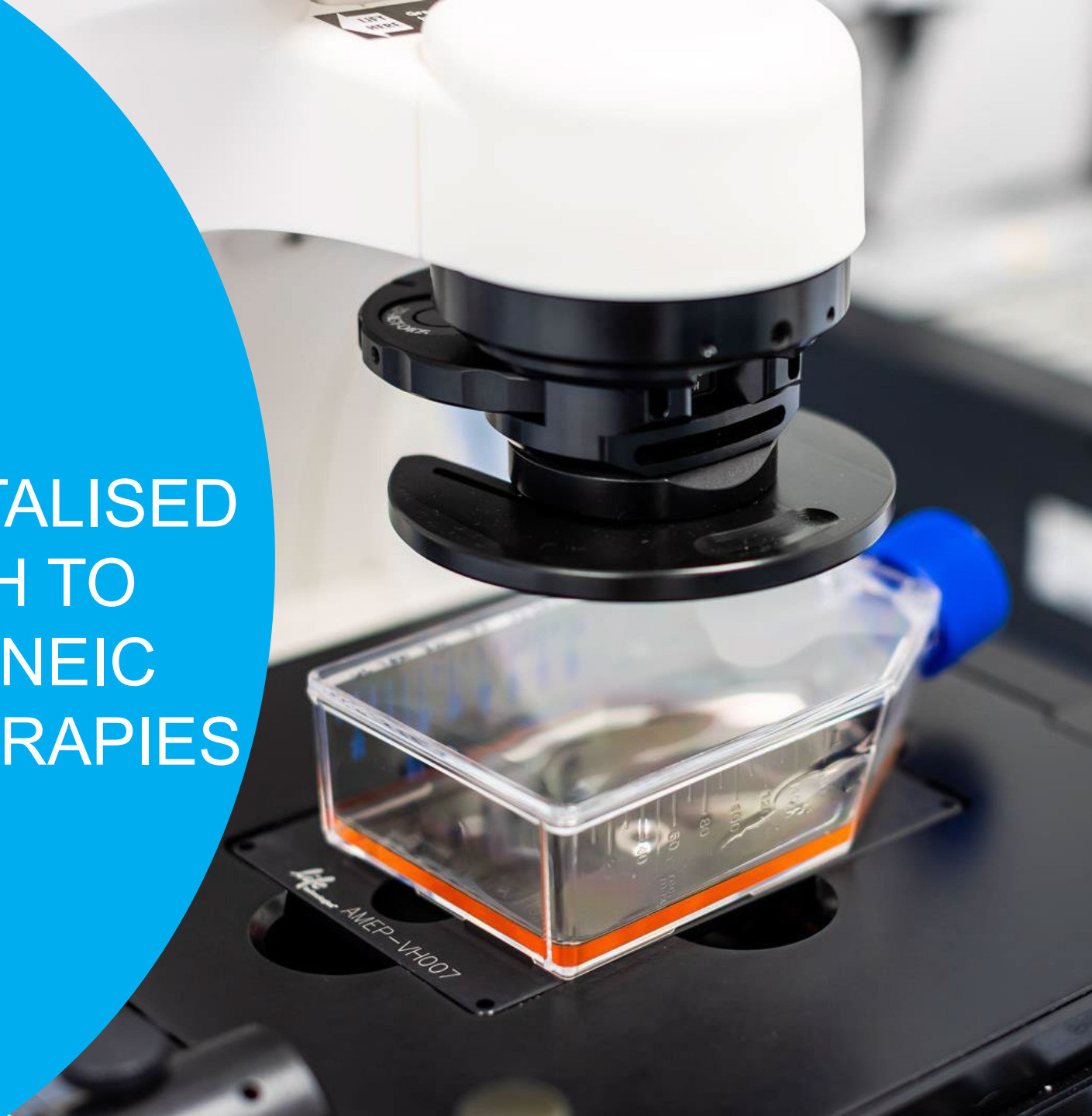


ReNeuron

CONDITIONALLY-IMMORTALISED IPSCS: A NEW APPROACH TO OFF-THE-SHELF ALLOGENEIC IPSC-DERIVED CELL THERAPIES

Steve Pells

Principal Investigator, Molecular Biology Group



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CELL THERAPY STRATEGY: ALLOGENEIC V. AUTOLOGOUS?

- Immunosuppression
- Off-the-shelf banks
- Amortise costs over treatments
- GMP source material
- Comprehensive QC
- Purification, QC, banks → consistency
- Scalability

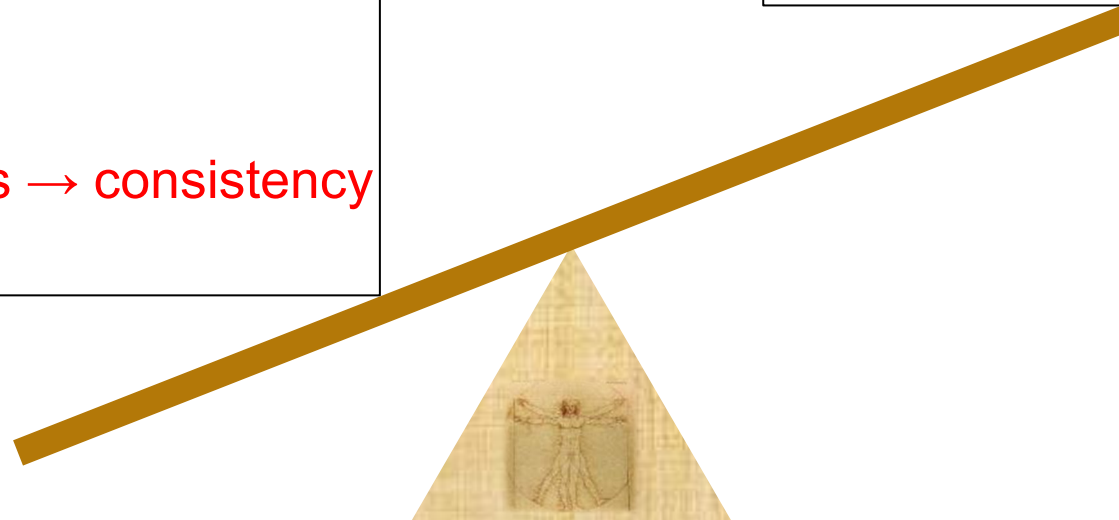
- Immune tolerance
- Lead time : Reprogramming, diffⁿ, QC
- Clinical grade iPSC lines expensive
- Inconsistent source material
- QC time, cost
- Patient : patient variability
- Bespoke “cottage industry”



CELL THERAPY STRATEGY: ALLOGENEIC V. AUTOLOGOUS?

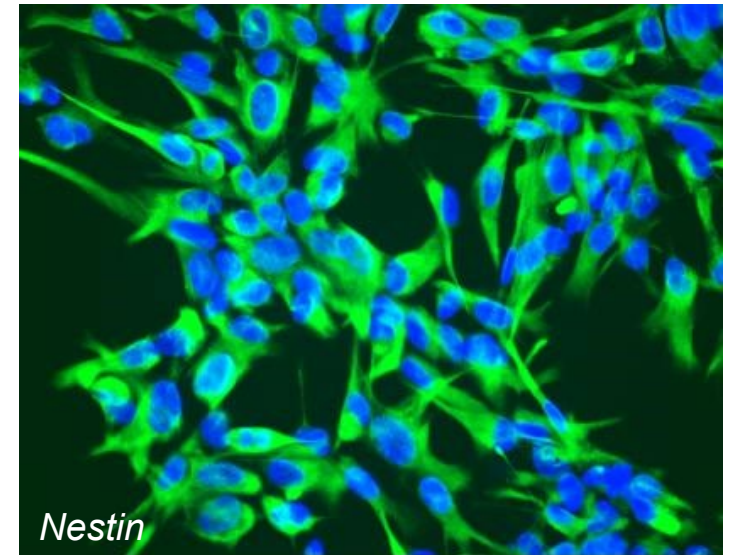
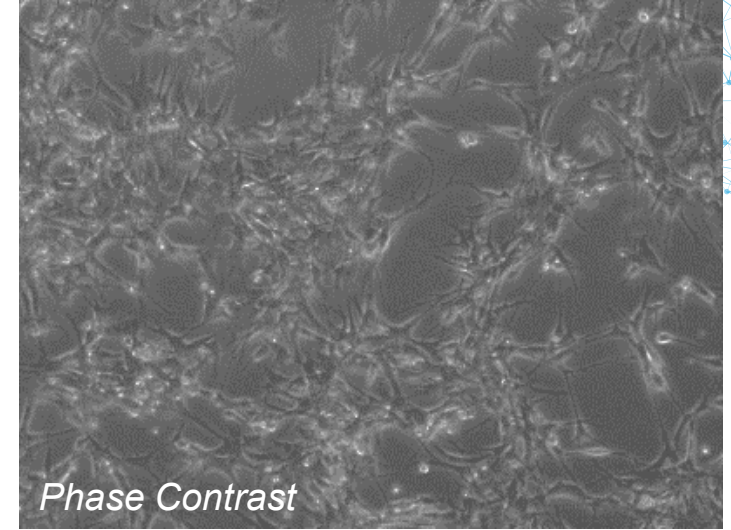
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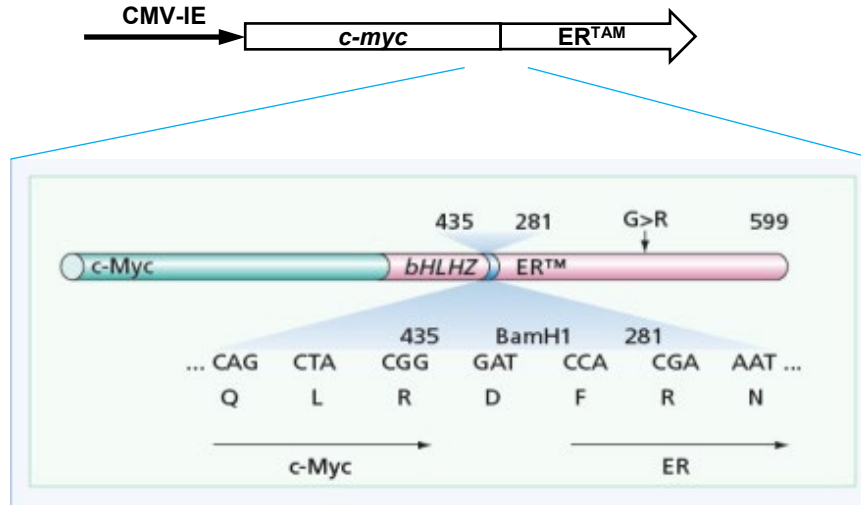


THE CTX HUMAN NEURAL STEM CELL LINE

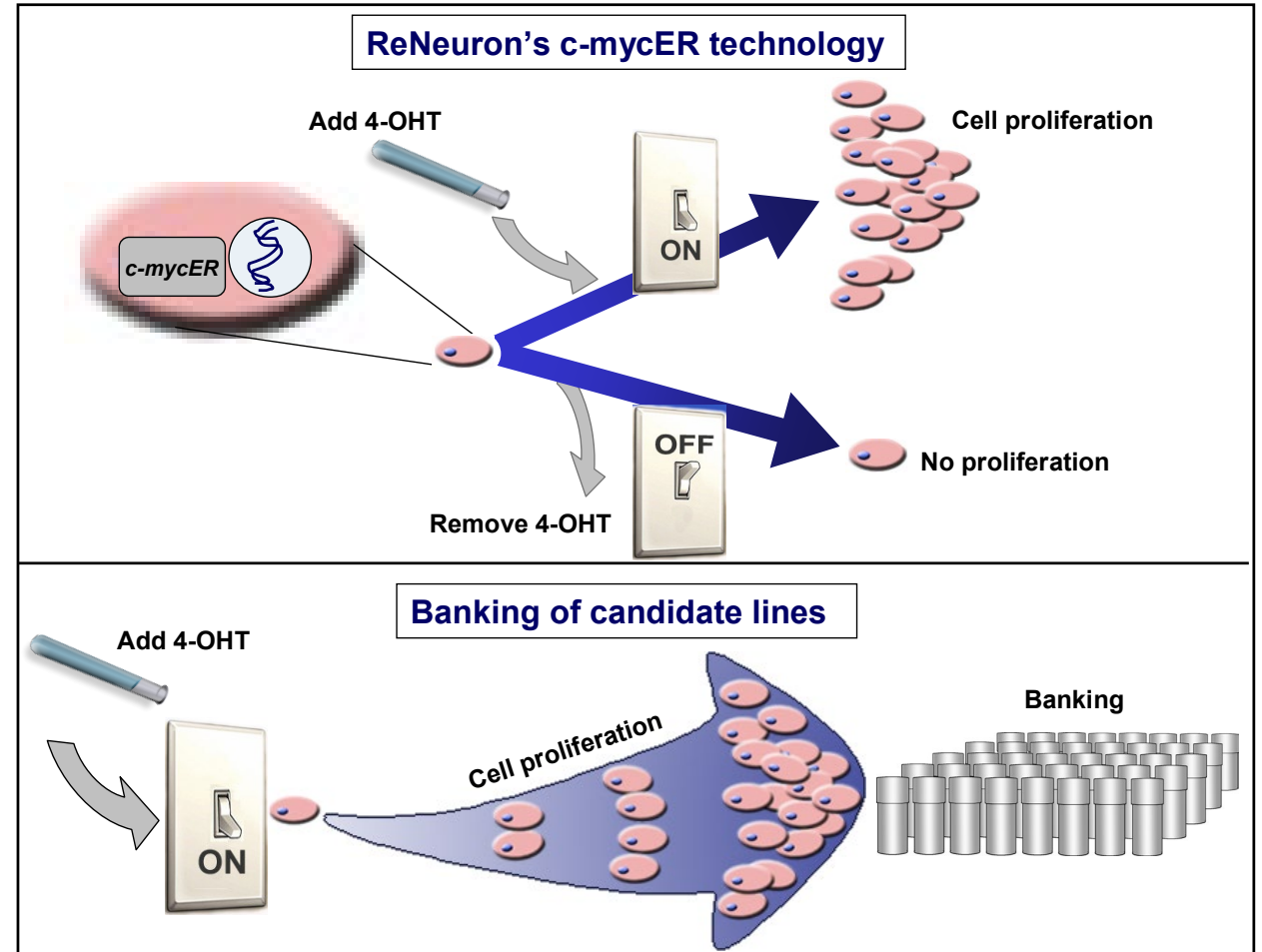
- Clonal cell line derived from human cortical tissue
- Multipotent neural progenitor with stable phenotype
- Conditionally-immortalised with *c-myc-ER^{TAM}*
- FDA, EMA, MHRA approved
- Allogeneic cell therapy – stroke, limb ischemia
- Fully qualified, xeno-free GMP cell manufacture process with stringent batch release criteria
- Scalable cell manufacture and cryo-banking



CONDITIONAL IMMORTALISATION



- *c-myc*-ER^{TAM} fusion protein
- ER^{TAM} moiety binds 4-OHT, not estrogen or tamoxifen
- Fusion protein inactive in cytoplasm
- Upon 4-OHT binding, translocates to the nucleus
- *c-myc* moiety promotes indefinite cell cycling:
CONDITIONAL IMMORTALISATION



* 4-OHT = 4-hydroxytamoxifen, a metabolite of tamoxifen

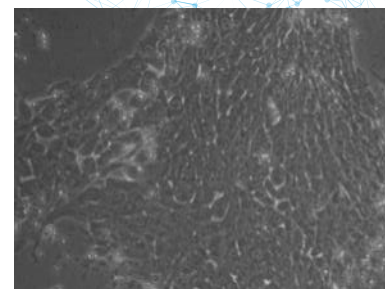
ANY CONDITIONALLY IMMORTALISED STEM CELL YOU LIKE?

- **Low-passage primary cells**

- Euploid
- Cycling

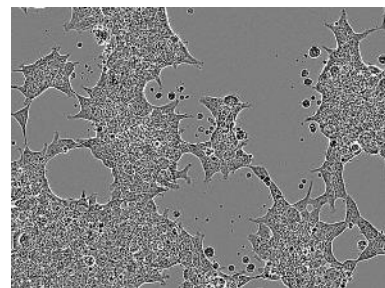


O K S M



- **Immortal cells**

- Aneuploid?
- 293FT (SV40LT)
- Lymphoblastoid



O K S M

[SV40LT
LIN28
p53sh]

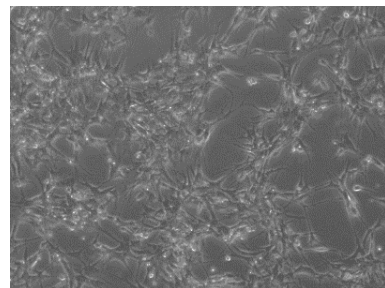


[MEK / ERK
GSK3
SMAD2—[Ⓟ]
EMT]

?

- **Adult stem cells**

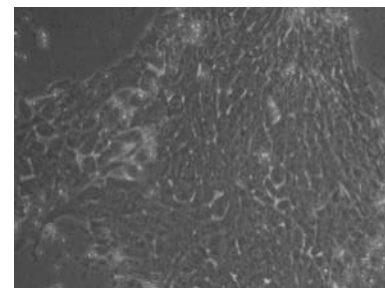
- Transcription factor expression
- Epigenome



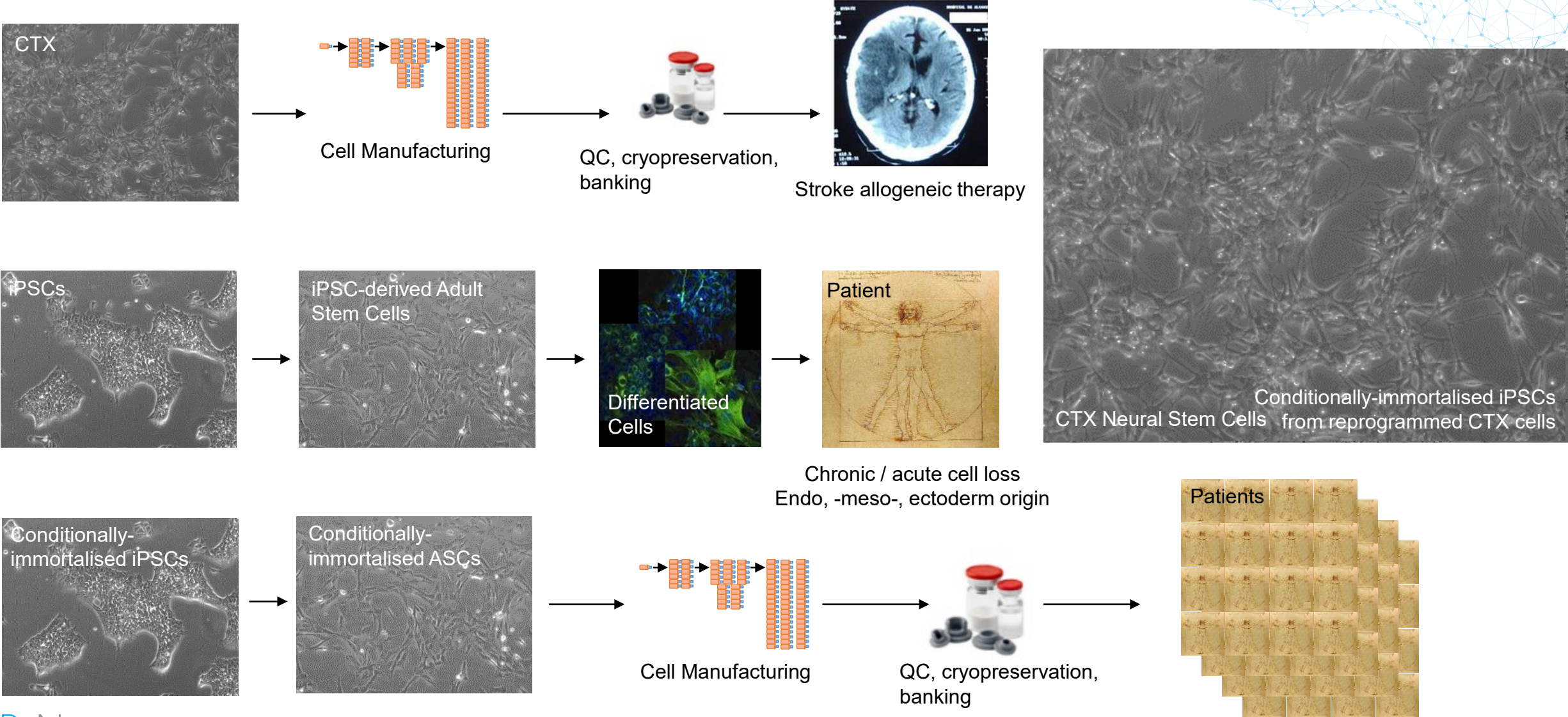
O [K]



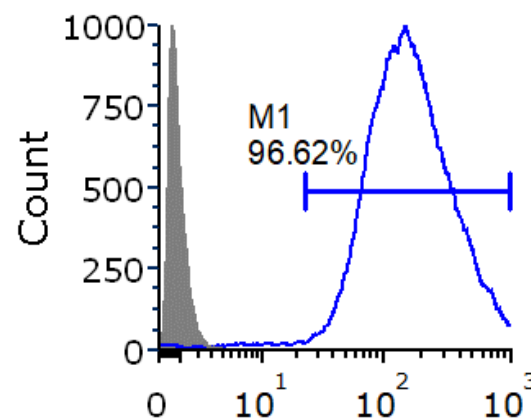
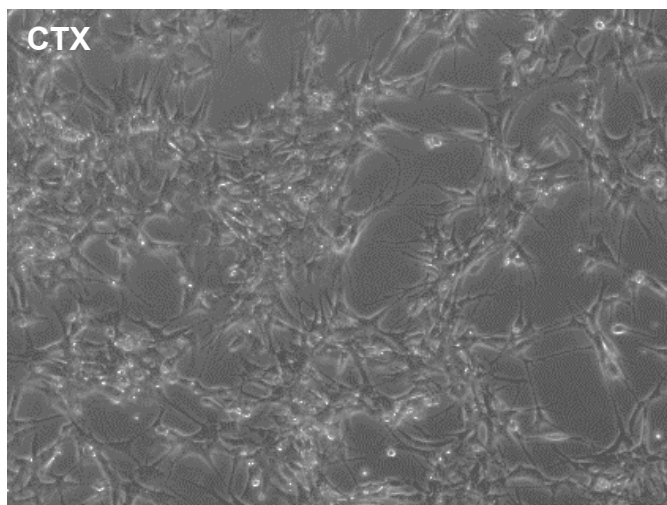
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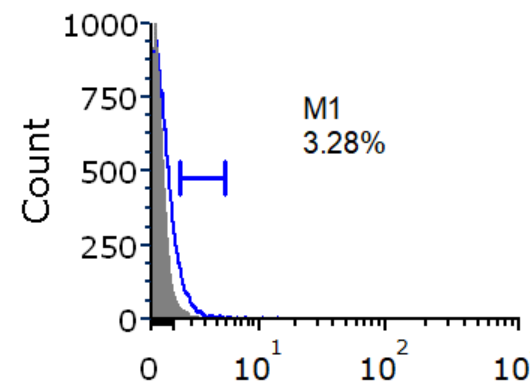
CONDITIONALLY IMMORTALISED IPSCS: A NEW CELL THERAPY PARADIGM



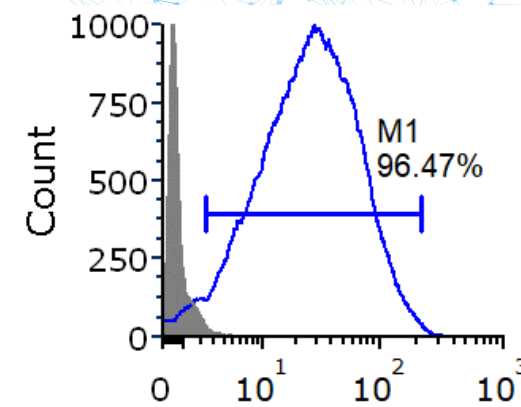
REPROGRAMMED CTX CELLS DISPLAY HPSC PHENOTYPE



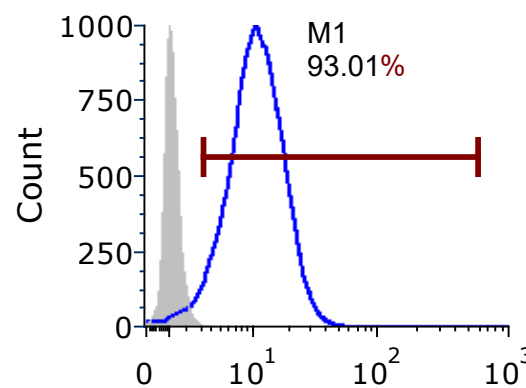
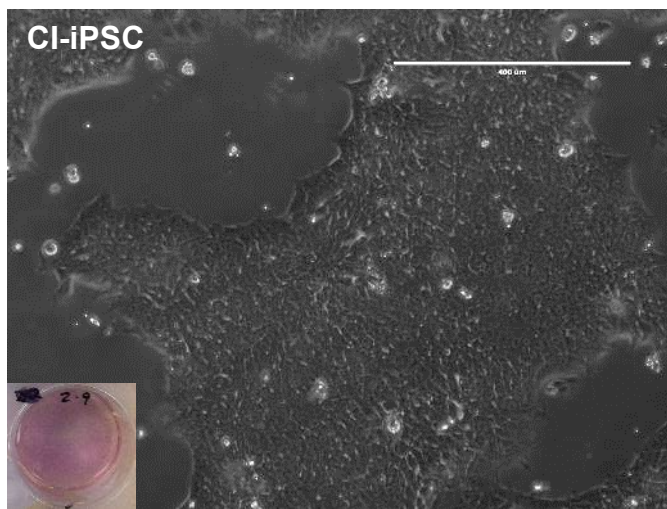
SSEA4



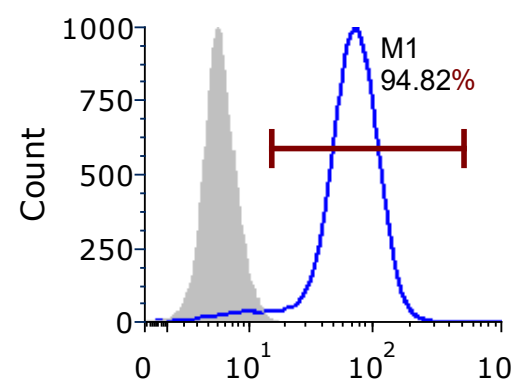
SSEA1



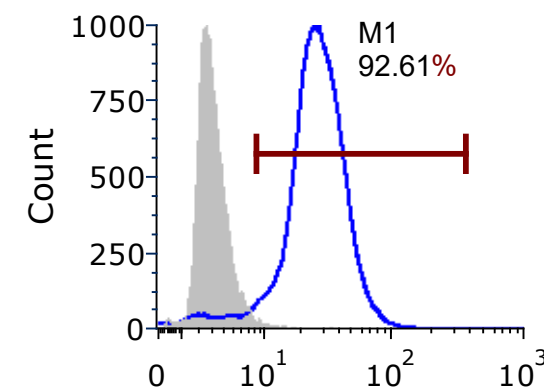
TRA-1-60



NANOG

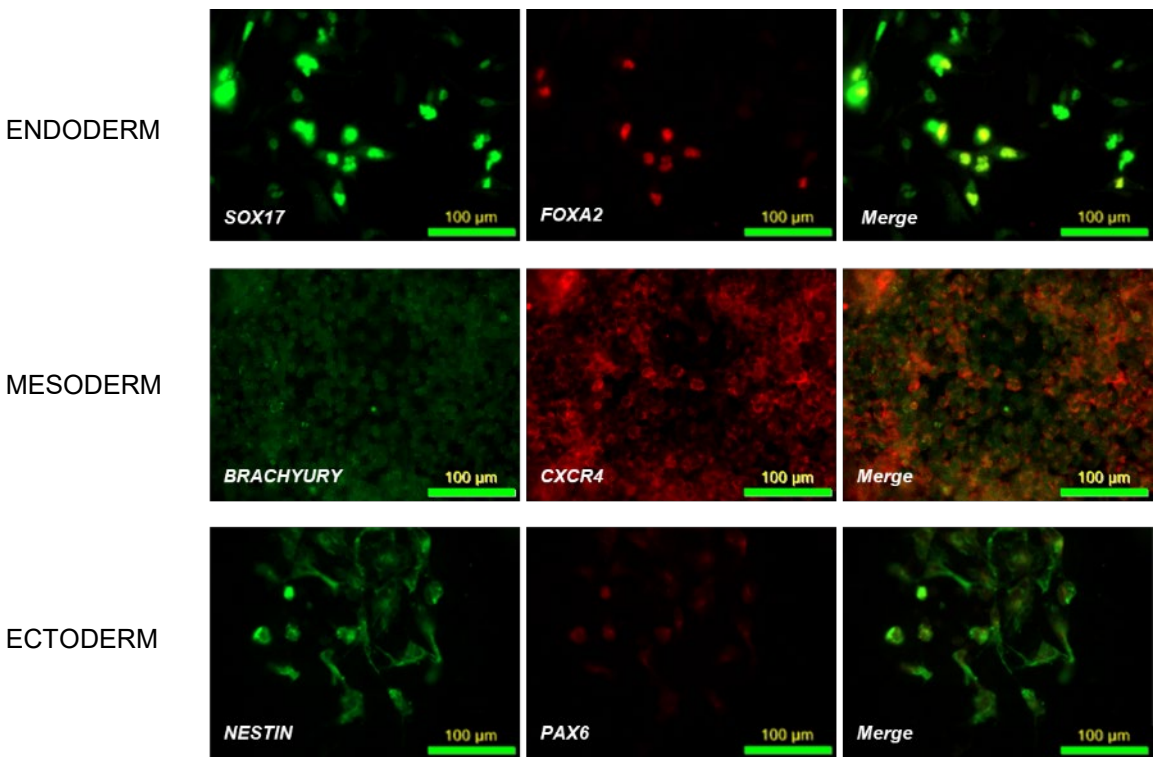
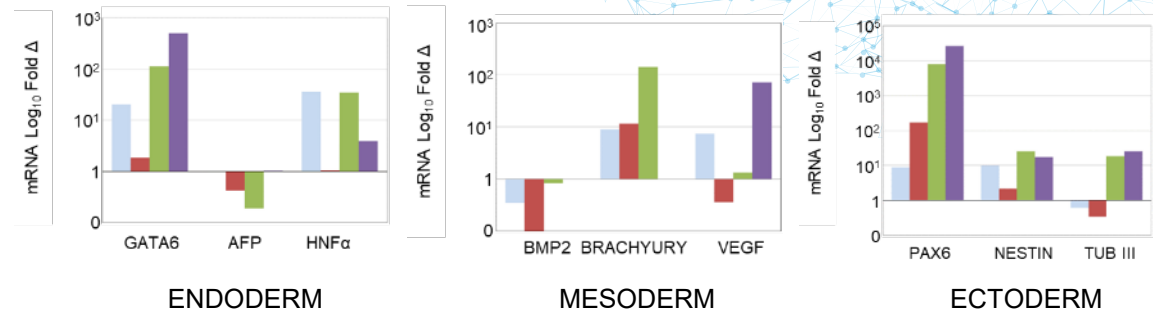
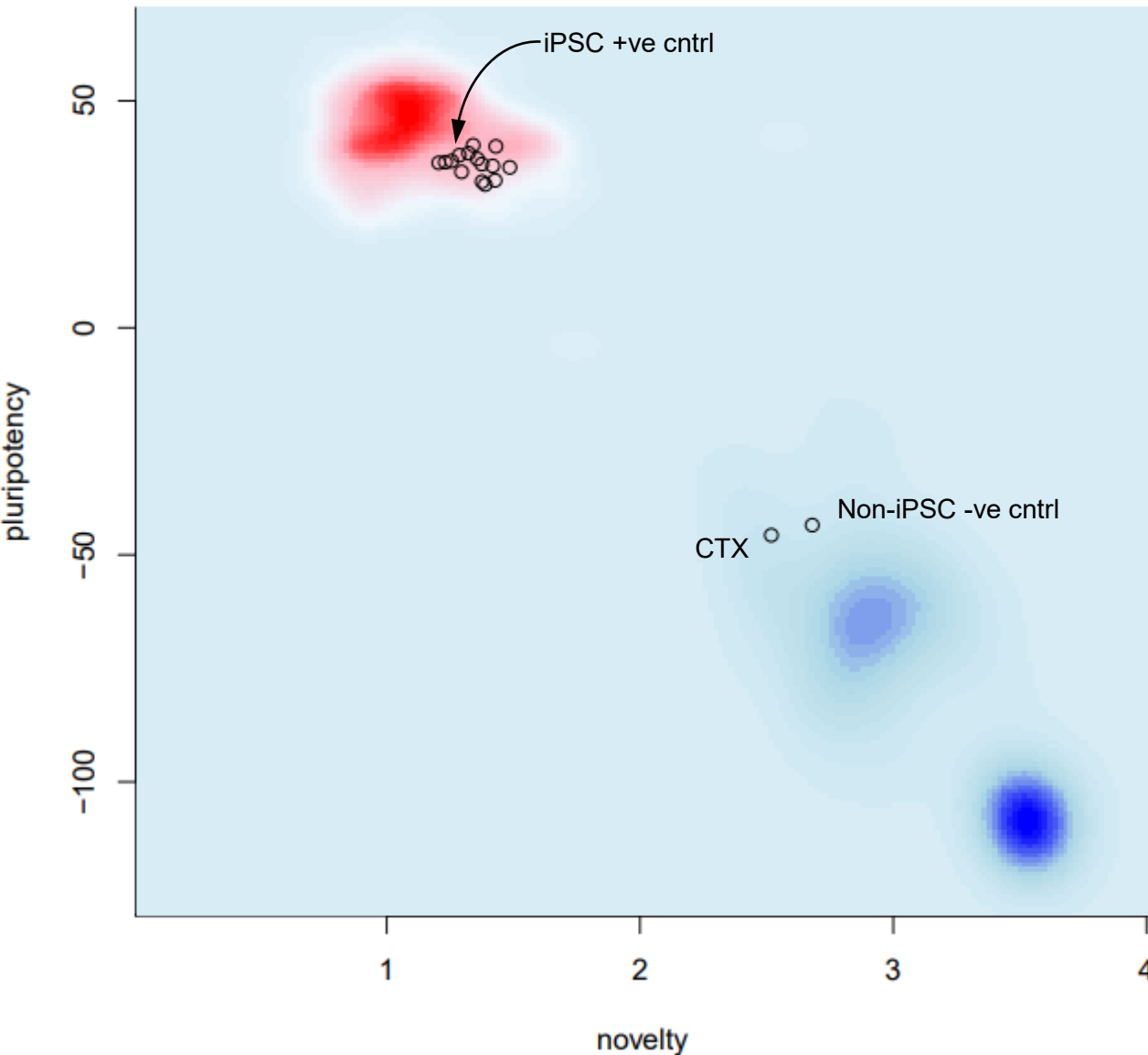


SOX2

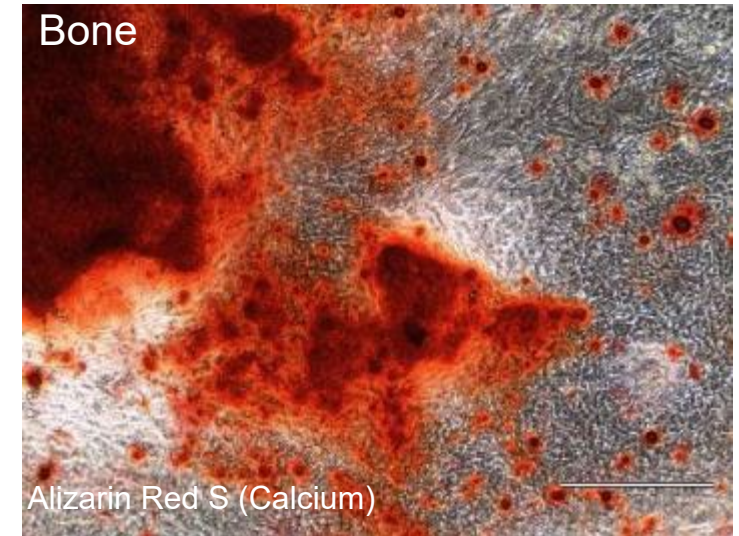
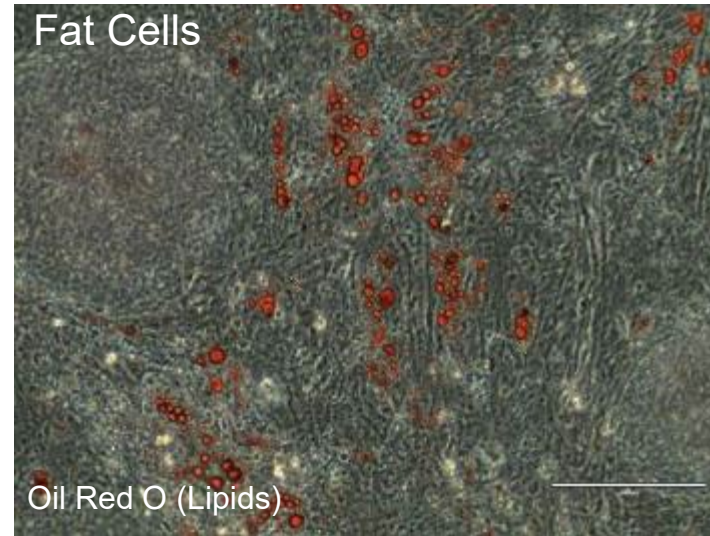
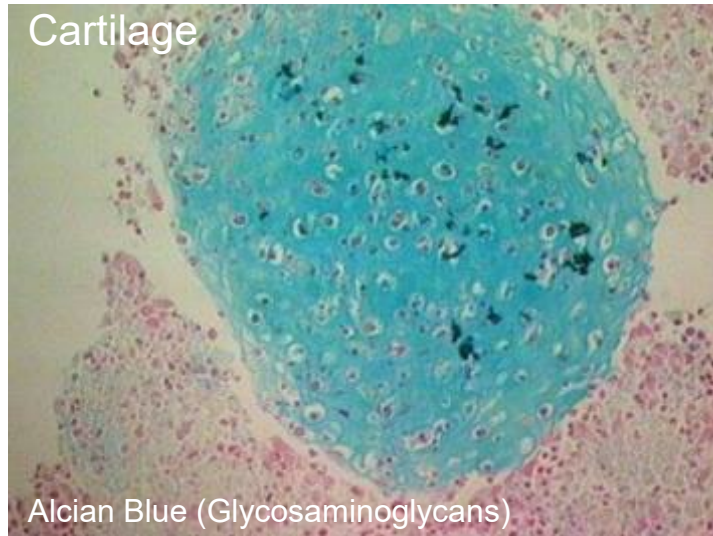
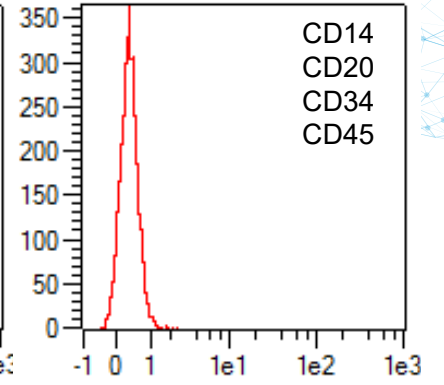
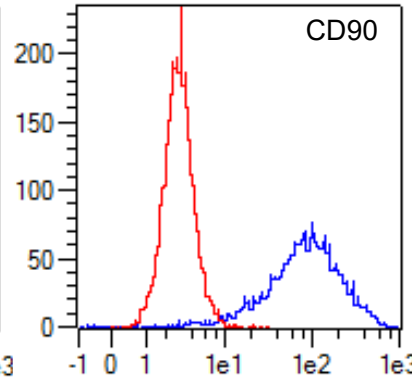
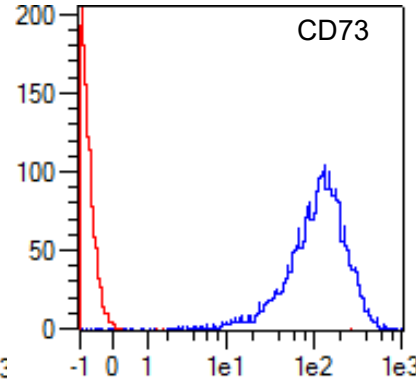
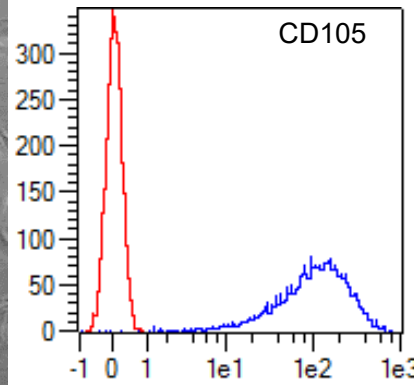
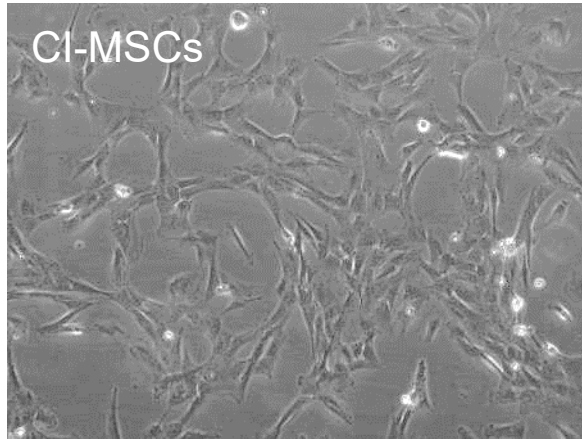


OCT4

CONDITIONALLY IMMORTALISED IPSCS ARE PLURIPOTENT

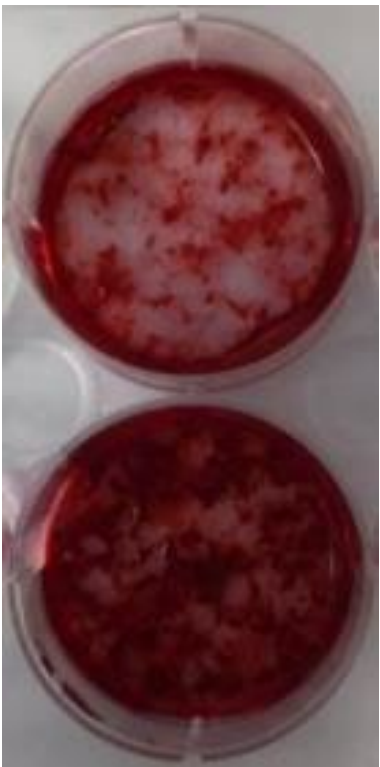
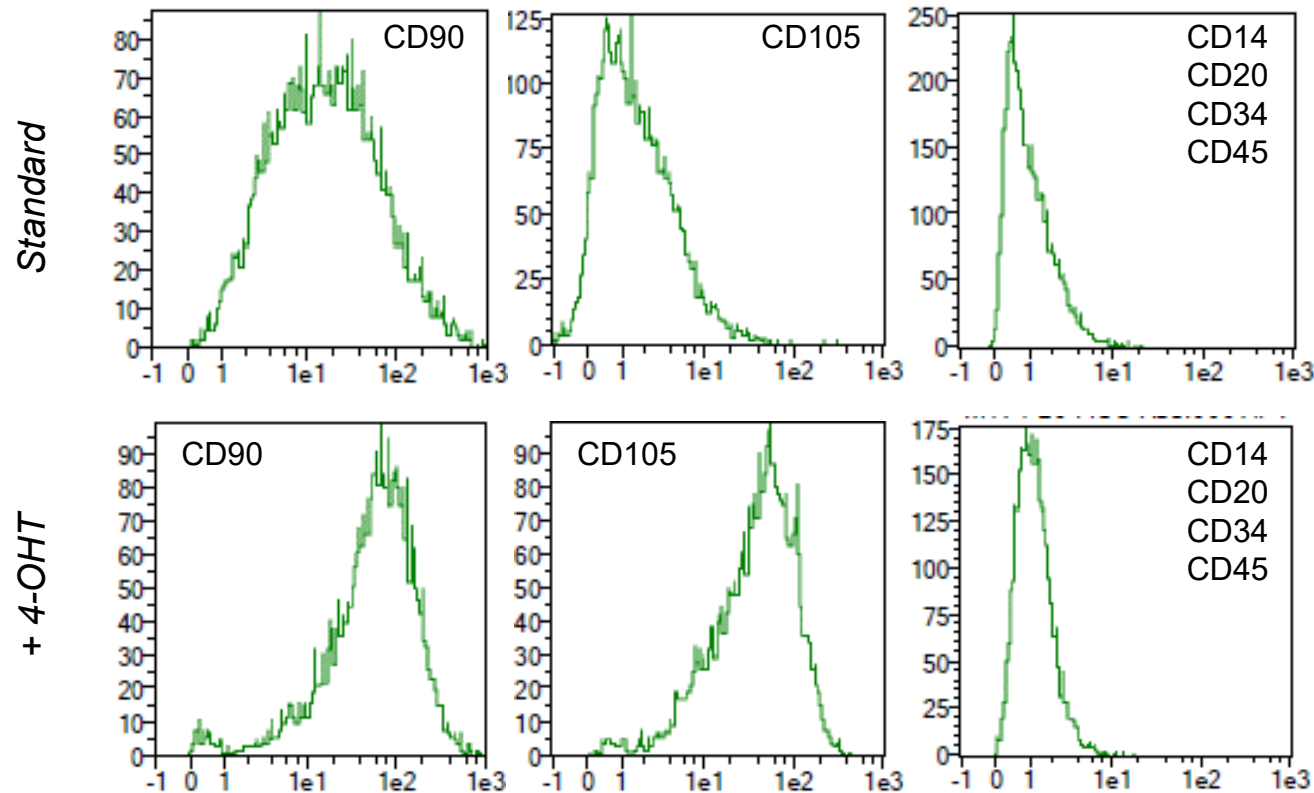


APPLICATION CASE 1: MESENCHYMAL STEM CELLS



CONDITIONAL IMMORTALISATION SUPPORTS PHENOTYPE MAINTENANCE IN ADULT STEM CELLS

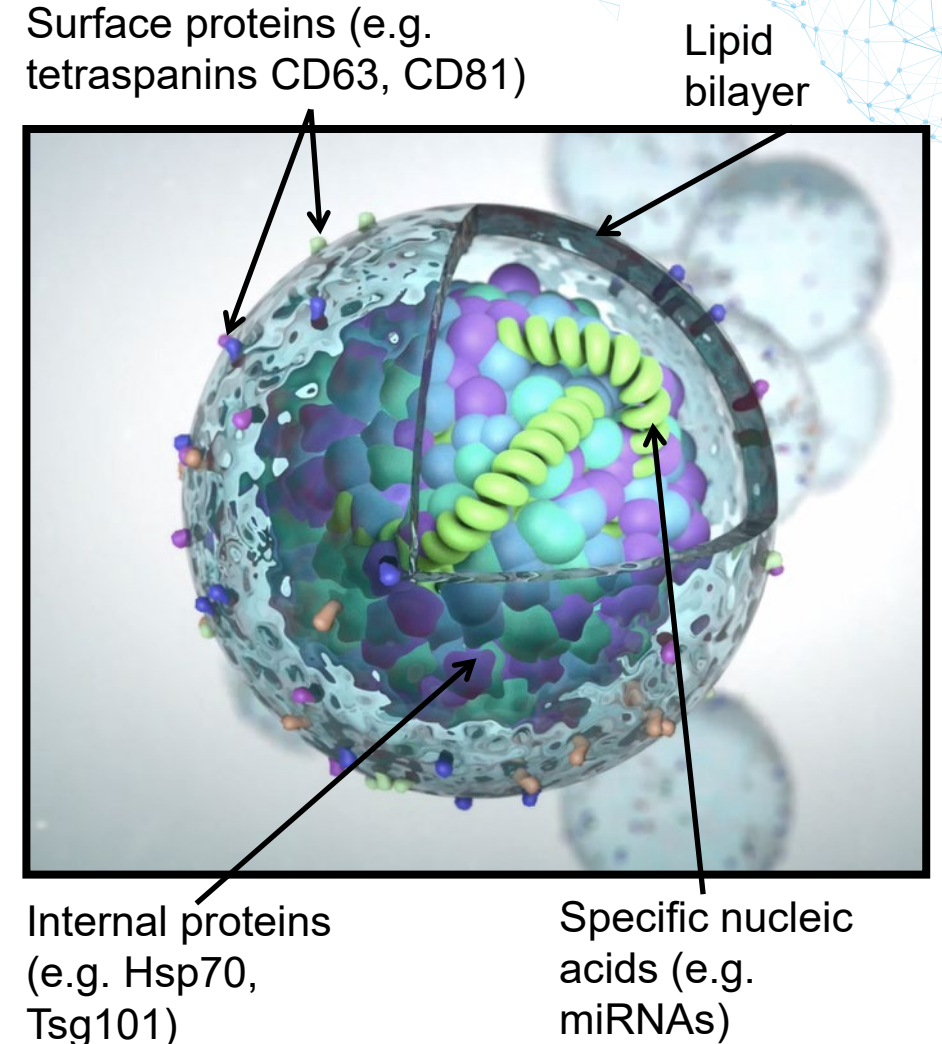
High Passage (P20) CI-MSCs



Osteogenesis

EXOSOMES – AN ALTERNATIVE ATMP MODALITY

- Naturally-occurring nanoparticles ($\varnothing \approx 100\text{nm}$) released by all mammalian cells as part of an intercellular communication system
- Contain and transport bio-active lipids, proteins and nucleic acids; payloads can be artificially modified
- Surface proteins target exosomes to recipient cells
- Tropisms vary between exosomes of different types; may be further modified e.g., biochemically or by gene editing of producer cells
- Biopharma interest in exosomes both as biomarkers and as native or engineered biotherapeutics
- An option as a cell-free ATMP modality



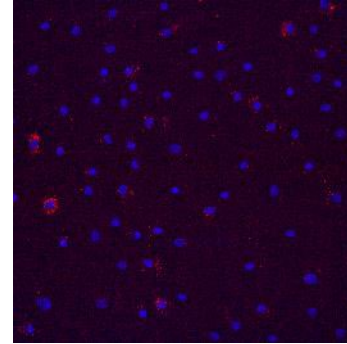
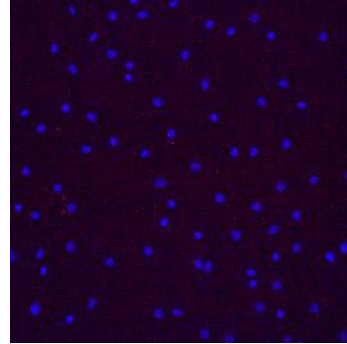
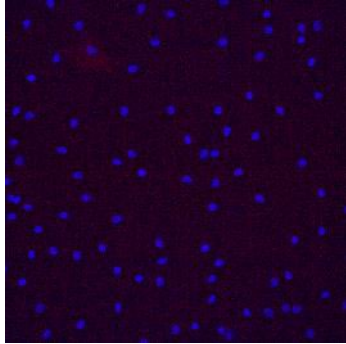
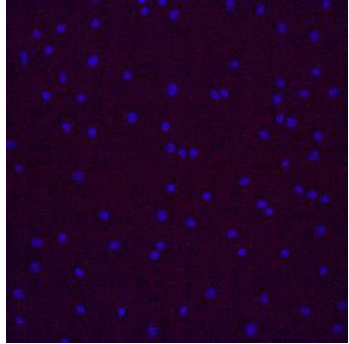
EXOSOMES FROM CI-ASCS HAVE NOVEL PROPERTIES

10 mins

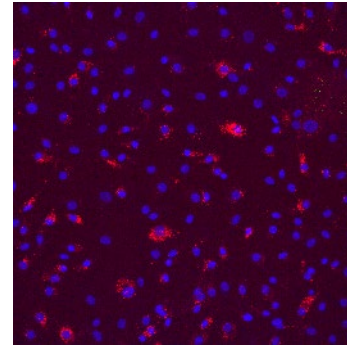
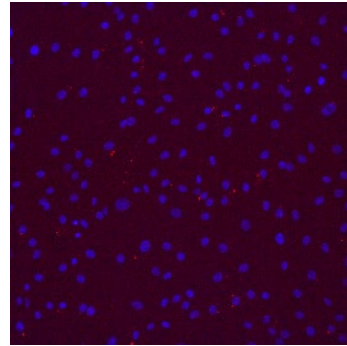
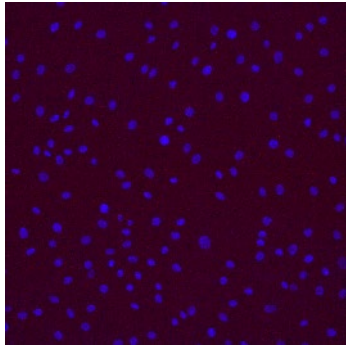
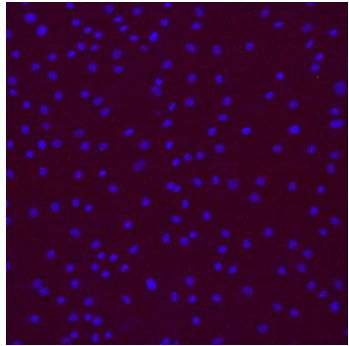
1 hr

4 hr

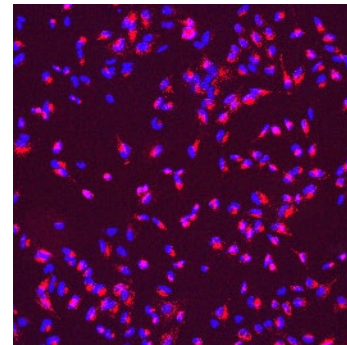
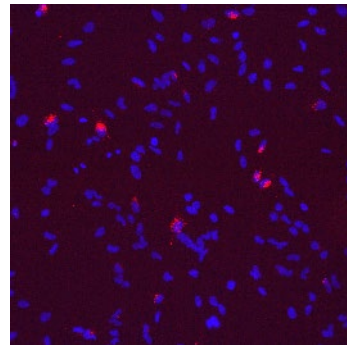
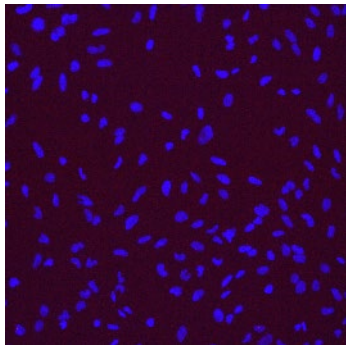
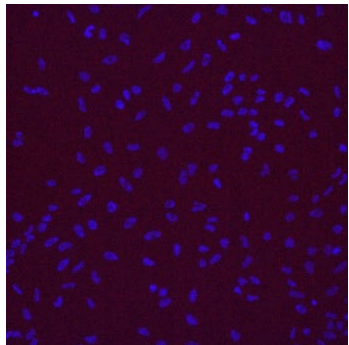
16 hr



HEK293C-derived exosomes uptake by normal adult HDFs



CI-MSC-derived exosomes uptake by normal adult HDFs



CI-MSC-derived exosomes uptake by HeLA cells

APPLICATION CASE 1: SUMMARY



CI-iPSCs can differentiate to cells fulfilling the ISCT criteria for **Mesenchymal Stem Cells**, an adult stem cell type with potential clinical applications



Conditional immortalisation **maintains adult stem cell phenotype** beyond what is normally observed



Conditional immortalisation of adult stem cells permits procedures often problematic with cell types such as primary multipotent ASCs, including **large scale banking, scalable cell manufacture** and **purification**



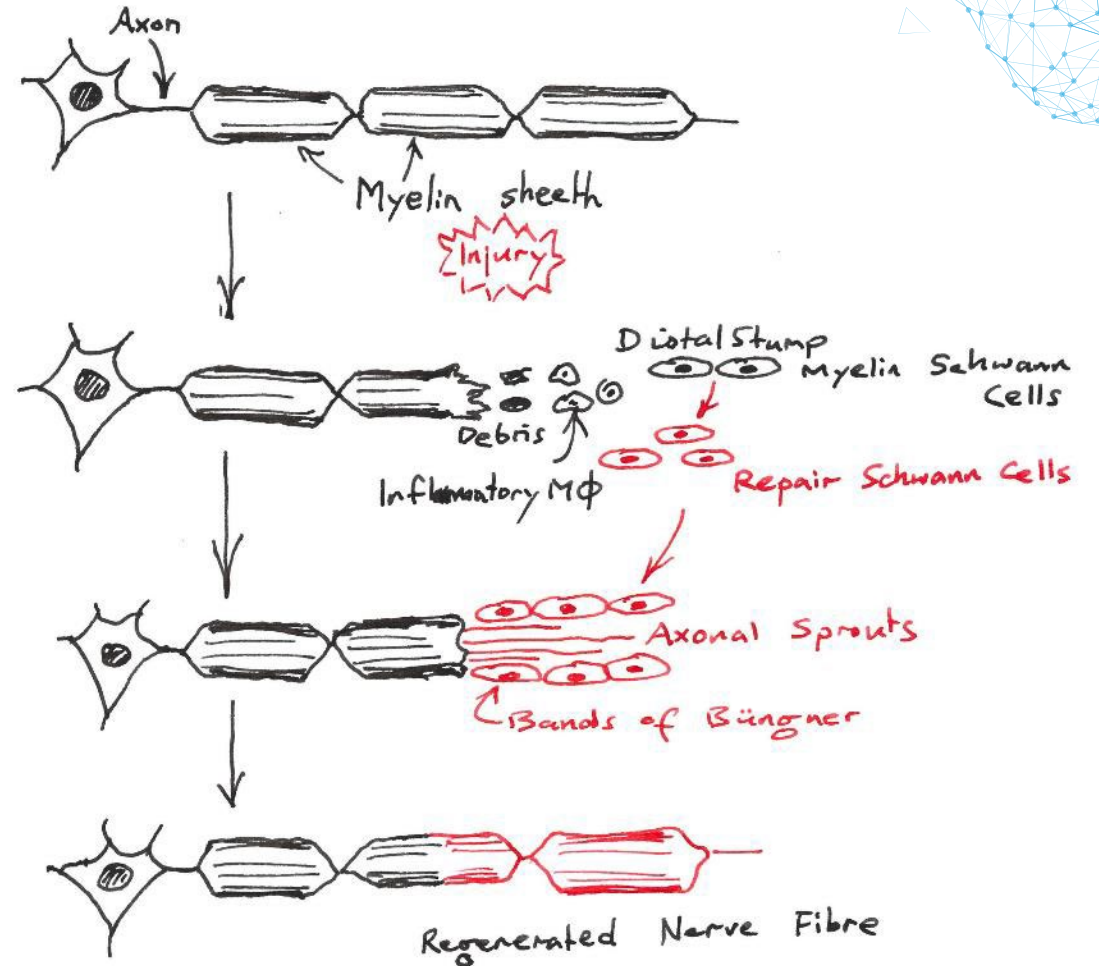
Scalable cell manufacture makes possible the production of cell products such as Adult Stem Cell-derived **exosomes**



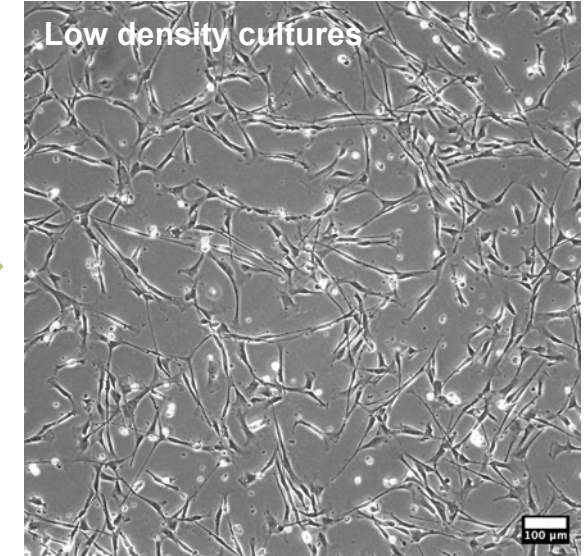
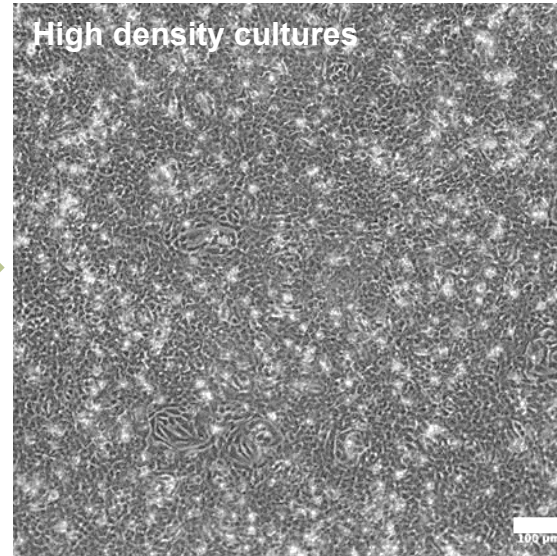
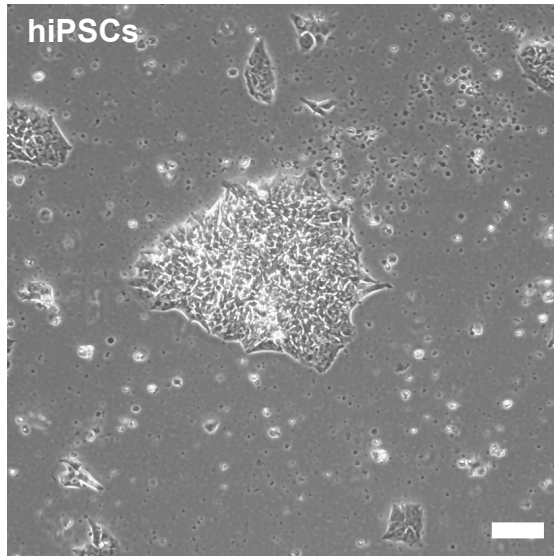
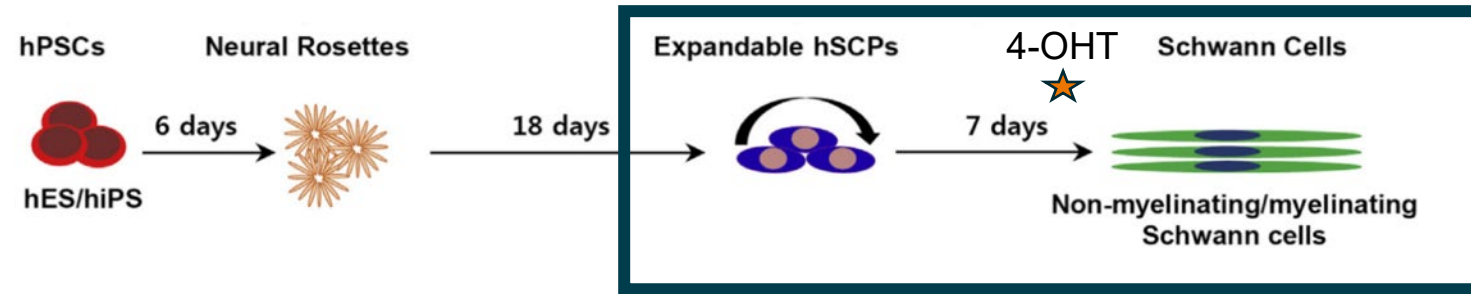
CI-MSD-derived exosomes display novel properties, such as enhanced target cell uptake, when compared to “industry standard” HEK-293-derived exosomal preparations

APPLICATION CASE 2: PNI & SCHWANN CELLS

- **Peripheral Nerve Injury** interrupts signal transmission to and from the CNS
- Causes loss of sensation, paralysis and pain which can become chronic
- Common: $\approx 1 / 1000$ per year
- High socioeconomic burden – patients often of working age
- Recovery and current treatment options unsatisfactory

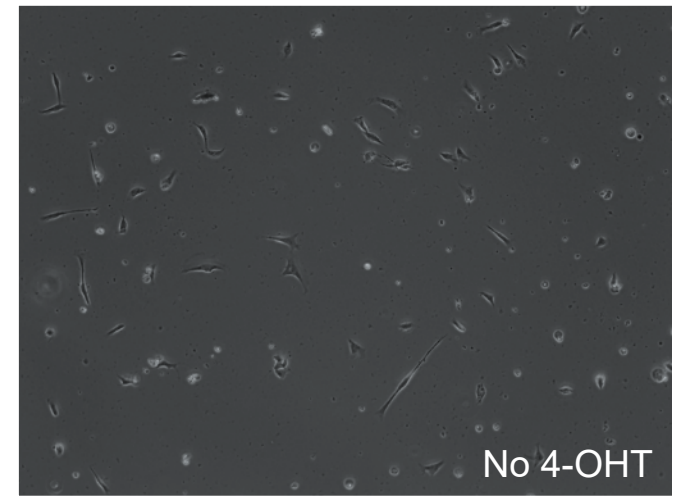
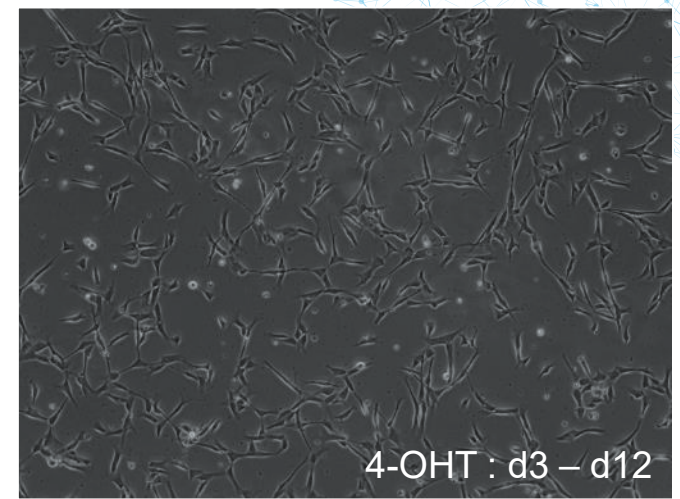
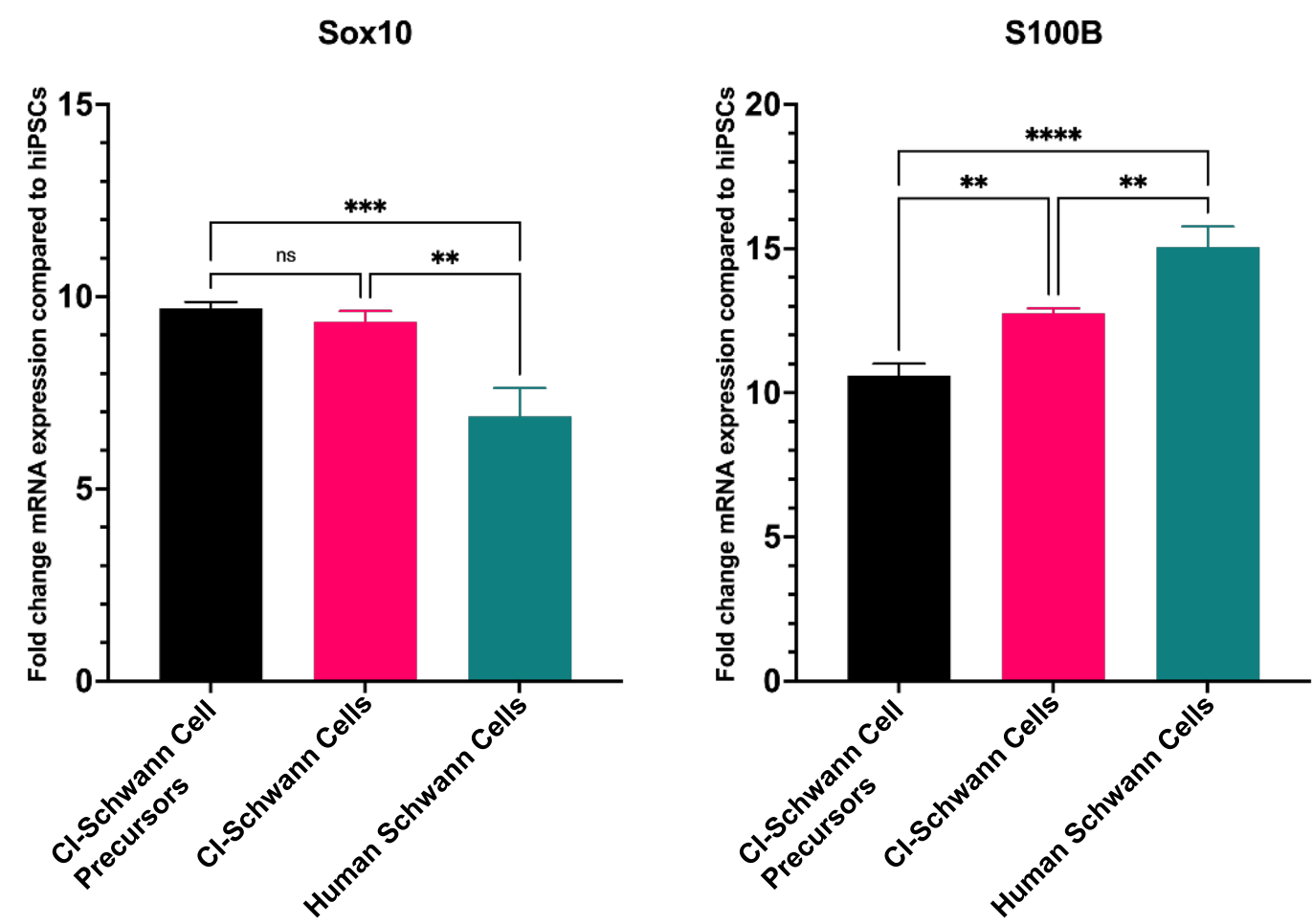


DIFFERENTIATION OF CI-IPCS TO SCHWANN CELLS



Kim, H.S et al., 2017; *Stem Cell Reports*; Powell, R. & Phillips, J.B., 2021; *In Vitro Models for Stem Cell Therapy*

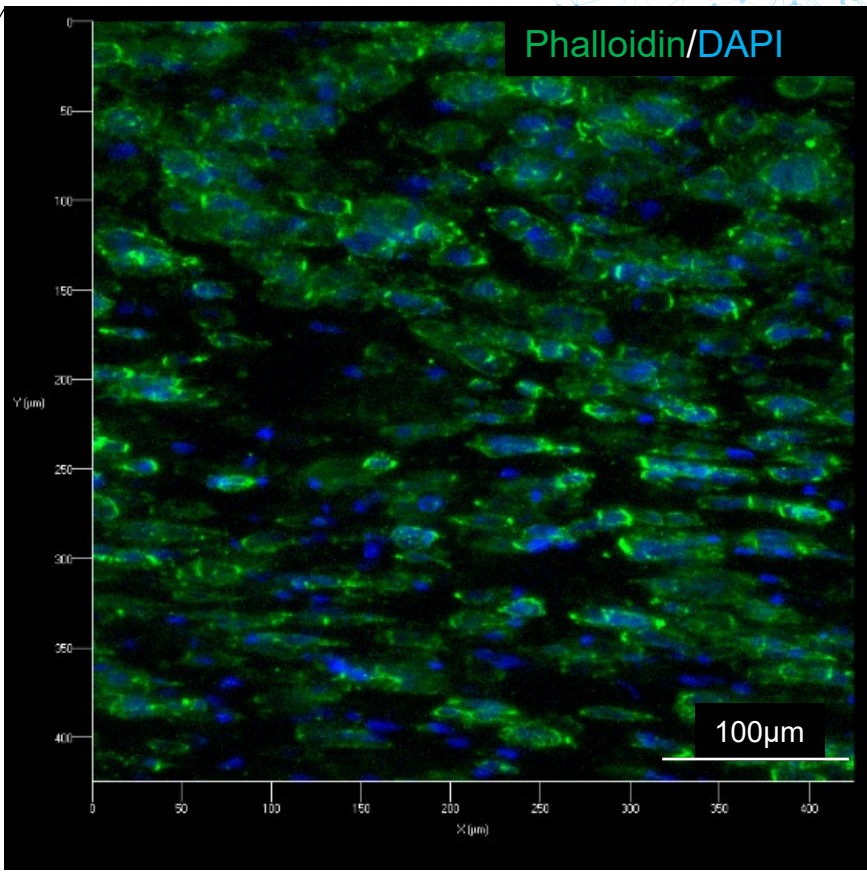
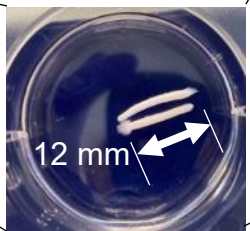
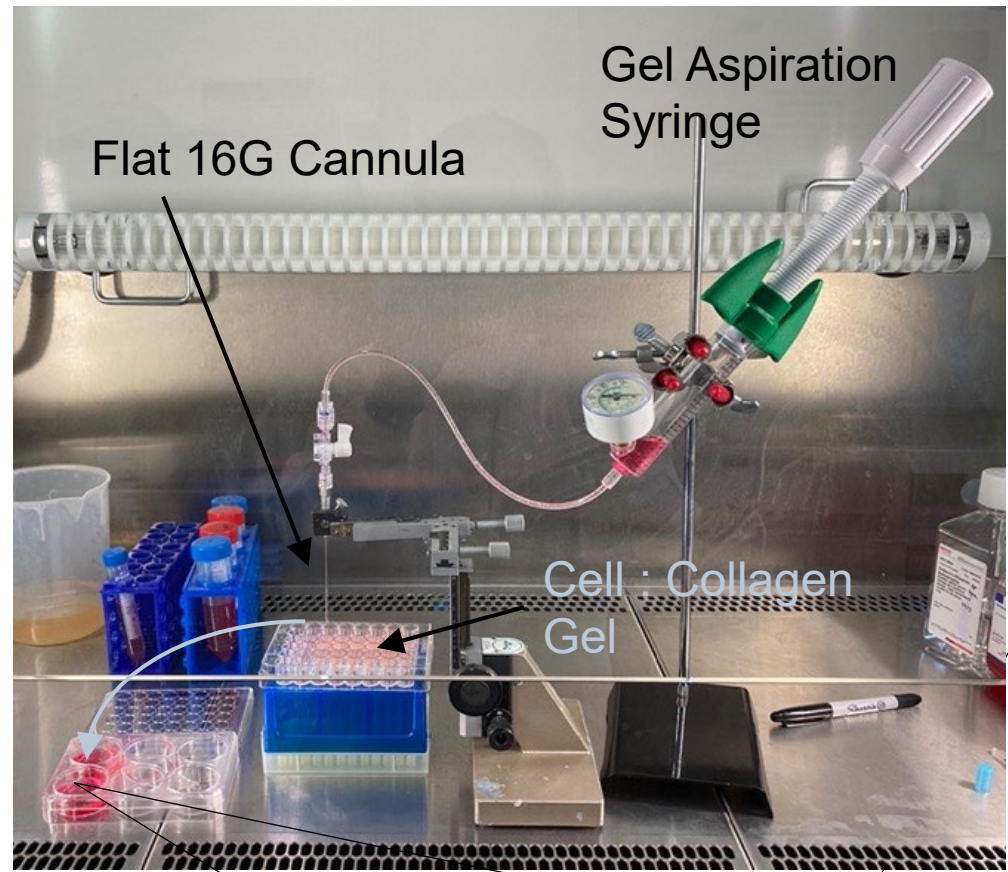
CI-IPSCS DIFFERENTIATE INTO SCHWANN CELLS AND CONDITIONAL IMMORTALISATION PROMOTES SURVIVAL



CI-Schwann Cells, Day 12

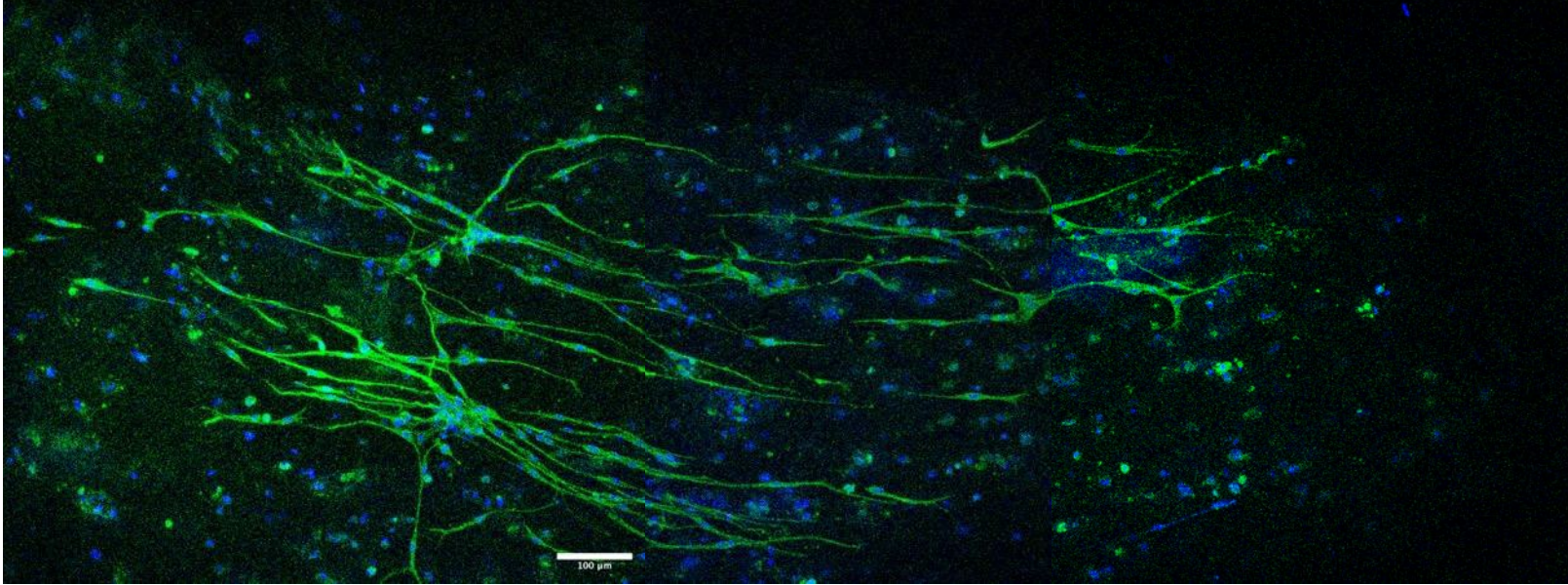
Rebecca Powell, UCL

GAE-ENGINEERED NEURAL TISSUE IS CREATED WITH CI-SCHWANN CELLS

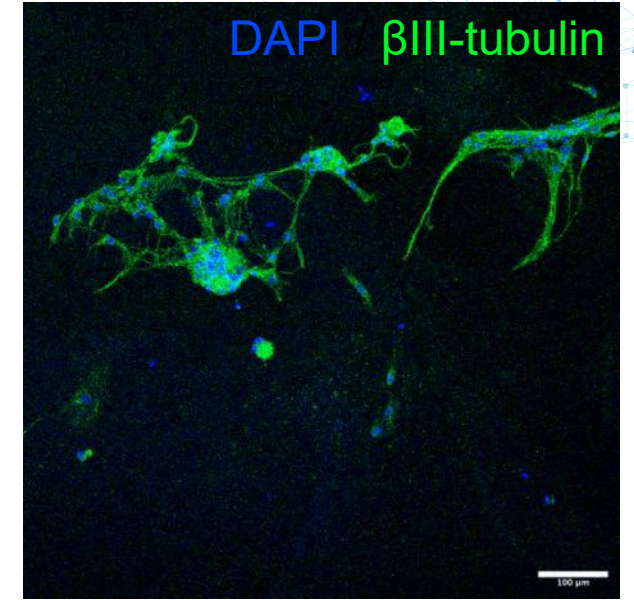


24 hrs: Schwann cell precursors have aligned in the GAE-engineered tissue

CI-SCHWANN CELLS IN THE CONTEXT OF EngNT SUPPORT NERVE OUTGROWTH AND EXTENSION



Dorsal Root Ganglion neuron outgrowth and neurite alignment on
CI-Schwann cell : collagen gel-engineered neural tissue



DRG neuron outgrowth on
acellular gel



Current experiments are directed towards assessing CI-Schwann cell behaviour and ability to promote neural outgrowth in a rat *in vivo* model

This experiment has not previously been possible, without conditional immortalisation to ensure the survival of hPSC-derived Schwann cells in the engineered neural tissue

APPLICATION CASE 2: SUMMARY



Peripheral Nerve Injury with catastrophic damage to axons lacks good treatment options



CI-iPSCs **differentiate** efficiently to form **Schwann cells**, the primary repair cell type for PNI



iPSC-derived Schwann cells survive very poorly without the **Conditional Immortalisation** system of CI-iPSCs



CI-SCs can be combined with protein matrices to form **3D Engineered Neural Tissue Structures** that recapitulate the Bands of Büngner cellular arrangements observed in naturally recovering nerve cells



In vivo testing is currently underway to assess the ability of CI-SCs to promote ingrowth of regenerating neurites in damaged nerve stumps, and cell survival, integration and phenotype

THE FUTURE: CI-IPSC V2.0



Starting Material

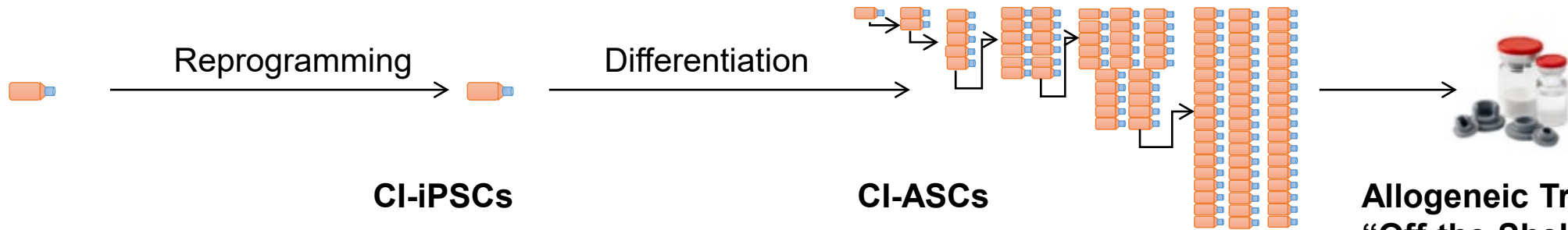
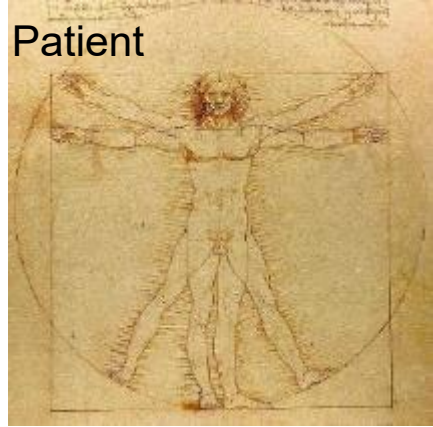
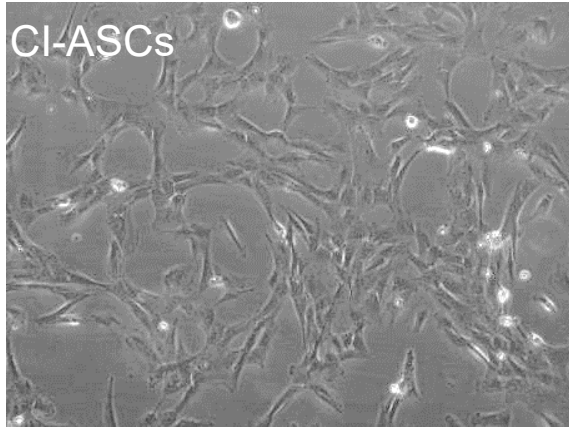
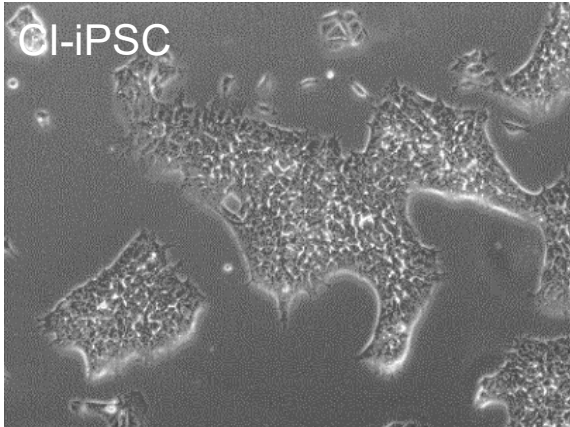
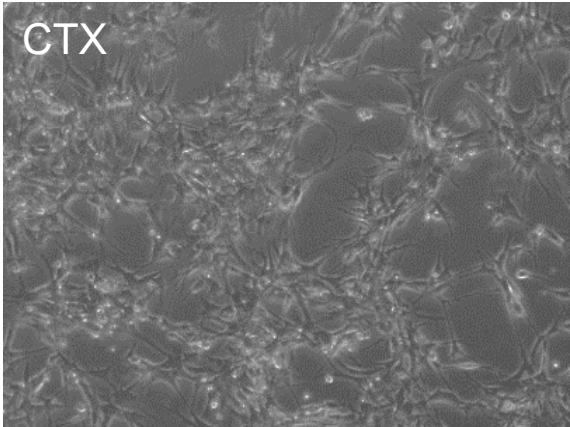
- Drug Product (GMP) CTX vial
- mRNA - based reprogramming
- IP Licensing in Place
- Full GMP process
- Collaborative project



CI-iPSC^{CLINIC}

- Clinical-grade
- Conditionally immortalised
- Banking
- Licensable

A NEW ATMP PLATFORM: CONDITIONALLY-IMMORTALISED IPSCS



CTX

- Multipotent – neural lineages
- Scalable production: 4-OHT / *c-myc*-ER^{TAM}

CI-iPSCs

- Pluripotent
- Teratoma
- Differentiation issues: time, efficiency, cost

CI-ASCs

- Multi- / oligo- / unipotent
- Pure populations
- Stable phenotype
- GMP scalable production

Allogeneic Treatments “Off-the-Shelf”

- Cell therapy
- Exosomes
- QC: Defined, safe & efficacious ATMPs

SUMMARY



Conditional Immortalisation makes possible processes such as scalable cell or cell product (e.g. exosomes) manufacture, purification and banking with labile or traditionally refractory cell types, e.g. adult stem cells such as Mesenchymal Stem Cells



Differentiation of certain cell types, e.g. Schwann cells, can be supported by conditional immortalisation where the differentiated cells might otherwise die, fail to grow or maintain phenotype



Our planned integration-free reprogramming of CTX neural stem cells via an end-to-end GMP reprogramming and line derivation pipeline will create a clinical grade, conditionally immortalised iPSC line ideal for at-scale biopharmaceutical and allogeneic therapeutic projects

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