
<th>CD3/CD28</th>
<th>CD8+ cells</th>
<th>CD4+ cells</th>
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<tr>
<td></td>
<td>% CD3+CD28* (of CD8+ cells)</td>
<td>% IFN+ (of CD8+ cells)</td>
</tr>
<tr>
<td>Untreated</td>
<td>[Graph]</td>
<td>[Graph]</td>
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<tr>
<td>MRx0518</td>
<td>[Graph]</td>
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**Treg differentiation assay**

<table>
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<tr>
<th>CD3/CD28, IL-2 and TGF-β</th>
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<tbody>
<tr>
<td>% CD25+Fox3 (of CD4+ cell population)</td>
</tr>
<tr>
<td>Untreated</td>
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<tr>
<td>YCFA</td>
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- MRx0518 increases % CD8+ T cells and activated IFN+ CD8+ T cells
- MRx0518 increases % CD4+ T cells and activated IFN+ Th1 CD4+ T cells
- MRx0518 reduces differentiation of CD4+CD25+FoxP3+ Tregs
Mode of action:
Bacterial effector molecules
Identification of immunostimulatory factors of LBPs: integrated host-microbe approach

Discovery-based target indicators

- Which host receptor(s) and pathways are stimulated?
- Which immune populations/mediators are induced and in which direction?
- What is the active bacterial fraction(s)?

Genus/species of interest

- Whole genome sequencing and mining
- Transcriptional analysis of effector genes
- Comparative genomics
- ID potential MAMPs

Immunomodulatory molecule ID

- ID potential MAMPs

Host response assays

Genome mining

Surfacome/Secretome

Molecular tools
Flagellin is a known TLR5 ligand, is it involved in MRx0518 immunostimulatory effect?

**Gene inactivation**

**Recombinant protein expression**

**Overall strategy**

MRx0518 activates NF-κB and TLR5 signalling

Trypsin treatment abolishes TLR5 activation
Flagellin in MRx0518\_SN activates NF-\(\kappa\)B and TLR5 signalling

**NF-\(\kappa\)B activation in response to SN**
- Little to no NF-\(\kappa\)B/TLR5 activation when flagellin gene is knocked out
- Supernatant of the reference strain does not activate TLR5
- Supernatant of reference strain does not contain flagellin

**MRx0518 flagellin is more potent than DSM100110 flagellin**

**Dose-response with recombinant flagellins**
- Purified recombinant flagellins activate TLR5
- MRx0518 flagellin is more potent than the reference flagellin at low concentrations
INACTIVATION OF MRX0518 FLAGELLIN GENE REDUCES IMMUNOSTIMULATORY EFFECTS

MRx0518 and its fliC mutant were tested in HT29-MTX cells to assess effects of the flagellin on immune gene transcription and IL-8 production

- Inactivation of the flagellin gene reduced the immunostimulatory host response
- Recombinant MRx0518 flagellin induced high levels of immunostimulatory gene expression

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<th>HT29-MTX gene expression</th>
<th>HT29-MTX IL-8 production</th>
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<td><strong>MRx0518</strong></td>
<td><strong>MRx0518 supernatant induces higher levels of IL-8 than the reference strain supernatant</strong></td>
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<td><strong>fliC&lt;sub&gt;SN&lt;/sub&gt;</strong></td>
<td><strong>IL-8 stimulatory effect abolished by flagellin gene inactivation</strong></td>
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- MRx0518 supernatant induces higher levels of IL-8 than the reference strain supernatant
- IL-8 stimulatory effect abolished by flagellin gene inactivation
- Recombinant MRx0518 flagellin induced high levels of IL-8
MRx0518 and DSM100110 both produce functional, but highly divergent, flagella

Genotypic and phenotypic characterization

- Highest level of sequence divergence between MRx0518 and DSM100110 FliC sequences located in central variable region
- Regions known to be critical for TLR5 interaction in other bacterial species were conserved between both strains

In vitro motility
- BBL Motility Medium supplemented with 0.005% TTC

MRx0518 – MoA
TLR5 AND FLAGELLIN IN CANCER – SUPPORTING LITERATURE

- TLR5 agonist Entolimod (a pharmacological derivative of Salmonella flagellin) activates NF-κB-, AP-1-, and STAT3-driven immunomodulatory pathways in the liver
- Anti-metastatic effect through stimulation of CD8+ T cells
- Expression and activation of TLR5-associated pathways is elevated in breast carcinomas
- Salmonella flagellin activation of TLR5 in breast cancer cells resulted in local cytokine release and inhibition of cell proliferation
- A Salmonella strain expressing Vibrio vulnificus flagellin had tumour-suppressive effects in a colon cancer model
- The engineered strain also decreased metastasis
MRx0518 monotherapy shows efficacy across different syngeneic tumour models

The proposed molecular/cellular events underlying the antitumorigenic effects of MRx0518 include:

- TLR5-mediated NF-κB activation
- Strong induction of chemokine (CCL20, CXCL1, -9, -10) and cytokine (IFNγ, TNFα, IL-8, IL-17) responses
- Activation and/or tumour trafficking of Th1 cells, CD8 cells, NK cells and MØs

TLR5 signalling via bacterial flagellin contributes to the immunostimulatory host-response profile

Additional bioactive molecules of MRx0518 are currently under investigation

Hajam et al., 2017
Clinical trials
Combination study with Keytruda®

- Assessing ability of MRx0518 to re-engage immune system to respond to checkpoint blockade
- Four different solid tumour types (melanoma, bladder, renal and NSCLC)
- Principal Investigator: Dr. Shubham Pant, MD Anderson (Houston, USA)

Trial design overview

- Primary endpoint: safety and tolerability
- Secondary and other endpoints:
  - Tumour response
  - Overall survival
  - Immunological biomarkers
  - Microbiome profile
Neoadjuvant monotherapy study

- Window study: dosing between diagnosis and surgery
- Up to 120 treatment-naïve patients
- Multiple solid tumour types (melanoma, prostate, breast, ovarian, urothelial, renal, lung, head & neck)
- Principal Investigator: Dr. Jonathan Krell, ICL (UK)

Trial design overview

- Primary endpoint: safety and tolerability
- Secondary and other endpoints:
  - Tumour response
  - Overall survival
  - Immunological biomarkers
  - Microbiome profile
**MRX0518: SUMMARY OF PRECLINICAL AND CLINICAL STUDIES**

- **MRx0518**
  - Genus: *Enterococcus*
  - Gram-positive, motile anaerobe

- **Primary MoA**
  - TLR5 agonist

- **Primary Effector Molecule**
  - FliC flagellin protein

- **Host-response**
  - Strong, consistent immuno-stimulatory profile

- **Efficacy**
  - Reduction of tumour growth; monotherapy/combination

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**Three clinical studies in 2019:**

- Solid tumours: Phase I/II combination with Keytruda®, open, USA
- Solid tumours: Phase I monotherapy neoadjuvant study in treatment-naïve patients, open, UK
- Pancreatic cancer: Phase I/II monotherapy neoadjuvant study to open 2019, USA
developing science
delivering therapies