The impact of IMI project outcomes in Industry

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Overview

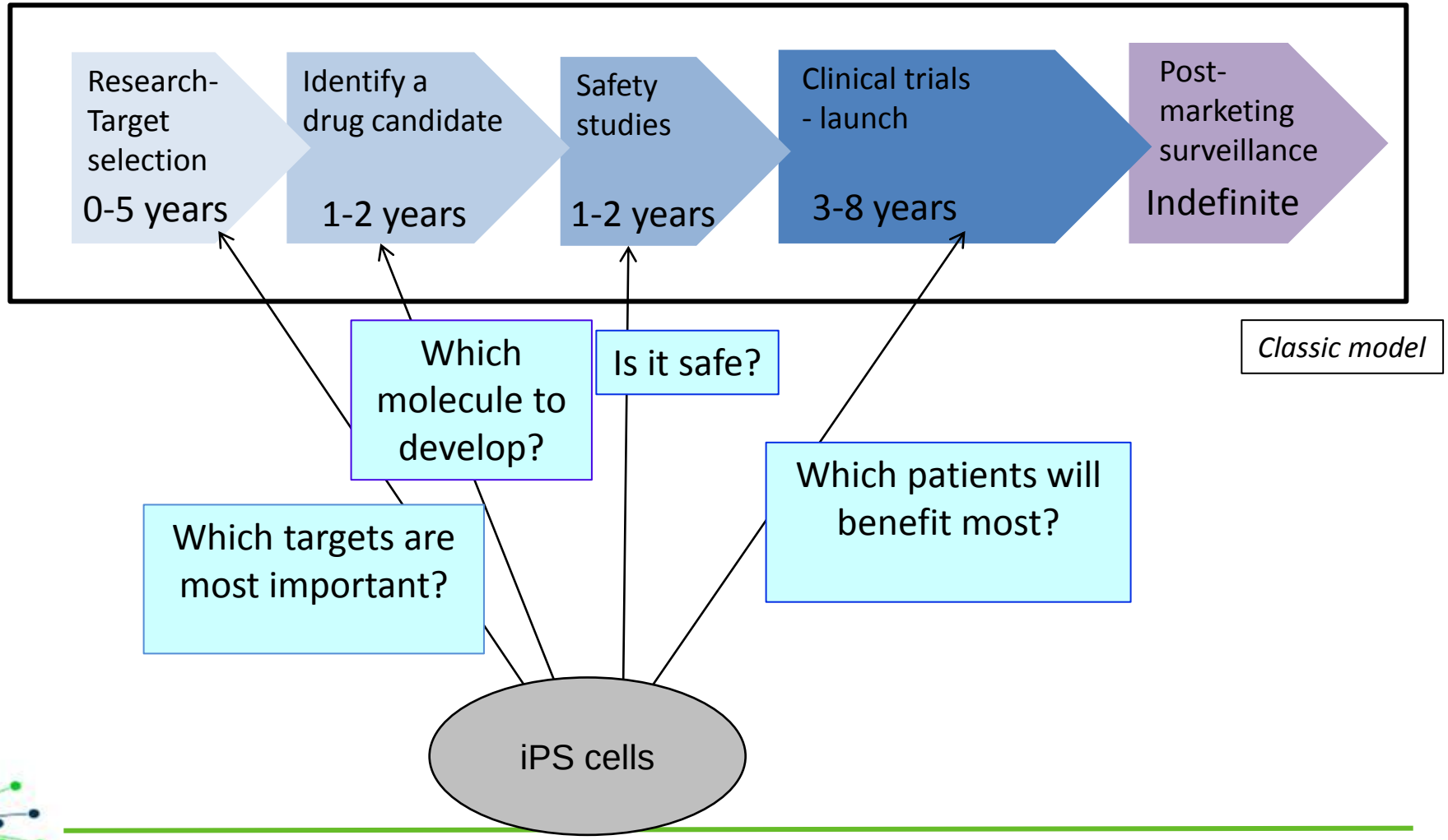
IMI initiatives using stem cells

– Present: enabling tools in classical drug discovery

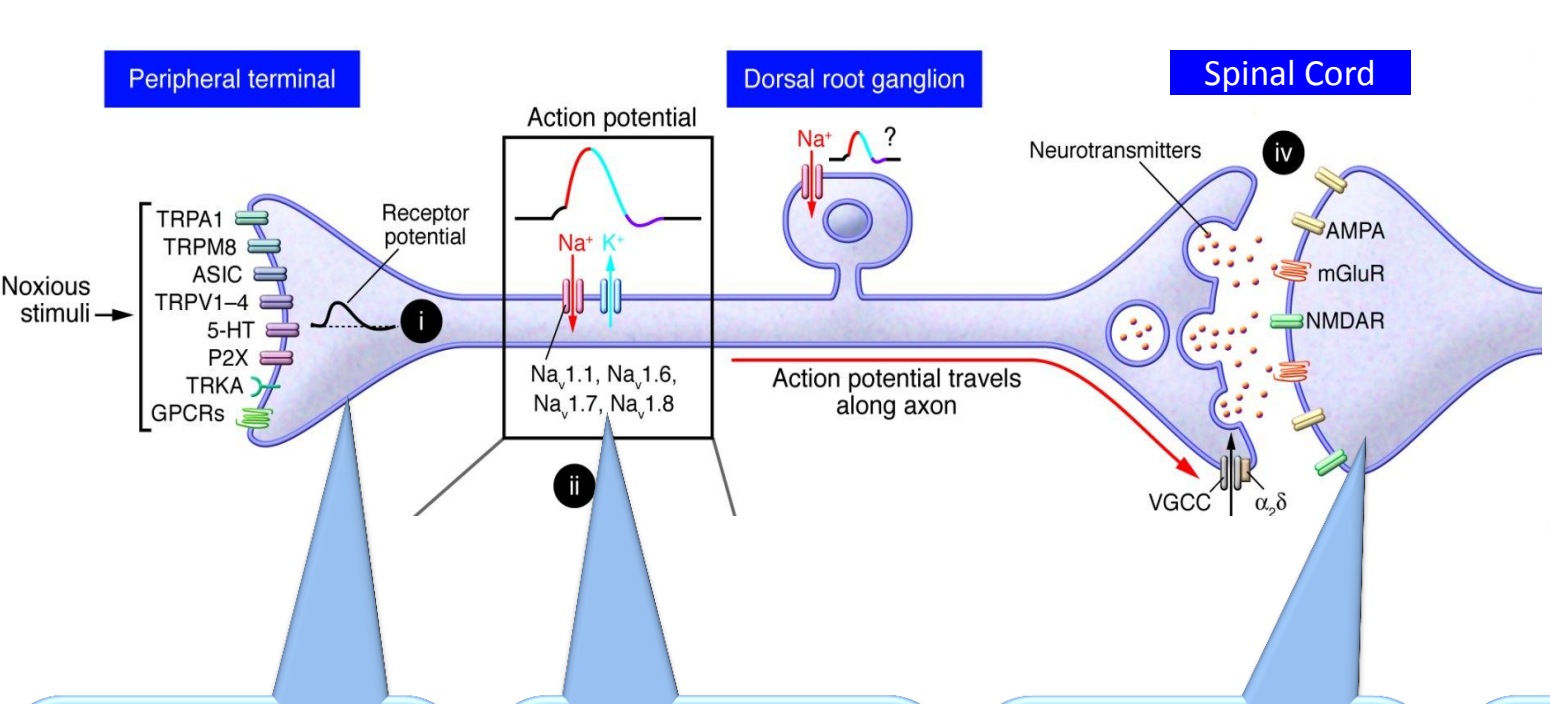
– Future : enabling precision medicine

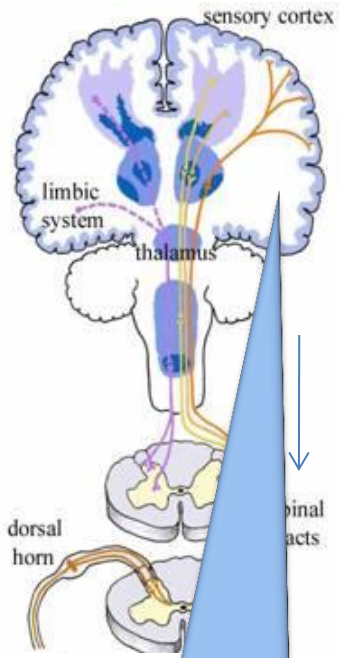
– Further future: Individualised therapy cells and diagnostics

iPS cells impact at all stages of drug development



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

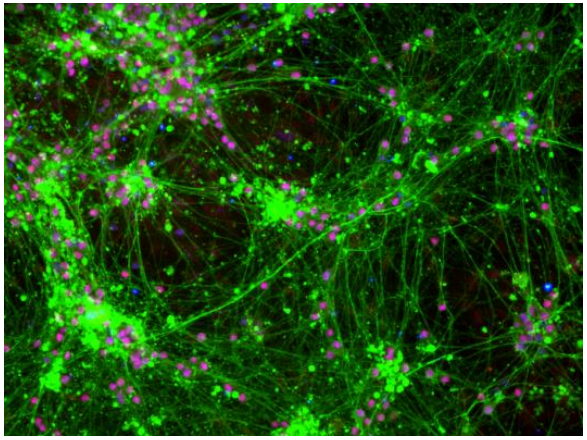
Using iPS cells in Target validation and screening for analgesics

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

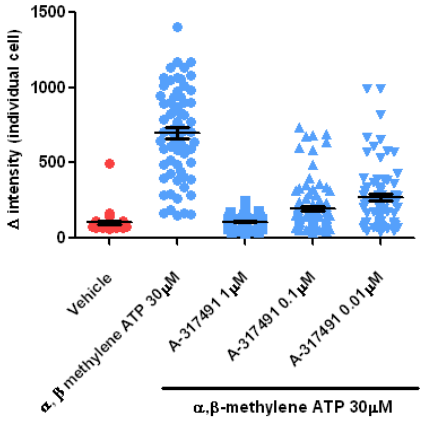
iPS cells make functional Sensory Neurones

Expression of sensory neurone markers

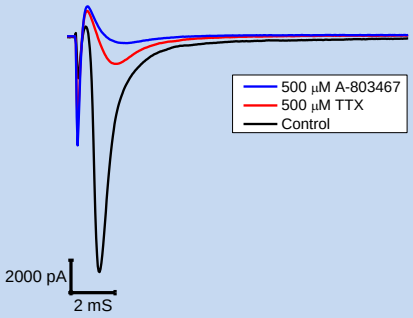
Peripherin / Brn3A / Islet-1

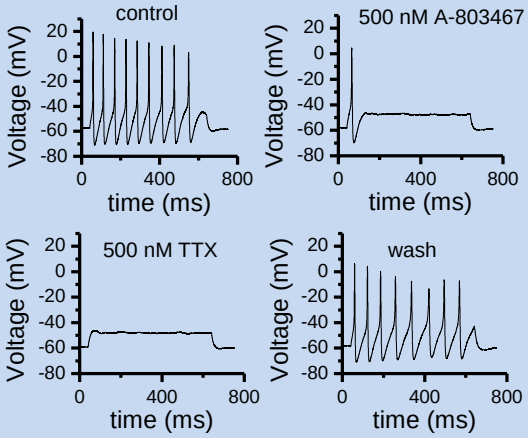


P2X3 - functional characterisation



NaV1.8 - functional characterisation



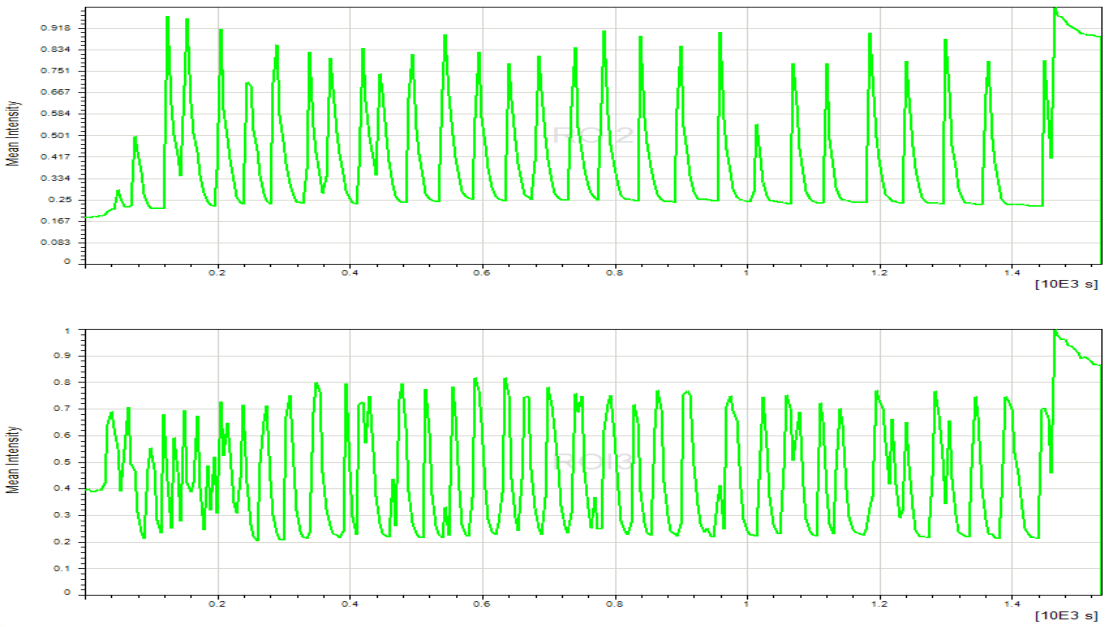


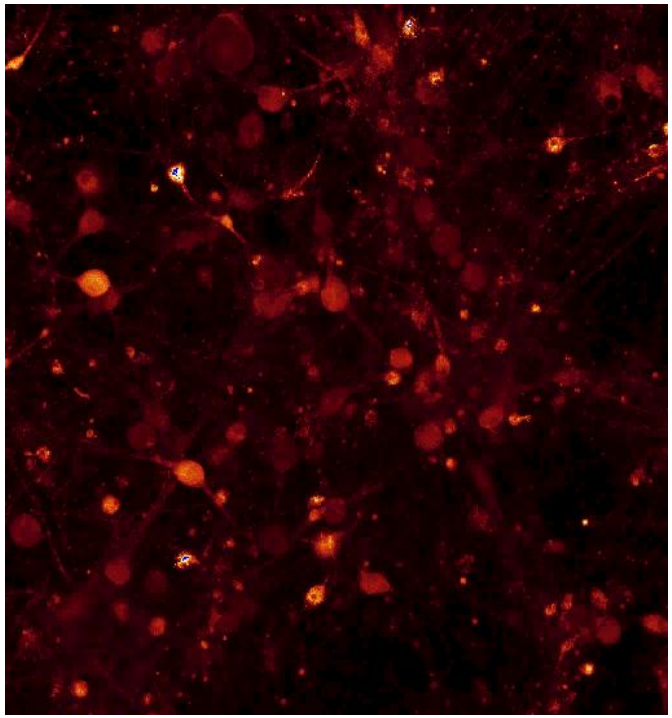
iPS cells recapitulate the majority of sensory neurone drug targets well

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

Higher throughput assays - Ca oscillations in sensory neurones

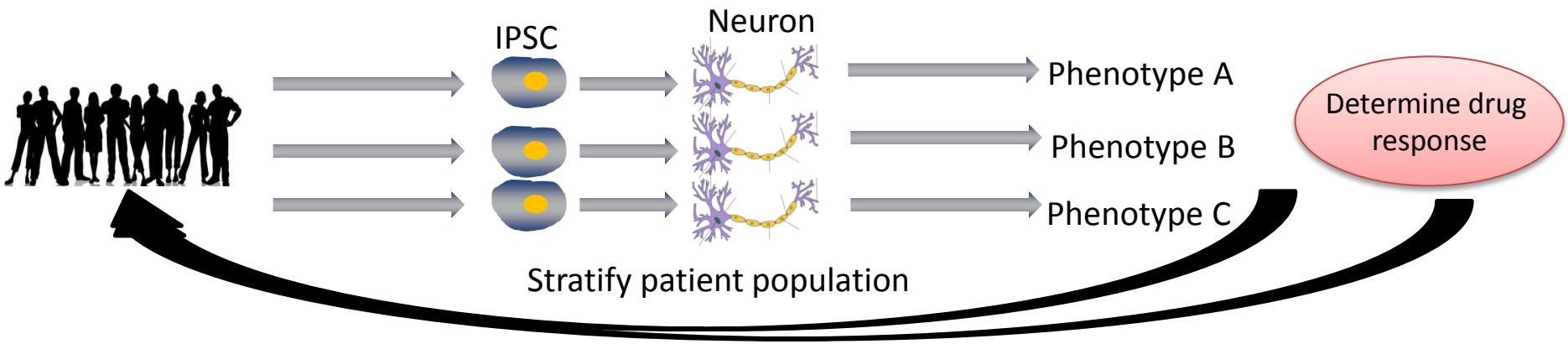
- Human iPS cells have very similar properties to normal neurones
- They form networks and respond synchronously





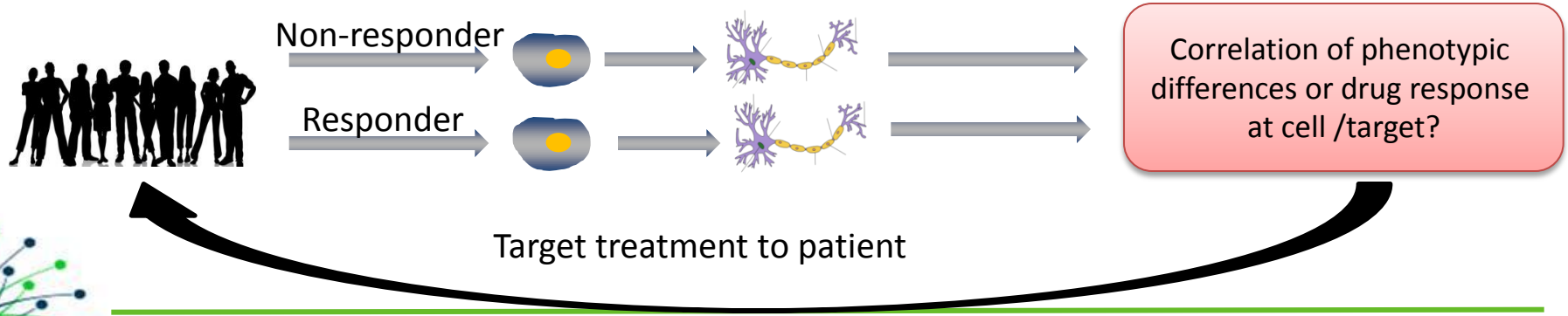
IPSC technology: potential as a future tool in precision medicine

Stratification based upon disease



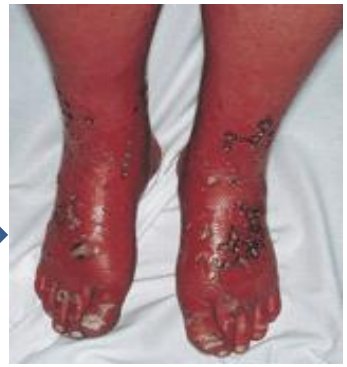
Benefits patients and results in lower costs

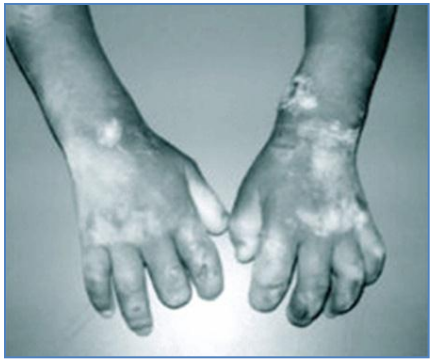
Stratification based upon drug response



Genetic Variation causes different sensitivity to pain

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



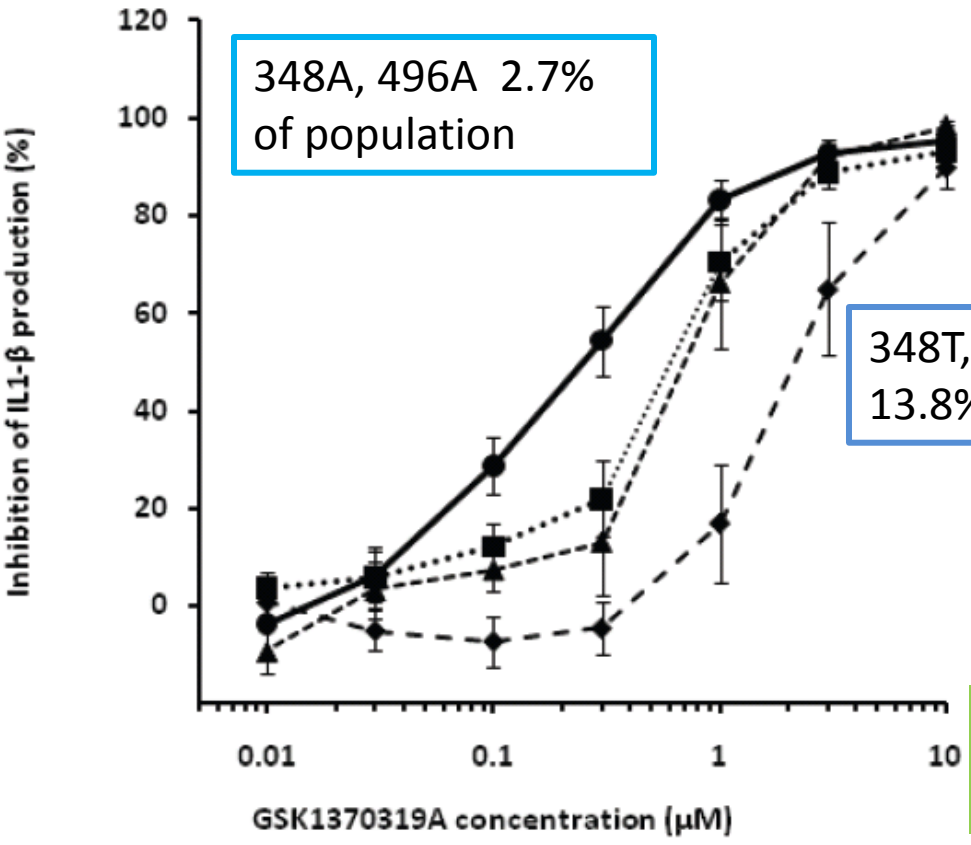


Do all patients respond the same way to analgesics?

Many genetically-defined cell types needed

[1]Ji et al 2008 Nature Genetics;[2] Winn et al 2005 Science; [7] Lin et al 2012 Am J Human Genetics; [8]Cox et al 2006 Nature 444(7121): 894-898; [9]Waxman 2007 Neurology (69(6): 505-507; [10]Kremeyer et al 2010 Neuron 66(5): 671-80; [11]LaFreniere et al 2010 Nat Med 16(10): 1157-60; [12]Singh et al 19989 Nat Genet 18(1): 25-29.

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



348A, 496A 2.7% of population

348T, 496E
13.8% of population

- P2X7channel is highly variant
- 29 SNPs
- Channel associated with multiple CNS disorders

In screening multiple genetically-defined cell types may be needed

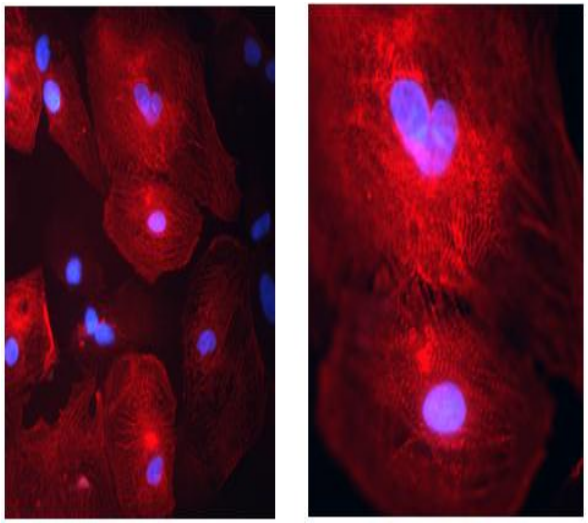
S MacHugh, S Roman, B Davis, A Koch, A Pickett, J Richardson, S Miller, S Wetten, C Cox, F Karpe, J Todd, E Bullmore. British Journal of Clinical Pharmacology (2012). Br J Clin Pharmacol 74:376-380.

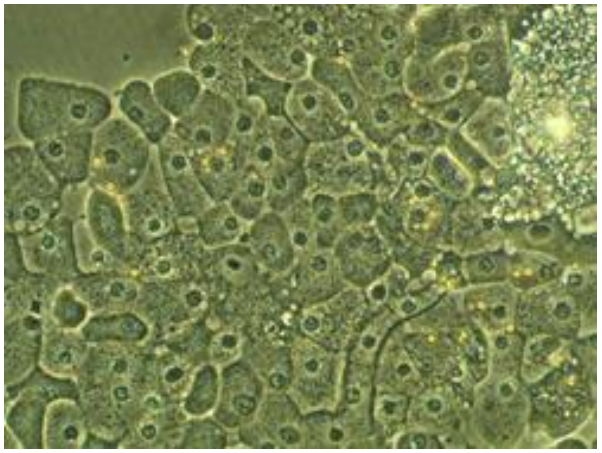
Using iPSCs in safety testing

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

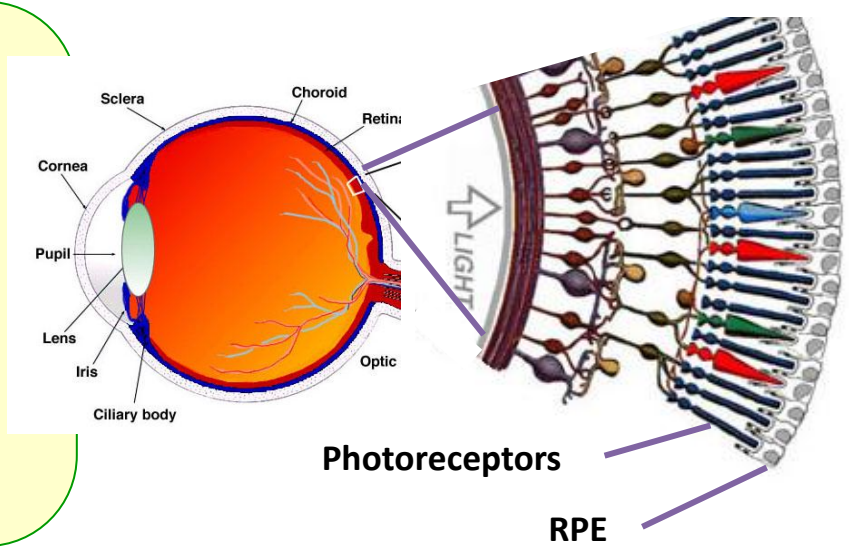
Differentiate Embryonic Stem Cells into RPE

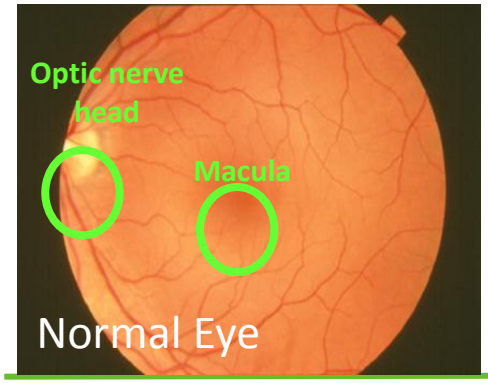
⬇

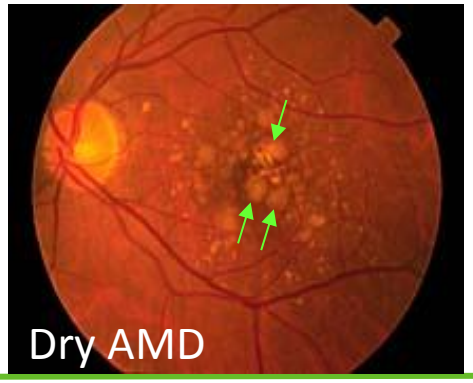
Seed RPE on coated polyester disc

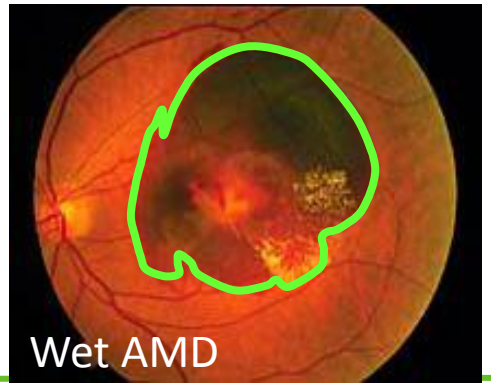
⬇

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

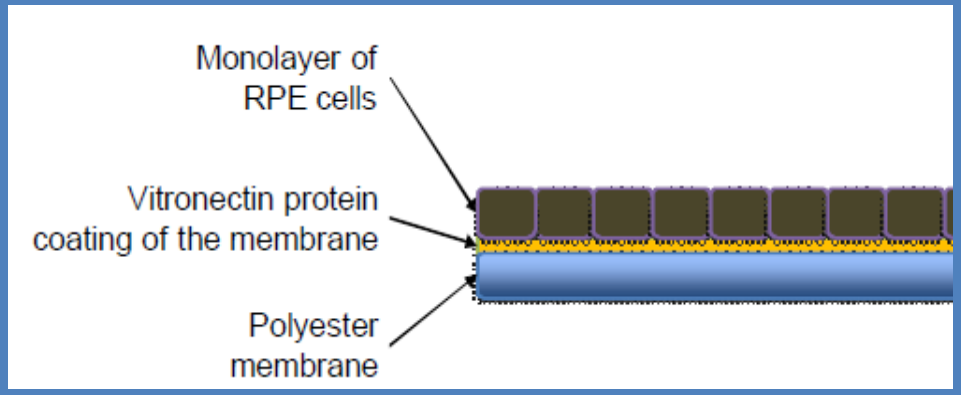


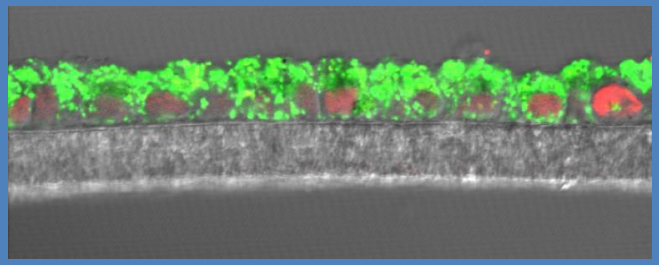






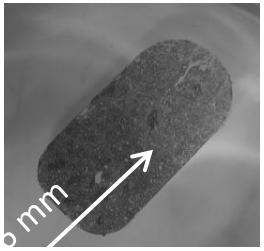
Status of therapeutic product





PAX6+PMEL17

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



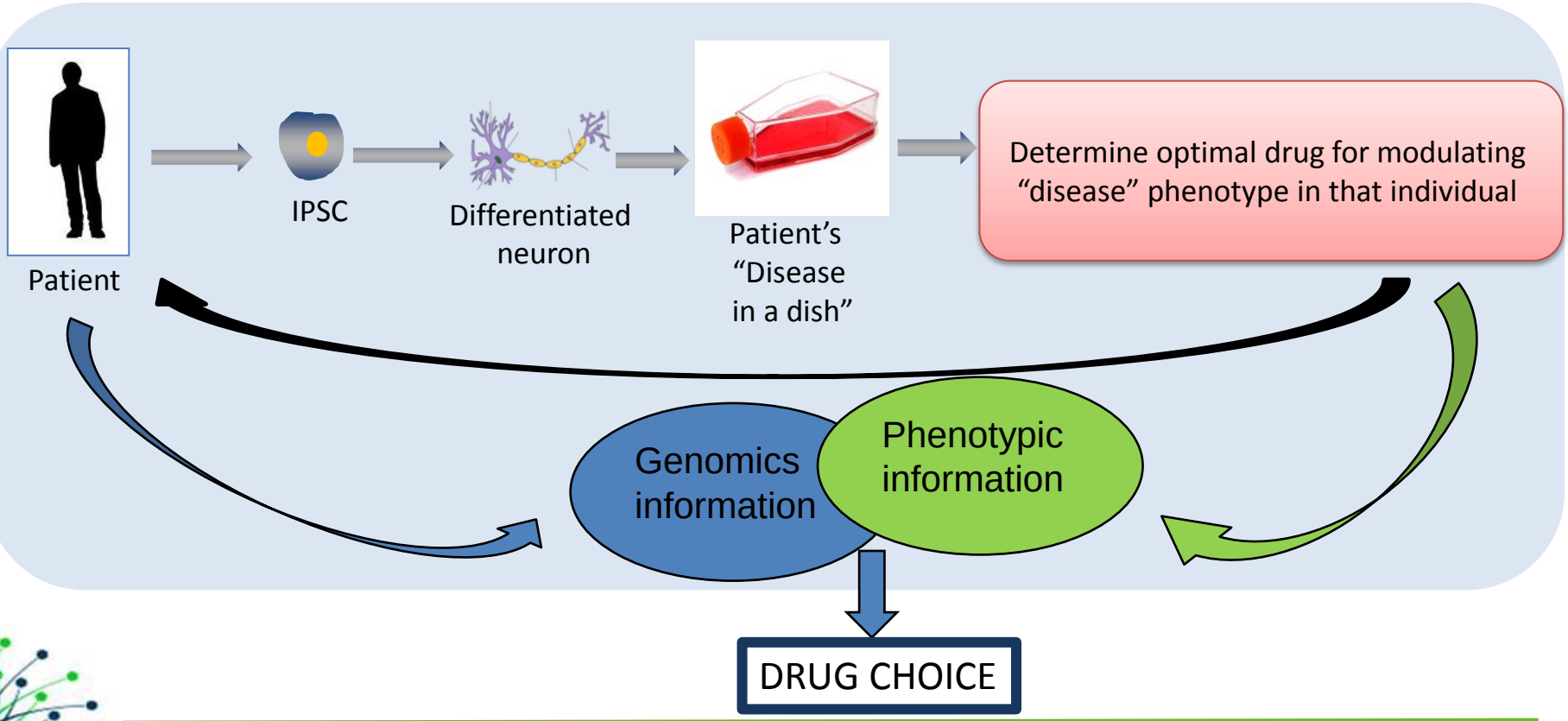
Bespoke device for insertion behind retina

Next iteration

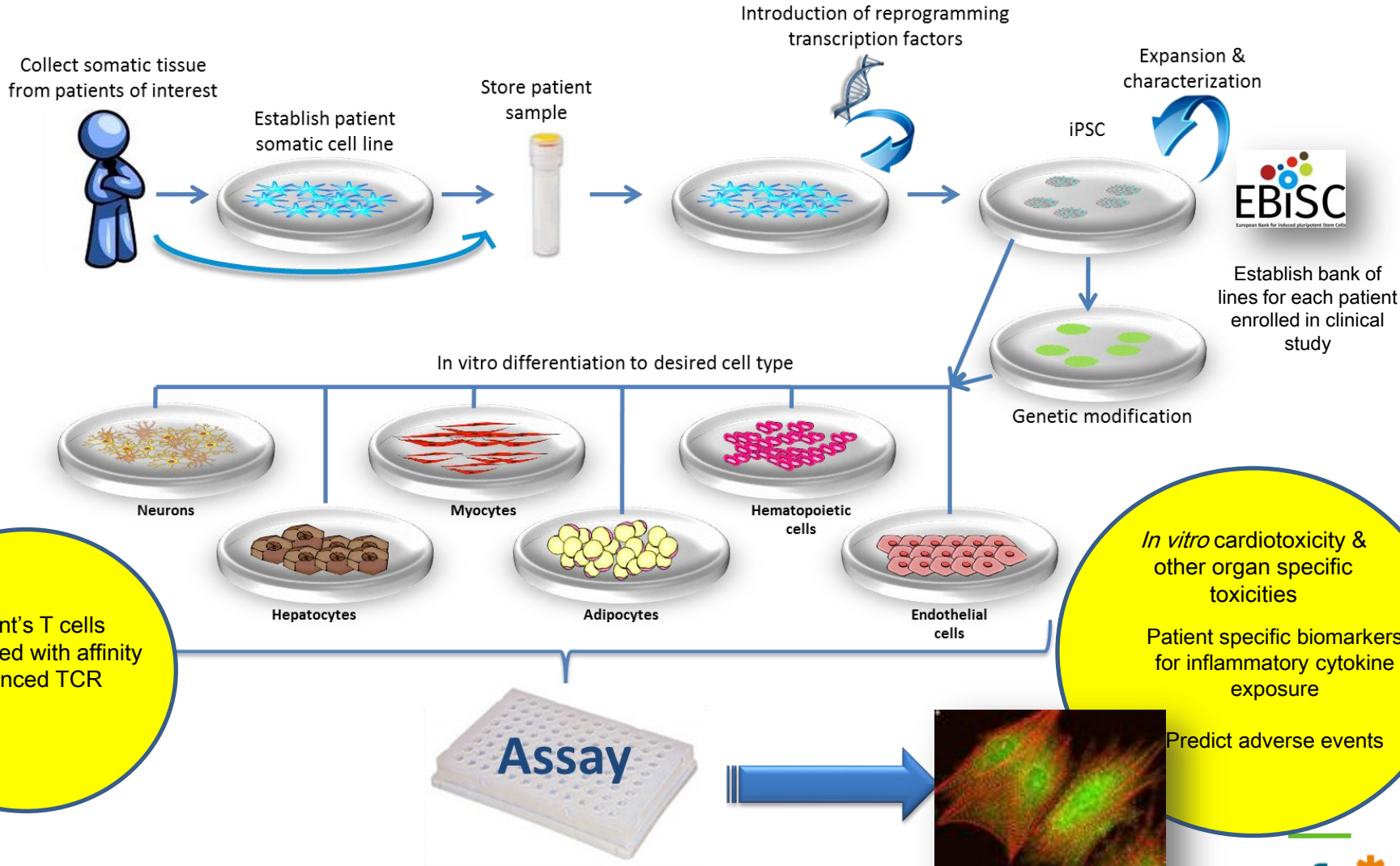
- Combination of RPE and neural net. HLA matched or individually-made iPS cells

Selecting the best drug for an individual

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



Predicting the safety of a drug for an individual patient - organ specific toxicity for novel cancer immuno-therapies



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.