The impact of IMI project outcomes in Industry

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Overview

IMI initiatives using stem cells
– Present: enabling tools in classical drug discovery
– Future: enabling precision medicine
– Further future: Individualised therapy cells and diagnostics
iPS cells impact at all stages of drug development

- Research-Target selection: 0-5 years
- Identify a drug candidate: 1-2 years
- Safety studies: 1-2 years
- Clinical trials - launch: 3-8 years
- Post-marketing surveillance: Indefinite

Which targets are most important?
Which molecule to develop?
Is it safe?
Which patients will benefit most?

Classic model
Developing analgesics drugs

Signal Detection
Peripheral terminals detect chemical, mechanical and temperature signals

Signal Transduction
Action potential carries nociceptive signal to spinal cord/brain

Pain Gating
Spinal pain gate modulates signal

Pain Perception
Brain interprets signal and produces conscious awareness
Using iPS cells in Target validation and screening for analgesics

1. Make sensory neurones from ES/iPS in vitro
2. Confirm phenotype - right receptors ion channels and enzymes and are function ‘normally’
   - high quality electrophysiology for ion channels
3. Convert to robust, higher throughput assays for screening
4. Identify potential new drugs
5. Confirm their activity in relevant genetically heterogeneous populations prior to clinical trials
iPS cells make functional Sensory Neurones

Expression of sensory neurone markers
Peripherin / Brn3A / Islet-1

P2X3 - functional characterisation

NaV1.8 - functional characterisation
iPS cells recapitulate the majority of sensory neurone drug targets well

<table>
<thead>
<tr>
<th>Target</th>
<th>mRNA</th>
<th>Functional response</th>
<th>Pharmacological validation</th>
<th>Target Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA-A</td>
<td></td>
<td>Electrophysiology</td>
<td>Benzo, selective PAMs</td>
<td>Mostly GABA a2/3 subtype</td>
</tr>
<tr>
<td>Trk-A</td>
<td></td>
<td>Phosphorylation assay – P-TrkA, P-ERK</td>
<td>Kinase inhibitor</td>
<td>Peaks early (5i - first week) in differentiation, then declines.</td>
</tr>
<tr>
<td>P2X3</td>
<td></td>
<td>Ca2+ flux, electrophysiology</td>
<td>Selective agonist, antagonist</td>
<td>Expressed early (3-4 weeks) on majority of neurones</td>
</tr>
<tr>
<td>TrpV1</td>
<td>Ca2+ flux</td>
<td>Capsaicin, selective antagonist</td>
<td>Requires long maturation. Present in lower than expected abundance</td>
<td></td>
</tr>
<tr>
<td>ASIC</td>
<td></td>
<td>Electrophysiology</td>
<td>Selective toxins</td>
<td>Mamalgin-1 blocks ASIC1a,1b,2a,2b heteromers: majority of response blocked</td>
</tr>
<tr>
<td>Nav1.8</td>
<td></td>
<td>Electrophysiology</td>
<td>TTX plus selective Nav1.8 blockers</td>
<td>Expressed on subpopulation of neurones; 15-20% of total sodium current. Population increases with maturation</td>
</tr>
<tr>
<td>Nav1.7</td>
<td></td>
<td>Electrophysiology</td>
<td>Selective Nav1.7 blockers</td>
<td>Blocks around 25-35% total sodium current.</td>
</tr>
<tr>
<td>HCN1</td>
<td></td>
<td>Electrophysiology</td>
<td>Forskolin</td>
<td>Current properties are most consistent with HCN1.</td>
</tr>
<tr>
<td>KCNQ2/3</td>
<td></td>
<td>Electrophysiology</td>
<td>Selective KCNQ2/3 opener</td>
<td>hyperpolarises membrane, and prevents firing of single and repetitive action potential firing.</td>
</tr>
</tbody>
</table>
Higher throughput assays
- Ca oscillations in sensory neurones

- Human iPS cells have very similar properties to normal neurones
- They form networks and respond synchronously
IPSC technology: potential as a future tool in precision medicine

Stratification based upon disease

- IPSC
- Neuron
- Phenotype A
- Phenotype B
- Phenotype C

Stratification based upon drug response

- Non-responder
- Responder

Determine drug response

Correlation of phenotypic differences or drug response at cell /target?

Benefits patients and results in lower costs

Target treatment to patient
Genetic Variation causes different sensitivity to pain

<table>
<thead>
<tr>
<th>Target</th>
<th>Channelopathy</th>
<th>Exploratory drug available</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN9A (NaV1.7)</td>
<td>Congenital Insensitivity to Pain(^8), Primary Erythromelalgia(^9), PEPD</td>
<td>yes</td>
</tr>
<tr>
<td>SCN10A (NaV1.8)</td>
<td>Increased sensitivity to pain</td>
<td>yes</td>
</tr>
<tr>
<td>TRPA1</td>
<td>Familial Episodic Pain Syndrome(^10)</td>
<td>yes</td>
</tr>
<tr>
<td>TPM8</td>
<td>Familial migraine</td>
<td>yes</td>
</tr>
<tr>
<td>KCNQ2/3</td>
<td>Benign Neonatal Convulsions(^12)</td>
<td>yes</td>
</tr>
<tr>
<td>P2X7</td>
<td>pain and neuro Inflammatory disorders</td>
<td>yes</td>
</tr>
</tbody>
</table>

Do all patients respond the same way to analgesics?
Many genetically-defined cell types needed

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Patient’s genetics influence whether drugs work at the drug target

- P2X7 channel is highly variant
- 29 SNPs
- Channel associated with multiple CNS disorders

348A, 496A 2.7% of population

348T, 496E 13.8% of population

In screening multiple genetically-defined cell types may be needed

Using iPSCs in safety testing

**Human cardiomyocytes**
Normal human cardiac muscle cells from stem cells
- Constant supply of human cardiomyocytes
- Channel proteins in cardiotoxicity
  - QT prolongation,
  - Conduction-arythmia

**Human liver cell**
Liver toxicity is very common with drugs
- Constant supply of human liver to test drugs in is not possible
- to develop the most predictive test that can be widely used and standardised
Patient’s genetics influence how well drugs work - through metabolism and immune reaction

- Polymorphisms in drug metabolising enzymes
  Cyp 2C9 and VKORC1 variants → Warfarin levels

- HLA-B 5701 → rare and potentially fatal hypersensitivity reaction to abacavir
  Screening for isoforms now required prior to administration

- Drugs metabolised by Cyp 2D6 and Cyp 2C19
  Avoided during discovery and development

For safety testing in liver and heart cells -

Many genetically-defined cell types needed
Cell therapy: retinal pigment epithelial cells to treat macular degeneration

Differentiate Embryonic Stem Cells into RPE

Seed RPE on coated polyester disc

Place matrix + cells behind neural retina under fovea (surgical procedure; ~ 45mins; local anesthesia)
Status of therapeutic product

- En face view of polyester membrane seeded with 100,000 RPE cells

Next iteration
- Combination of RPE and neural net. HLA matched or individually-made iPS cells
Selecting the best drug for an individual

For severe genetic conditions - e.g. Epilepsy, CF, cerebellar ataxias

Patient → IPSC → Differentiated neuron → Patient’s “Disease in a dish”

Genomics information → Phenotypic information → DRUG CHOICE

Determine optimal drug for modulating “disease” phenotype in that individual
Predicting the safety of a drug for an individual patient - organ specific toxicity for novel cancer immuno-therapies

- Collect somatic tissue from cancer patients preparing to undergo immunotherapy
- Establish patient somatic cell line
- Store patient sample
- In vitro cardiotoxicity & other organ specific toxicities
- Establish bank of lines for each patient enrolled in clinical study
- Introduction of reprogramming transcription factors
- Expansion & characterization

Patient specific biomarkers for inflammatory cytokine exposure

Predict adverse events

Patient’s T cells engineered with affinity enhanced TCR

Assay

In vitro cardiotoxicity & other organ specific toxicities

Patient specific biomarkers for inflammatory cytokine exposure

Predict adverse events
Summary

The impact of IMI projects using iPS cells for Industry:

• To develop methods and tools to help identify better drug target drugs.

• To provide better technologies to ensure they are safe

• To underpin precision medicine approaches

• To invent new diagnostics and ‘personalised’ therapy.