Identification and Profiling of Biomarkers in Neurodegeneration

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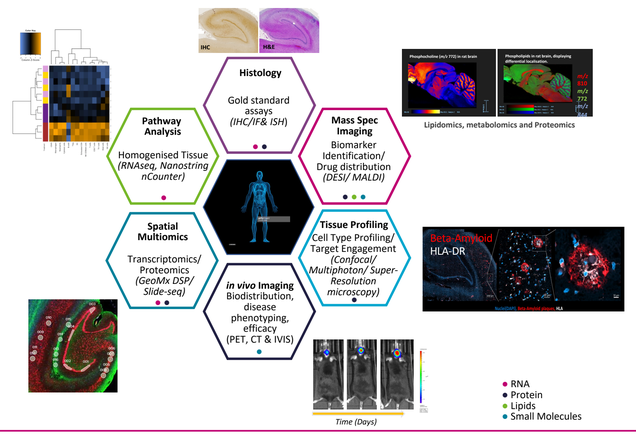
Introduction

- Novel biomarkers are necessary to further the development of treatments for neurodegenerative diseases like Alzheimer's Disease, Amyotrophic Lateral Sclerosis (ALS) and Parkinson's Disease with advanced and emerging technologies being crucial for their identification and validation in both tissue and fluid samples.
- Tissue samples can be spatially profiled using a range of techniques, including the GeoMx Digital Spatial Profiler to examine transcriptomic or proteomic profiles of specific regions and mass spectrometry imaging to examine a range of molecules including metabolites and lipids. The outputs from these technologies can be overlaid to examine variations between diseased and control samples.
- Liquid samples like plasma or cerebrospinal fluid can be used to track biomarker profiles longitudinally and can be interrogated using Luminex or Simoa technology alongside mass spectrometry for a wide range of different molecules. Again, the highly multiplexed readouts can be interrogated for a deeper understanding of drug and disease.

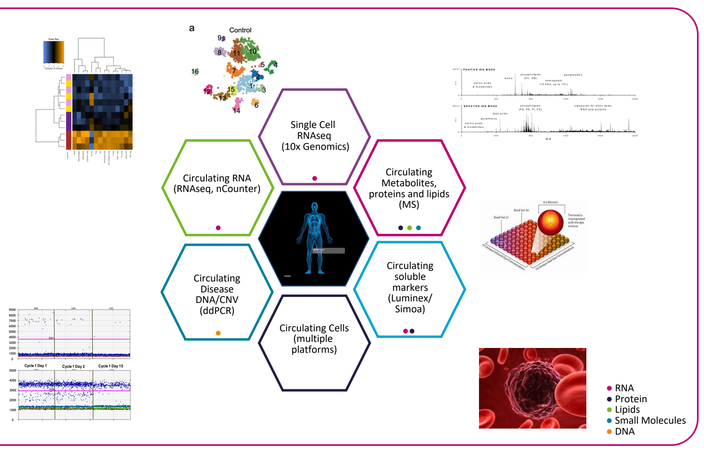
Deeper understanding of drug and disease – **Tissue**

- Advancing Biomarker Discovery and validation through combining standard and emerging technologies
- Highly multiplexed readouts can be interrogated for a deeper understanding of drug and disease
- Hypothesis free and hypothesis driven biomarker analysis workflows are being established to compare gold standard methods to emerging multiplexing platforms



Deeper understanding of drug and disease – **Circulating**

- Liquid biopsies are just as valuable as they give a real time insight into the patient's disease and response
- Opportunity to utilise longitudinally
- Additional multiplexed readouts can be interrogated/overlaid to support hypothesis observed
- Circulating factors can provide information on the heterogeneity of the disease along with early response or resistance biomarkers, often before clinical responses are observed



Technologies and Platforms

Sample Acquisition

- We can run analysis on your own samples, assist with acquiring relevant human samples or run in vivo models to acquire animal samples if required.

Human Samples

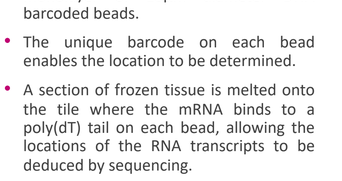
- A dedicated samples team that has built connections with various sample providers including biobanks with global networks – this enables us to access 'hard to find' samples at competitive rates.
- Extensive experience in sourcing a variety of high-quality samples including brain tissue, spinal cord and biofluids from donors with conditions such as Amyotrophic Lateral Sclerosis, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease.
- Experience in establishing new bespoke sample collections in a timely manner based on the needs of our collaborators.
- Our samples team is well versed in managing applications for external ethical approvals to ensure compliance with HTA guidelines.

Samples from *in vivo* Models

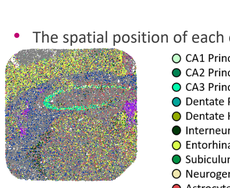
- In addition to validating new models and extensive experience of neurosurgical techniques we have experience of running numerous mouse models of disease:
  - Alzheimer's Disease (5xFAD model)
  - Huntington's Disease (R6/1 model)
  - Orthotopic Mouse brain tumour model (G261-luc)
  - Acute LPS induced model of acute neuroinflammation
  - U87-luc subcutaneous xenograft model

SlideSeq Platform

- Spatial transcriptomics method with single cell resolution for use on frozen tissues

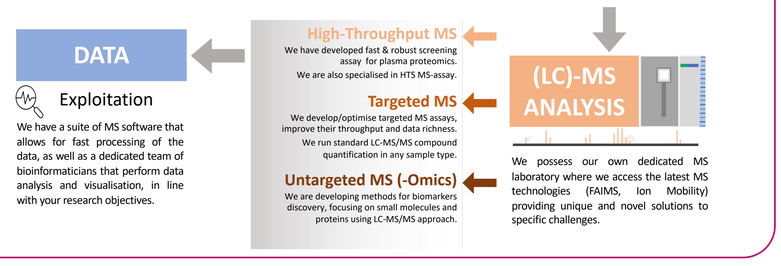


- 3 x 3mm coverslip coated with a monolayer of 10µm diameter DNA barcoded beads.
- The unique barcode on each bead enables the location to be determined.
- A section of frozen tissue is melted onto the tile where the mRNA binds to a poly(dT) tail on each bead, allowing the locations of the RNA transcripts to be deduced by sequencing.



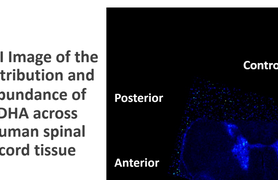
- Clustering analysis identifies groups of cells present with similar expression profiles.
- Robust Cell Type Decomposition (RCTD) uses single-cell RNA-Seq datasets to identify the cell types present in each cluster.
- The spatial position of each cell type can then be mapped.

Mass Spectrometry Capabilities



Protein Multiplexing (Luminex® and Quanterix™ Simoa ® Platforms)

- Luminex and Simoa platforms perform bead-based immunoassays, enabling simultaneous multiplex detection of up to 100 analytes



Pre-validated Assays

- Various commercially pre-validated assays are available and can be run in house on the Luminex MAGPIX instrument
- Catalogues with >600 validated human analytes (includes several multiplex neuroscience/ neurodegeneration panels)
  - NF-H (but not NF-L), Tau (Total and phospho (pT181 + pT231)), TDP-43

Customised Assays

- Assays to targets not commercially available can be built and validated in house (E.g. NF-L assay validated in house)
- Several neuro focused assays are currently being validated for use with both human and mouse CSF and plasma samples

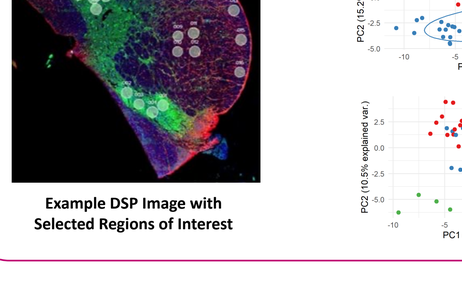
Pre-validated Assays

- NF-L
- Total Tau
- pTau (181 or 231)
- GFAP
- TDP-43
- Aβ40 or Aβ42

GeoMx® Digital Spatial Profiler

nanoString

- Quantitative, non-destructive, spatial proteomics and transcriptomics from FFPE or fresh frozen tissue sections or TMAs



Human and mouse (and selected canine) probe panels available

Profiling Panels

Transcriptome Profiling

- Whole Transcriptome Atlas- profiling of >18,000 protein coding genes

Multiplexed Protein Panel

- Panels of 20 – 80 proteins (Immuno-oncology and neuroscience focused)
- Customised assays, barcoding bespoke antibodies can additionally be validated and added into the multiplex panels

Morphology Markers

- Can be selected from a host of pre-validated antibodies (E.g. GFAP (Astrocytes); Iba1 (Microglia), NeuN (Neurons), Amyloid-β (Plaques))
- New markers bespoke to each project can additionally be validated in house

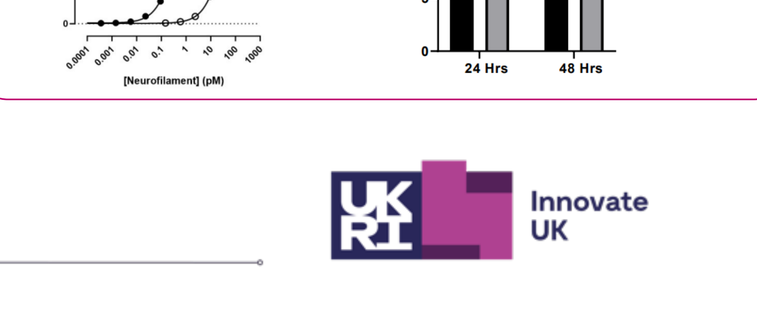
Mass Spectrometry Imaging

- Label free surface analysis of samples (E.g. tissue/ dried blood spots) for a variety of analytes
- Possible to analyse different molecular classes from a single section.
- Relative/semi-quantitation
- Can be correlated and overlaid with microscopy or DSP images (on same or consecutive sections)

MSI Analytes

- Small molecules
- Metabolites
- Lipids
- Peptides/proteins

What anatomical structures can we see with each technique?



Lipidomic Profiling in Human Spinal Cord Tissue

Queen Mary University of London

UK SPINE Knowledge Exchange

- Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterised by the loss of motor neurons in the brain and spinal cord, with approximately 15% of patients additionally developing a form of Frontotemporal Dementia (FTD)
- The dysregulation of lipid metabolism is a common hallmark of a range of neurodegenerative diseases, including ALS.

Mass Spectrometry Imaging (MSI)

- A collection of lipids and metabolites previously recognised to be associated with anti-inflammatory pathways was spatially profiled across human spinal cord tissue using MSI.
- The snap frozen tissue was additionally H&E stained in order to define the anterior and posterior orientation.
- One of the lipids, the omega-3 polyunsaturated fatty acid Docosahexaenoic acid (DHA), was found to be increased in the grey matter of the spinal cord in ALS and FTD-ALS patients.

MSI Image of the distribution and abundance of DHA across human spinal cord tissue

Spatial Protein Profiling in Neurodegeneration

UK SPINE Knowledge Exchange

- The aim of this project was to examine differences in protein profiles in neurodegenerative disease, (specifically in this case, Amyotrophic Lateral Sclerosis (ALS)), and aging.
- A panel of 74 proteins was spatially profiled across 6 spinal cord tissues (3 from control donors and 3 from patients suffering from ALS) using the GeoMx DSP platform.
- Data from 4 Regions of each spinal cord were collected, 2 from the grey matter (posterior horn, anterior horn) and 2 from the white matter (lateral funiculus, outer white matter).
- Data from the various regions of each tissue were collected and analysed.

Example DSP Image with Selected Regions of Interest

Luminex Neurofilament-Light Assay

ALCHEMAB THERAPEUTICS

- Neurofilament light is an established biomarker for neuronal damage in a range of neurodegenerative diseases.
- A Luminex assay was developed and validated to quantify NF-L in a variety of samples, including cerebrospinal fluid, cell lysates and tissue homogenates.
- Multiplexing of this assay with a selection of other proteins has additionally been tested and validated.

Specificity of the assay for NF-L over NF-H

Knockdown of NF-L in SH-SY5Y cells

UKRI Innovate UK