Background

Contract research services company

Established in 1994

Located in Glasgow, UK
Overview

Custom Reagent Development

- Recombinant protein production
- Cell line development

Drug Discovery Services

- Screening assays (PDEs, Ion Channels, Nuclear Receptors etc)
- Proof-of-concept in vivo models

- Internal cell line development program
- 70 ion channel cell lines developed to date
- Drug discovery screening & safety services
- Custom ion channel cell line development
Ion Channel Services

Ion channel cell line development

- Classical cell line development methods
- Constitutive / inducible expression, co-expression, viral transduction
- Approximately 50 clones per target assessed by fluorescence assay
- Top clones selected for further manual & automated patch clamp validation

Ion channel screening services

- HTS fluorescence assays
- Manual & automated (QPatch, IonWorks) patch clamp electrophysiology
- Primary cell electrophysiology
<table>
<thead>
<tr>
<th>Channel Type</th>
<th>Example Channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGSC</td>
<td>Nav1.1, Nav1.2, Nav1.3, Nav1.4, Nav1.5, Nav1.6, Nav1.7, Nav1.8</td>
</tr>
<tr>
<td>TRP</td>
<td>TRP1.1, TRP1.2, TRP1.3, TRP1.4, TRP1.5, TRP1.6, TRP1.7, TRP1.8</td>
</tr>
<tr>
<td>P2X</td>
<td>P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, P2X7</td>
</tr>
<tr>
<td>Kv</td>
<td>Kv2.1, Kv7.2, Kv7.3, Kv2.1/9.3, Kv11.1, Kv2.2, Kir2.1, Kir2.2, Kir2.4, Kv3.1a, Kir3.1/3.4, Kv3.4, TREK1, Kv7.1, TRESK, TASK1</td>
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<td>Cav</td>
<td>Cav1.2, Cav2.2, Cav3.2</td>
</tr>
<tr>
<td>Other</td>
<td>Ano1, Ano2, NMDA, HCN2, HCN4, ENaC</td>
</tr>
</tbody>
</table>

**Transporters**
- NCX1
- Na+/K+ ATPase

**SB Ion Channels Portfolio**
TRP channels

• 6 mammalian sub-families (TRPA, TRPC, TRPM, TRPV, TRPML, TRPP)
• Expressed in many cell types & tissues and possess diverse functions
• Polymodal activation properties
  ➢ Signalling mediators & cascades
  ➢ pH
  ➢ Temperature
  ➢ Membrane voltage
  ➢ Mechanostimulation
TRP channels as drug targets

• Pathological implications include pain, kidney disease, CNS disorders, cardiovascular abnormalities

• Attractive drug targets
  
  ➢ Less homology between subtypes compared to e.g. sodium & calcium channels
  
  ➢ TRP channel mutations are sufficient to cause disease in humans (validated targets in translational medicine models)

• Challenging targets to work with
  
  ➢ Functional expression
  
  ➢ Signal rundown
  
  ➢ Platform compatibility
Functional TRP channel expression

• Unable to detect TRP currents under standard conditions

• Serum starvation, cell synchronisation & optimization of assay conditions (e.g. time post re-addition of serum) results in detection of robust, repeatable signals
TRPC Assays

**EC$_{50}$ for Carbachol, TRPC3**

**IC$_{50}$ for Pyr3, TRPC3**

**EC$_{50}$ for Carbachol, TRPC5**

**IC$_{50}$ for ML 204, TRPC5**

**EC$_{50}$ for Carbachol, TRPC6**

**IC$_{50}$ for ML 204, TRPC6**

**EC$_{50}$ for Carbachol, TRPC7**

**IC$_{50}$ for SKF 96365, TRPC7**
TRPC6

• Found in a broad range of tissues including brain, heart, kidney, lung & intestine

• Associated with several pathological processes including Focal Segmental Glomerulosclerosis (FSGS), cardiac hypertrophy, pulmonary hypertension & glaucoma

• Lack of specific agonists / antagonists, can be activated by OAG or indirectly by Carbachol or internal calcium and blocked by ML204, SKF96365, Lanthanum & Gadolinium

• Reported difficulties in developing automated electrophysiology assays for compound assessment due to inability to reactivate channels
TRPC6 Cell Line Validation

<table>
<thead>
<tr>
<th></th>
<th>Rosiglitazone</th>
<th>Riluzole</th>
<th>OAG</th>
<th>Carbachol</th>
<th>La(^{3+})</th>
<th>ML204</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPC6</td>
<td>EC(_{50}) (µM)</td>
<td>EC(_{50}) (µM)</td>
<td>EC(_{50}) (µM)</td>
<td>EC(_{50}) (µM)</td>
<td>IC(_{50}) (µM)</td>
<td>IC(_{50}) (µM)</td>
<td>IC(_{50}) (µM)</td>
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<tr>
<td>no effect</td>
<td>no effect</td>
<td>12</td>
<td>6</td>
<td>385.61</td>
<td>4.11</td>
<td>1440</td>
<td></td>
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<tr>
<td>WT</td>
<td>*EC(_{50}) (µM)</td>
<td>*EC(_{50}) (µM)</td>
<td>*EC(_{50}) (µM)</td>
<td>*EC(_{50}) (µM)</td>
<td>*IC(_{50}) (µM)</td>
<td>*IC(_{50}) (µM)</td>
<td>*IC(_{50}) (µM)</td>
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<tr>
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<td>no effect</td>
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</table>
TRPC6 – QPatch

Challenges

• TRPC6 activation (and decay of signal)

- Repeat activation of TRPC6

- Measuring activation & compound addition in same well
Repeated stimulations with 30uM OAG resulted in distinct TRPC6 current rundown.

- Initial QPatch experiments suggested EC50 of OAG to be 30uM.
• Reduced OAG application to EC20 concentration (10uM)

• Slower run-down & increased response to 100uM OAG
Cell culture optimisation

- Cell splitting / plating density
- Time post-plating prior to assay
- General TLC

By controlling the culture conditions we can reduce EC50 of OAG
TRPC6 QPatch Optimization

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**Graphs:**

1. Graph 1: Y-axis labeled as 'X' (unit unspecified), X-axis labeled as 'Sweep Time (s)'.
2. Graph 2: Y-axis labeled as 'MgC1/VI', X-axis labeled as 'Sweep Time (s)'.
3. Graph 3: Y-axis labeled as 'X' (unit unspecified), X-axis labeled as 'Sweep Time (s)'.
4. Graph 4: Y-axis labeled as 'MgC1/VI', X-axis labeled as 'Sweep Time (s)'.

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**Legend:**

- Green dots
- Purple dots
- Orange dots
- Blue dots
- Red dots
- Grey dots
- Black dots

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**Notes:**

- The graphs show changes in MgC1/VI and X over time.
- The data points are plotted at different time intervals.
- The graphs indicate a trend in the data over the sweep time.
TRPC6 QPatch Stats

• 15/16 whole cell
• 12/15 achieved gigaseal
• 70% success rate
TRPC6 inhibitor studies

Standard assay DMSO concentrations are suitable for TRPC6 QPatch studies

Result: Robust Qpatch assay for TRPC6 compound dose-response studies

IC50 = 4nM
TRPC5 Assay Development

<table>
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<th>Carbachol</th>
<th>La^{3+}</th>
<th>ML204</th>
<th>RR</th>
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<tbody>
<tr>
<td>TRPC5</td>
<td>EC_{50} (µM)</td>
<td>IC_{50} (µM)</td>
<td>IC_{50} (µM)</td>
<td>IC_{50} (µM)</td>
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<td>HEK293</td>
<td>EC_{50} (µM)</td>
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<td>IC_{50} (µM)</td>
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![Graph 1](Saline ML204 1.00nM ML204 10.0nM ML204 100nM ML204 1.00µM ML204 100nM ML204 Saline Sweep Time [s])

![Graph 2](4nM 8nM 10nM 100nM Saline 100nM 100nM 100nM 100nM ML204 1.00nM ML204 1.00nM ML204 1.00nM ML204 1.00nM 100nM 100nM 100nM 100nM Sweep Time [s])
TRPC5 QPatch Success Rate

- 15/16 whole cell
- 9/15 achieved gigaseal
- 75% success rate
TRP channel cell lines & assays

SB has developed a comprehensive panel of functional validated TRP cell lines & assays

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<thead>
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<th>TRPA Ion Channel Cell Lines</th>
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<tr>
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<td>Species</td>
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<td>TRPA1</td>
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<td>Fluorescence Assay</td>
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<td>TRPC3</td>
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</table>
TRP screening in practice

**DISCO**

- EU grant funded 3 year research program, research teams from 6 European & 1 South American countries
- Bioprospecting of untapped, rare and underutilised plant species and ecotypes
- Identify and develop new sustainable biosources of plant-derived products of pharmaceutical and industrial interest
- Screen 2000 extracts against a broad range of targets (ion channels, GPCRs, PDEs, nuclear receptors etc)
TRPA1 DISCO screen

- 2000 plant extracts were screened against human TRPA1
- 5 extracts were identified following hit confirmation

Dose Response Curve of Agonist AITC against hTRPA1

Dose response curve of agonist AITC against human TRPA1 using calcium sensitive dye in the flexstation assay. EC₅₀ value was 1.81µM. Dose response curve and EC₅₀ value were generated by Prism software (GraphPad Inc).

Dose Response Curve of Antagonist Ruthenium Red against hTRPA1

Dose response curve of antagonist ruthenium red against human TRPA1 using calcium sensitive dye in the flexstation assay. IC₅₀ value was 14.8µM. Dose response curve and IC₅₀ value were generated by Prism software (GraphPad Inc).
• Hit extracts were assessed for TRP selectivity
TRPA1 DISCO screen

TRP Channel Profiling

% Inhibition

Extract 1
Extract 2
Extract 3
Extract 4
Extract 5
TRPC6 DISCO screen

- 2000 plant extracts were screened against human TRPC6
- 38 extracts were identified following hit confirmation

• Next step: selectivity profiling
Summary

• Generated an extensive panel of functional TRP channel cell lines

• Validated electrophysiological alternatives to fluorescence assays

• Significantly increases capabilities in screening & profiling against TRP channels

Ongoing developments:

• TRPC6 podocyte electrophysiology – assay development underway

• TRPC species variants – mouse and rat TRPC5 & TRPC6 in development