

**Modelling late-onset diseases with induced pluripotent stem cells:**

**A matter of time management**

Charlotte Cossins<sup>1</sup> and Paul J. Fairchild<sup>1\*</sup>

<sup>1</sup>University of Oxford, Sir William Dunn School of Pathology,  
South Parks Road, Oxford, OX1 3RE, U.K.

\*Correspondence to:

Professor Paul J. Fairchild, Sir William Dunn School of Pathology,  
University of Oxford, South Parks Road, Oxford OX1 3RE, UK.

E-mail: [Paul.Fairchild@path.ox.ac.uk](mailto:Paul.Fairchild@path.ox.ac.uk)

## Abstract

The advent of induced pluripotent stem cells (iPSC) has revolutionised *in vitro* modelling of many intractable human diseases. By deriving iPSC from individuals with progressive disease, it has been possible to capture disease-associated genes and study their impact on the downstream function of terminally-differentiated cell types. Nevertheless, late onset diseases pose particular challenges for disease modelling given the protracted time frame over which they normally develop. Such practical issues are accentuated by the propensity for iPSC to produce cell types of foetal origin rather than their adult counterparts and by the rejuvenation of the resulting cells, thereby erasing any physiological evidence of ageing. Here we review progress in overcoming these issues and argue that achieving a combination of maturation and ageing will enable better recapitulation of all features of late-onset diseases.

## Key Words:

Induced Pluripotent Stem Cells; Disease Modelling; Ageing; Rejuvenation; Epigenetic Memory; Transdifferentiation

## Introduction

Current estimates suggest that one fifth of the world's population will be over 60 years of age by 2050 [1]. Furthermore, the incidence of age-related diseases is expected to rise, with the resulting burden of disease already impacting healthcare systems in developed countries [2]. For many late-onset diseases there are no effective treatments, a situation epitomised by amyotrophic lateral sclerosis (ALS), for which hundreds of promising drugs based on animal models have failed subsequent clinical trials [3]. There is, therefore, a strong imperative to better understand disease pathogenesis in order to develop more targeted interventions. Nevertheless, the study of late-onset diseases has proven difficult due to the limitations of disease modelling. Animal models rarely develop age-related pathologies unless induced by genetic modification or pharmacologic intervention [4]. Furthermore, insights from studies of patient tissue are typically only applicable to the final stages of disease, particularly if it is obtained post-mortem [5].

The advent of human induced pluripotent stem cells (hiPSCs) has provided a promising new approach to disease modelling: by reprogramming patient-derived somatic cells to pluripotency, cell lines may be generated in perpetuity that preserve the disease-associated genotype [6–9]. Importantly, these patient-derived hiPSCs can be differentiated into cell types affected by the disease, which may be subject to genome editing to investigate disease pathogenesis [10–12].

Although hiPSC have proven successful in modelling developmental disorders [13], recapitulating disease phenotypes of late-onset diseases has proven rather more challenging due, in part, to the foetal profile of cell types differentiated from them [14]. For example,

maturation of erythrocytes is characterised by enucleation and expression of adult haemoglobin, however protocols for their differentiation typically achieve enucleation in only a proportion of hiPSC-erythrocytes which persist in their expression of foetal haemoglobin [15]. Furthermore, cellular rejuvenation induced through the process of reprogramming prevents the development of late-stage phenotypes of age-related pathologies in hiPSC-based models. For example, key features of Parkinson's Disease (PD), such as neurodegeneration and alpha synuclein aggregation, are only observed if toxic stressors are used to mimic aspects of ageing [16–18]. Here we discuss recent advances in addressing these limitations by inducing maturation and physiological ageing among the progeny of hiPSC, largely drawing on examples from neurons and cardiomyocytes, and argue that achieving both processes together will enable better recapitulation of all features of late-onset diseases.

### **The immaturity of iPSC-derived lineages**

The ability of cell types differentiated from hiPSC to faithfully recapitulate the equivalent adult cell type was first questioned following transcriptomic analysis of progeny spanning all three germ layers which suggested that, regardless of cell type, differentiated cells more closely resemble cells from very early human development rather than their adult counterparts [19]. Subsequent studies have reported similar findings in cell types as diverse as pancreatic beta cells and hepatocytes [20,21], suggesting that the progeny of hiPSC may perform functions essential to the foetus rather than the adult. For example, iPSC-derived neurons have been shown to lack extensive dendrite branching and produce action potentials characteristic of late embryonic stages of development [22]. Furthermore, hiPSC-derived cardiomyocytes (hiPSC-CM) are mononuclear in nature and lack the anticipated ultrastructure of adult cardiomyocytes, such as T-tubules, thereby affecting their capacity for calcium handling [23]. Moreover, a lack

of Ik1 expression leads to a foetal-like action potential profile [24, 25]. The impact of this immature profile on attempts to restore heart function after myocardial infarction in mice was evident from studies by Liao and colleagues who demonstrated their inability to electrically couple with existing myocardium, resulting in fatal arrhythmias [26]. These findings involving unrelated cell types therefore highlight the need to develop protocols to promote maturation from a foetal to an adult state in order to accurately model adult-onset diseases.

### **Improving cell maturation**

One approach to promoting the maturation of hiPSC-derived cells types which has enjoyed some success has been to extend the duration of cell culture. For example, long-term culture of hiPSC-CM has been shown to lead to improved maturation, as evidenced by sarcomere organisation, calcium handling and action potential amplitudes that are more typical of mature cardiomyocytes [27]. Nevertheless, extended culture periods alone are insufficient to achieve full maturation since the local microenvironment *in vitro* may fail to elicit the necessary signalling pathways, suggesting the need for further forms of intervention (Table 1).

### ***Guiding maturation in vitro***

In order to address deficiencies in the culture microenvironment, many have sought to supplement the culture medium with small molecules, successfully reducing the time required for maturation in some cases [28, 29]. However, with only limited knowledge of the final stages of ontogeny in the human, many protocols use small molecules necessary for successful embryonic development to direct differentiation of hiPSC, rather than promoting maturation into fully adult cells [30, 31]. Identification of signalling molecules responsible for the later

stages of maturation is therefore essential, for which various candidate genes in the mouse have been identified. For example, Guo and colleagues recently demonstrated the importance of serum response factor (SRF) in supporting cardiomyocyte maturation in mice, its deletion through genome editing disrupting sarcomere expansion, T-tubule formation and mitochondrial biogenesis [32]. Should these findings prove applicable to the human, the addition of SRF to cultures of hiPSC during differentiation may enhance hiPSC-CM maturation.

An alternative approach which may provide a source of signalling molecules, even in cases where they have yet to be identified, has been to recapitulate the complex microenvironment in which cells mature *in vivo* by co-culturing them with accessory cell types. For example, independent studies have demonstrated how co-culture with astrocytes improves maturation of iPSC-derived neurons, including increased expression of neuronal markers [33] and synapse formation [34], resulting in improvements in electrophysiology [35]. Furthermore, the advent of organoid technology has overcome many of the physical limitations of 2D culture, such as the periodic depletion of secreted molecules upon routine replacement of the medium, and has enabled the structural complexity encountered *in vivo* to be more faithfully recapitulated in three dimensions. That these structures support maturation of hiPSC progeny is evident from cardiac organoids that display up-regulation of maturation-associated genes, such as those involved in sarcomere structure and calcium handling, with concurrent down-regulation of foetal-associated genes [36]. That such improvements in culture techniques may translate to better modelling of late-onset diseases is supported by the finding that beta amyloid aggregation may be observed in organoid models of Alzheimer's Disease (AD) yet is rarely evident in 2D culture [37].

### ***Combining hiPSCs and animal models***

Despite attempts to improve culture conditions to promote maturation *in vitro*, hiPSC-derived progeny continue to resemble foetal cells more closely than their adult counterparts, suggesting that it may prove difficult to recapitulate *in vitro* all signalling pathways required to support full maturation. Transplanting the products of hiPSC differentiation into animal recipients may, therefore, provide an *in vivo* environment conducive to maturation (Figure 1). Accordingly, transplantation of hiPSC-derived microglia into neonatal mouse brains enhanced their maturation so that they closely resembled primary human microglia [38]. This strategy may support maturation by supplying organoids with soluble plasma-derived factors, the complexity of which cannot be adequately recapitulated in culture medium. In support of this contention, Liu and colleagues successfully restored some cardiac function in a macaque model of myocardial infarction (MI) by transplanting human embryonic stem cell-derived cardiomyocytes (hESC-CMs), which subsequently showed enhanced maturation, including aligned cytoplasmic myofibrils and cardiomyocyte hypertrophy three months post-transplant [39]. The authors reported that all donor cardiomyocytes electrically coupled to host myocardium, unlike similar studies in the guinea pig, where only 60% electrically coupled [39, 40], possibly due to the greater disparity between the *in vivo* microenvironment of the guinea pig and human.

### ***Exploiting epigenetic memory***

Induced pluripotency initially generates iPSC with an epigenetic memory reminiscent of the somatic cells of origin [41], although continuous passaging eventually erases this epigenetic signature, leaving iPSC largely indistinguishable from ESC [42]. Although transient, the epigenetic memory of iPSC has been exploited to generate cells of an adult phenotype:

terminally-differentiated dendritic cells (DC) from either mouse or man have been reprogrammed to pluripotency and re-differentiated to yield highly-immunogenic DC expressing abundant co-stimulatory molecules and secreting high levels of interleukin (IL)-12 [43]. Such cells are indistinguishable from primary DC, unlike those differentiated from conventional iPSC derived from unrelated cell types such as fibroblasts, which display poor co-stimulatory capacity and a cytokine profile strongly favouring IL-10, undermining their immunogenicity. Although this study suggests, in principle, that the transient epigenetic memory of hiPSC may be exploited to generate mature progeny, the viability of this method remains to be seen for the generation of other cell types: while routine access to adult neurons and cardiomyocytes may prove challenging, attempts to generate mature B cells from B cell-derived iPSC have proven unsuccessful [44], as have efforts to generate mature iPSC-CM using cardiac progenitor cells (CPCs) [45].

<b>Maturation Strategy</b>	<b>Advantages</b>	<b>Disadvantages</b>	<b>Example studies</b>
Extended cell culture	Inexpensive	Limited maturation	[27]
Addition of small molecules to culture medium	Inexpensive Quick	Limited maturation	[28] [29]
Co-culture	Provide a 2D environment of multiple cell types.	Limited maturation.  Limited contact between iPSC-progeny and accessory cells.	[33] [34] [35]
3D culture	Provide a 3D environment of multiple cell types. Organized structure. Useful in	Highly variable. Need for improved vascularization and optimization	[36] [37]

	screening compounds. Enhanced maturation.	of differentiation protocols. Time-consuming and expensive. May raise ethical issues	
Combining hiPSCs and animal models	Provide a 3D environment of multiple cell types. Interaction with plasma-derived factors by vascularisation. Enhanced maturation.	Highly variable. Time-consuming and expensive. Raises ethical issues.	[38] [39] [40]
Exploiting epigenetic memory of iPSC	Faithfully recapitulates features of adult cells.	Success is highly variable. Difficult to access all adult cell types.	[43]

**Table 1.** Summary of the advantages and disadvantages of different strategies to induce maturation together with examples of studies employing such approaches.

### Rejuvenation of iPSC progeny

As advancing age increases the likelihood of developing a late-onset disease [46], it is imperative that we recapitulate ageing in hiPSC-based models. However, accumulating evidence suggests that the age signature of iPSC is erased upon reprogramming to pluripotency, resulting in restoration of telomere length, mitochondrial function and nuclear morphology, as well as reversal of cellular senescence [47]. Importantly, rejuvenation is not merely a characteristic of iPSC, but also their differentiated progeny [47]. This finding has been actively exploited for certain cell types: cytotoxic T lymphocytes (CTL) are, for instance, prone to

exhaustion in response to chronic viral infection by organisms such as HIV-1, losing their capacity to eliminate virally-infected cells. Nevertheless, by reprogramming antigen-specific CTL to pluripotency and re-differentiating the resulting iPSC along the T cell lineage, abundant CTL can be produced which, having been rejuvenated, are able to efficiently kill target cells in an antigen-specific manner [48, 49]. Nevertheless, the impact of rejuvenation can prove a significant obstacle to the acquisition of late-stage features of age-related disease, such as the neurodegeneration associated with PD [50]. It is, therefore, necessary to recapitulate physiological ageing *in vitro* in order to investigate why ageing results in progression of the disease from a compensated dysfunction to a progressive, uncontrollable decline in the elderly.

### **Can ageing be induced *in vitro*?**

Patients typically present with symptoms of degenerative diseases that reflect the later stages of disease progression. Given that rejuvenated iPSC and their progeny may only provide insight into early disease mechanisms that would likely occur in the pre-symptomatic adult, there is a pressing need to develop protocols to accelerate ageing *in vitro* (see Table 2) in order to achieve clinically-relevant disease models [51].

### ***Manipulating age-related pathways***

Attempts to induce ageing *in vitro* have generally focussed on manipulating specific hallmarks of ageing such as mitochondrial dysfunction, characteristic of the later stages of neurodegenerative disease. This has been achieved by treatment of cells with toxins such as hydrogen peroxide, leading to an increase in oxidative stress [16], which has been implicated in both ageing and age-related disease [52]. Since genomic damage is also characteristic of ageing, the administration of toxins such as hydroxyurea [53] has been shown to recapitulate

218 this aspect of cellular ageing. A more physiological approach involves passaging iPSC  
219 multiple times prior to differentiation which was shown in a recent study to lead to DNA  
220 damage in iPSC-derived neurons, accelerating the ageing process [54]. Interestingly, Vera and  
221 colleagues exploited the pharmacological inhibition of telomerase to further modify the  
222 biological age of hESC-CM and neurons differentiated from iPSC from an individual with PD  
223 [55]. In both cell types, they observed telomere shortening and acquisition of some age-related  
224 markers while a progressive loss of tyrosine hydroxylase (TH) expression was reported among  
225 iPSC-neurons, characteristic of the early stages of PD [55].

226  
227 While these studies successfully induce some of the recognised hallmarks of ageing, they do  
228 not promote acquisition of the full spectrum of age-related markers, adversely impacting the  
229 ability of iPSC to recapitulate all features of late-onset diseases. Indeed, it is debateable  
230 whether it could ever prove feasible to advance the biological age of cell cultures by targeting  
231 a single consequence of ageing. Instead, it may be necessary to modulate ageing at source by  
232 targeting pathways that broadly influence all aspects of ageing, insight into which may be  
233 gleaned from disorders associated with rapid, premature ageing, such as Hutchinson-Gilford  
234 progeria syndrome (HGPS).

### 235 236 ***Targeting the underlying mechanisms of ageing***

237 In 2013, Miller and colleagues reported successfully modelling the later stages of PD *in vitro*  
238 by inducing ageing in hiPSC-midbrain dopaminergic (hiPSC-mDA) neurons [50]. Given that  
239 the accumulation of progerin, a truncated form of lamin A associated with the inner nuclear  
240 membrane, causes cells to age prematurely in individuals with HGPS, patient-derived iPSC-  
241 mDA neurons were genetically modified to over-express the gene. An array of different

markers associated with neuronal morphology, mitochondrial function and gene expression were found to be suggestive of accelerated ageing. In addition, markers specific to neuronal ageing were observed, including loss of TUJ1 expression and neuromelanin accumulation. Increased apoptosis and dendrite degeneration were also observed, both consistent with enhanced neurodegeneration, while the appearance of Lewy-body-precursor inclusions was consistent with the acquisition of a disease-related phenotype. Accordingly, progerin-expressing hiPSC-mDA neurons failed to rescue disease among a subset of Parkinsonian mice, which was attributed to loss of tyrosine hydroxylase (TH) expression, characteristic of early PD [50]. This study demonstrates the need to target the underlying mechanisms of ageing in order to better recapitulate the later stages of age-related diseases. Furthermore, given that progerin accumulation is a biomarker of vascular ageing [56] and has recently been observed in the hearts of patients with dilated cardiomyopathy [57], progerin-induced ageing of iPSC progeny may also provide much-needed *in vitro* models of cardiovascular disease.

### ***Transdifferentiation***

An alternative approach, which may circumvent the need to intervene in ageing-related pathways, is to exploit the process of transdifferentiation in which patient-derived cells are directly reprogrammed into an alternative cell type relevant to disease progression, thus bypassing the need for pluripotency (Figure 2) [58]. Many protocols have been developed to reprogram fibroblasts into an extensive array of cell types, including neurons [59], osteoblasts [60], endothelial cells [61], leukocytes [62] and cardiomyocytes [63]. These cells might be expected to appropriately model ageing, as directly converting fibroblasts into neurons has been shown to preserve the biological age of the parent cells in both mice [64] and humans [65]. Importantly, there is no longer the need to rapidly accelerate ageing *in vitro*, which is unlikely

to completely recapitulate physiological ageing that takes place over a much longer time scale. Developing such overarching strategies that target all age-related markers is, therefore, of great interest when investigating advanced stages of late-onset diseases, although by-passing pluripotency necessarily sacrifices the capacity for genome editing, as well as the scale-up and quality control of differentiated progeny, posing challenges of consistency and reproducibility.

Ageing Strategy	Advantages	Disadvantages	Examples
Toxin treatment e.g. hydrogen peroxide, hydroxyurea, sodium arsenite	Can mimic oxidative stress. Can mimic genomic damage. Quick and inexpensive.	Does not induce all age-related markers. Age-related disease models only show earlier aspects of disease.	[16], [53], [66]
Telomerase inhibition	Induce telomere shortening and genomic damage.	Does not induce all age-related markers. Age-related disease models only show earlier aspects of disease.	[55]
Passaging at pluripotency	More physiologically-relevant. Induces several DNA markers of ageing.	Does not induce all age-related markers.	[54]
Progerin expression	Induces more than one hallmark of ageing. Age-related disease models show	Limited understanding of physiological role of progerin in ageing	[50]

	later stages of disease.		
Transdifferentiation	Retains age-related markers	Unable to perform genome editing. Highly variable. Cannot divide indefinitely	[64], [65]

**Table 2.** Summary of the advantages and disadvantages of different strategies to induce ageing together with examples of relevant studies.

### Translational Insight

While cellular maturation enables cells to perform the specialised functions of the adult cell type, cellular ageing is marked by a progressive decline in this ability. That these are distinct processes which would both normally occur in disease, suggests that current models of age-related pathogenesis, in which immature iPSC are induced to age, may be flawed (Figure 3). For example, while over-expression of progerin in iPSC-mDA neurons induced rapid ageing *in vitro*, the maturity of the resulting cells was not investigated, raising the possibility that ageing had been induced in foetal-like neurons, possibly contributing to the lack of some age-related features in this model [50]. Similarly, the issue of developmental immaturity of hiPSC progeny is raised in a recent study investigating ALS, which is overcome by inducing cellular stress, targeting immaturity with a solution more suitable to cellular rejuvenation [66]. Applying ageing strategies to immature cells may contribute to the limited translatability of our current *in vitro* models. In order to address these deficiencies, it may prove necessary in future to combine existing approaches to disease modelling. For instance, the epigenetic memory of early passage iPSC [43] may be exploited to generate organoids consisting of mature adult cell

types whose subsequent ageing may be induced by transplantation into ageing mice. In this context, rapid physiological ageing was recently achieved in mice by ablation of a specific neuronal subtype [67], providing an animal model of accelerated ageing and an appropriate *in vivo* environment in which to investigate the physiological ageing of fully matured cells. Whilst hiPSC progeny have the potential to revolutionise modelling of late-onset diseases in the human, it is only through such innovative approaches that it may be possible to address the combined limitations posed by maturation and rejuvenation in order to fully understand disease pathogenesis and develop effective treatments.

## **Acknowledgements**

Research into iPSC in the authors' laboratory is supported by grants from the Edward Penley Abraham (EPA) Trust (Grant: RF278) and the Guy Newton Translation Fund (Grant GN05(10)).

## **Financial and Competing Interests Disclosure**

P.J.F holds intellectual property relevant to the directed differentiation of human iPSC but has no other relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript. C.C. has no conflicts of interest to declare.

## References

1. Lutz W, Sanderson W, Scherbov S. The coming acceleration of global population ageing. *Nature*. 2008. 451, 716-719 doi:10.1038/nature06516
2. Dall TM, Gallo PD, Chakrabarti R, West T, Semilla AP, Storm M V. The care span: An aging population and growing disease burden will require a large and specialized health care workforce by 2025. *Health Aff.* 2013;32,2013-2020 doi:10.1377/hlthaff.2013.0714
3. Ziff OJ, Patani R. Harnessing cellular aging in human stem cell models of amyotrophic lateral sclerosis. *Aging Cell*. 2019; 18. doi:10.1111/accel.12862
4. Jucker M. The benefits and limitations of animal models for translational research in neurodegenerative diseases. *Nat Med*. 2010; 6, 1210-1214 doi:10.1038/nm.2224
5. Ferrer I, Martinez A, Boluda S, Parchi P, Barrachina M. Brain banks: benefits, limitations and cautions concerning the use of post-mortem brain tissue for molecular studies. *Cell Tissue Bank*. 2008; 9, 181-194. doi:10.1007/s10561-008-9077-0
6. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126(4), 663-676 doi:10.1016/j.cell.2006.07.024
7. Takahashi K, Tanabe K, Ohnuki M *et al*. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007; 131, 861-872. doi:10.1016/j.cell.2007.11.019
8. Yu J, Vodyanik MA, Smuga-Otto K *et al*. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; 318, 1879-1880. doi:10.1126/science.1151526
9. Park IH, Arora N, Huo H *et al*. Disease-specific induced pluripotent stem cells. *Cell*. 2008; 134, 877-886. doi:10.1016/j.cell.2008.07.041
10. Srikanth P, Young-Pearse TL. Stem cells on the brain: modeling neurodevelopmental and

neurodegenerative diseases using human induced pluripotent stem cells. *J Neurogenet.* 2014; 28, 5-29.  
doi:10.3109/01677063.2014.881358

11. Bellin M, Marchetto MC, Gage FH, Mummery CL. Induced pluripotent stem cells: the new patient? *Nat. Rev. Mol. Cell Biol.* 2012; 13(11), 713-726. doi:10.1038/nrm3448

12. Laurent LC, Ulitsky I, Slavin I *et al.* Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. *Cell Stem Cell.* 2011; 8(1), 106-118. doi:10.1016/j.stem.2010.12.003

13. Ardhanareeswaran K, Mariani J, Coppola G, Abyzov A, Vaccarino FM. Human induced pluripotent stem cells for modelling neurodevelopmental disorders. *Nat. Rev. Neurol.* 2017; 13, 265-278.  
doi:10.1038/nrneurol.2017.45

14. Cornacchia D, Studer L. Back and forth in time: Directing age in iPSC-derived lineages. *Brain Res.* 2017; 1656, 14-26. doi:10.1016/j.brainres.2015.11.013

15. Kobar L, Yates F, Oudrhiri N *et al.* Human induced pluripotent stem cells can reach complete terminal maturation: in vivo and in vitro evidence in the erythropoietic differentiation model. *Haematologica.* 2012; 97(12), 1795-1803. doi:10.3324/haematol.2011.055566

16. Cooper O, Seo H, Andrabi S *et al.* Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. *Sci. Transl. Med.* 2012; 4(141).  
doi:10.1126/scitranslmed.3003985

17. Byers B, Cord B, Nguyen HN *et al.* SNCA triplication parkinson's patient's iPSC-derived DA neurons accumulate  $\alpha$ -synuclein and are susceptible to oxidative stress. *PLoS One.* 2011; 6(11).  
doi:10.1371/journal.pone.0026159

18. Nguyen HN, Byers B, Cord B *et al.* LRRK2 mutant iPSC-derived DA neurons demonstrate increased

susceptibility to oxidative stress. *Cell Stem Cell*. 2011; 8, 267-280. doi:10.1016/j.stem.2011.01.013

19. Patterson M, Chan DN, Ha I *et al*. Defining the nature of human pluripotent stem cell progeny. *Cell Res*. 2012; 22(1), 178-193. doi:10.1038/cr.2011.133

20. Hrvatin S, O'Donnell CW, Deng F *et al*. Differentiated human stem cells resemble fetal, not adult,  $\beta$  cells. *Proc Natl Acad Sci U S A*. 2014; 111,3038-3034.. doi:10.1073/pnas.1400709111

21. Baxter M, Withey S, Harrison S *et al*. Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes. *J Hepatol*. 2015; 62(3), 581-589. doi:10.1016/j.jhep.2014.10.016

22. Weick JP. Functional properties of human stem cell-derived neurons in health and disease. *Stem Cells Int*. 2016;2016. doi:10.1155/2016/4190438

23. Koivumäki JT, Naumenko N, Tuomainen T *et al*. Structural immaturity of human iPSC-derived cardiomyocytes: in silico investigation of effects on function and disease modeling. *Front Physiol*. 2018; 9, 80. doi:10.3389/fphys.2018.00080

24. Hoekstra M, Mummery CL, Wilde AAM, Bezzina CR, Verkerk AO. Induced pluripotent stem cell derived cardiomyocytes as models for cardiac arrhythmias. *Front Physiol*. 2012; 3, 346. doi:10.3389/fphys.2012.00346

25. Shimoni Y, Clark RB, Giles WR. Role of an inwardly rectifying potassium current in rabbit ventricular action potential. *J Physiol*. 1992; 448, 709-727. doi:10.1113/jphysiol.1992.sp019066

26. Liao SY, Liu Y, Siu CW *et al*. Proarrhythmic risk of embryonic stem cell-derived cardiomyocyte transplantation in infarcted myocardium. *Heart Rhythm*. 2010.; 7(12), 1852-1859 doi:10.1016/j.hrthm.2010.09.006

27. Lundy SD, Zhu WZ, Regnier M, Laflamme MA. Structural and functional maturation of

cardiomyocytes derived from human pluripotent stem cells. *Stem Cells Dev.* 2013; 22(14), 1991-2002.  
doi:10.1089/scd.2012.0490

28. Chambers SM, Qi Y, Mica Y *et al.* Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. *Nat Biotechnol.* 2012; 30, 715-720. doi:10.1038/nbt.2249

29. Zhang Y, Pak CH, Han Y *et al.* Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron.* 2013; 78(5), 785-798. doi:10.1016/j.neuron.2013.05.029

30. Marchianò S, Bertero A, Murry CE. Learn from your elders: developmental biology lessons to guide maturation of stem cell-derived cardiomyocytes. *Pediatr Cardiol.* 2019; 40(7), 1367-1387. doi:10.1007/s00246-019-02165-5

31. Arbab M, Baars S, Geijsen N. Modeling motor neuron disease: the matter of time. *Trends Neurosci.* 2014; 37, 642-652. doi:10.1016/j.tins.2014.07.008

32. Guo Y, Jardin BD, Zhou P *et al.* Hierarchical and stage-specific regulation of murine cardiomyocyte maturation by serum response factor. *Nat. Commun.* 2018; 9(1), 3837. doi:10.1038/s41467-018-06347-2

33. Muratore CR, Srikanth P, Callahan DG, Young-Pearse TL. Comparison and optimization of hiPSC forebrain cortical differentiation protocols. *PLoS One.* 2014; 9(8). doi:10.1371/journal.pone.0105807

34. Jones EV., Cook D, Murai KK. A neuron-astrocyte co-culture system to investigate astrocyte-secreted factors in mouse neuronal development. *Methods Mol Biol.* 2012; 814, 341-352. doi:10.1007/978-1-61779-452-0\_22

35. Tang X, Zhou L, Wagner AM *et al.* Astroglial cells regulate the developmental timeline of human neurons differentiated from induced pluripotent stem cells. *Stem Cell Res.* 2013; 11(2), 743-757.

doi:10.1016/j.scr.2013.05.002

36. Giacomelli E, Bellin M, Sala L *et al.* Three-dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. *Dev.* 2017; 144(6), 1008-1017. doi:10.1242/dev.143438
37. Penney J, Ralvenius WT, Tsai LH. Modeling Alzheimer's disease with iPSC-derived brain cells. *Mol Psychiatry.* 2020; 25, 148-167. doi:10.1038/s41380-019-0468-3
38. Svoboda DS, Barrasa MI, Shu J *et al.* Human iPSC-derived microglia assume a primary microglia-like state after transplantation into the neonatal mouse brain. *Proc. Natl. Acad. Sci. USA* 2019; 116, 25293-25303. doi:10.1073/pnas.1913541116
39. Liu YW, Chen B, Yang X *et al.* Human embryonic stem cell-derived cardiomyocytes restore function in infarcted hearts of non-human primates. *Nat. Biotechnol.* 2018; 36, 597-605. doi:10.1038/nbt.4162
40. Shiba Y, Fernandes S, Zhu WZ *et al.* Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature.* 2012; 498, 322-325. doi:10.1038/nature11317
41. Kim K, Doi A, Wen B *et al.* Epigenetic memory in induced pluripotent stem cells. *Nature.* 2010; 467, 285-290. doi:10.1038/nature09342
42. Nishino K, Toyoda M, Yamazaki-Inoue M *et al.* DNA methylation dynamics in human induced pluripotent stem cells over time. *PLoS Genet.* 2011; 7(5). doi:10.1371/journal.pgen.1002085
43. Horton C, Davies TJ, Lahiri P, Sachamitr P, Fairchild PJ. Induced pluripotent stem cells reprogrammed from primary dendritic cells provide an abundant source of immunostimulatory dendritic cells for use in immunotherapy. *Stem Cells.* 2020; 38, 67-79. doi:10.1002/stem.3095
44. Wada H, Kojo S, Kusama C, et al. Successful differentiation to T cells, but unsuccessful B-cell generation, from B-cell-derived induced pluripotent stem cells. *Int. Immunol.* 2011; 23(1), 65-74.

doi:10.1093/intimm/dxq458

45. Sanchez-Freire V, Lee AS, Hu S *et al.* Effect of human donor cell source on differentiation and function of cardiac induced pluripotent stem cells. *J. Am .Coll. Cardiol.* 2014; 64, 436-448. doi:10.1016/j.jacc.2014.04.056

46. Niccoli T, Partridge L. Ageing as a risk factor for disease. *Curr Biol.* 2012; 22(17), 741-752. doi:10.1016/j.cub.2012.07.024

47. Lapasset L, Milhavet O, Prieur A *et al.* Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. *Genes Dev.* 2011; 25, 2248-2253. doi:10.1101/gad.173922.111

48. Nishimura T, Kaneko S, Kawana-Tachikawa A *et al.* Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation. *Cell Stem Cell.* 2013; 12(1), 114-126 doi:10.1016/j.stem.2012.11.002

49. Minagawa A, Yoshikawa T, Yasukawa M *et al.* Enhancing T cell receptor stability in rejuvenated iPSC-derived T cells improves their use in cancer immunotherapy. *Cell Stem Cell.* 2018; 23(6), 850-858. doi:10.1016/j.stem.2018.10.005

50. Miller JD, Ganat YM, Kishinevsky S, et al. Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell.* 2013; 13, 691-705. doi:10.1016/j.stem.2013.11.006

51. Klimmt J, Dannert A, Paquet D. Neurodegeneration in a dish: advancing human stem-cell-based models of Alzheimer's disease. *Curr. Opin. Neurobiol.* 2020; 61, 96-104. doi:10.1016/j.conb.2020.01.008

52. Luo J, Mills K, le Cessie S, Noordam R, van Heemst D. Ageing, age-related diseases and oxidative stress: what to do next? *Ageing Res Rev.* 2020; 57, 100982. doi:10.1016/j.arr.2019.100982

446 53. Tan Y, Ke M, Huang Z *et al.* Hydroxyurea facilitates manifestation of disease relevant phenotypes in  
447 patients-derived iPSCs-based modeling of late-onset Parkinson's disease. *Aging Dis.* 2019; 10, 1037-  
448 1048. doi:10.14336/AD.2018.1216

449 54. Tagliafierro L, Zamora ME, Chiba-Falek O. Multiplication of the SNCA locus exacerbates neuronal  
450 nuclear aging. *Hum Mol Genet.* 2019; 28, 407-421. doi:10.1093/hmg/ddy355

451 55. Vera E, Bosco N, Studer L. Generating late-onset human iPSC-based disease models by inducing  
452 neuronal age-related phenotypes through telomerase manipulation. *Cell Rep.* 2016; 17, 1184-1192.  
453 doi:10.1016/j.celrep.2016.09.062

454 56. Ragnauth CD, Warren DT, Liu Y *et al.* Prelamin a acts to accelerate smooth muscle cell senescence  
455 and is a novel biomarker of human vascular aging. *Circulation.* 2010; 121, 2200-2210.  
456 doi:10.1161/CIRCULATIONAHA.109.902056

457 57. Messner M, Ghadge SK, Goetsch V *et al.* Upregulation of the aging related LMNA splice variant  
458 progerin in dilated cardiomyopathy. *PLoS One.* 2018; 13(4). doi:10.1371/journal.pone.0196739

459 58. Zhang Y, Xie X, Hu J, et al. Prospects of directly reprogrammed adult human neurons for  
460 neurodegenerative disease modeling and drug discovery: iN vs. iPSCs Models. *Front Neurosci.* 2020;  
461 14, 546484. doi:10.3389/fnins.2020.546484

462 59. Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M. Direct conversion of  
463 fibroblasts to functional neurons by defined factors. *Nature.* 2010; 463, 1035-1041.  
464 doi:10.1038/nature08797

465 60. Chang Y, Cho B, Kim S, Kim J. Direct conversion of fibroblasts to osteoblasts as a novel strategy for  
466 bone regeneration in elderly individuals. *Exp. Mol. Med.* 2019; 51, 1-8. doi:10.1038/s12276-019-0251-  
467 1

61. Han JK, Chang SH, Cho HJ *et al.* Direct conversion of adult skin fibroblasts to endothelial cells by defined factors. *Circulation*. 2014; 130, 1168-1178. doi:10.1161/CIRCULATIONAHA.113.007727
62. Szabo E, Rampalli S, Risueño RM *et al.* Direct conversion of human fibroblasts to multilineage blood progenitors. *Nature*. 2010; 468, 521-526. doi:10.1038/nature09591
63. Chang Y, Lee E, Kim J, Kwon YW, Kwon Y, Kim J. Efficient in vivo direct conversion of fibroblasts into cardiomyocytes using a nanoparticle-based gene carrier. *Biomaterials*. 2019; 192, 500-509. doi:10.1016/j.biomaterials.2018.11.034
64. Yang Y, Jiao J, Gao R *et al.* Enhanced rejuvenation in induced pluripotent stem cell-derived neurons compared with directly converted neurons from an aged mouse. *Stem Cells Dev*. 2015; 24, 2767-2777. doi:10.1089/scd.2015.0137
65. Mertens J, Paquola ACM, Ku M *et al.* Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. *Cell Stem Cell*. 2015; 17(6), 705-718. doi:10.1016/j.stem.2015.09.001
66. Jutzi D, Campagne S, Schmidt R *et al.* Aberrant interaction of FUS with the U1 snRNA provides a molecular mechanism of FUS induced amyotrophic lateral sclerosis. *Nat Commun*. 2020; 11(1), 6341. doi:10.1038/s41467-020-20191-3
67. Zhang Y, Kim MS, Jia B *et al.* Hypothalamic stem cells control ageing speed partly through exosomal miRNAs. *Nature*. 2017; 548, 52-57. doi:10.1038/nature23282

## Figure Legends

**Figure 1** | Maturation of hiPSC progeny can be improved by combining them with appropriate animal models. Schematic demonstrating that maturation of hiPSC progeny can be induced *in vitro*

with co-culture and organoid technology. However, maturation can be further enhanced by engrafting these cellular aggregates into an appropriate recipient species. Orange cells represent iPSC progeny; green and yellow cells represent accessory cells in co-culture and in organoids. The origin and species of these may differ.

**Figure 2** | A summary of the different protocols developed to generate aged cells from a somatic cell. One strategy is to directly convert the somatic cell to the desired cell type, thereby retaining the hallmarks of ageing. Alternatively, different methods may be applied to hiPSC progeny to induce rapid ageing *in vitro*, such as the induction of oxidative stress or genomic damage, although this approach typically targets a single hallmark of ageing.

**Figure 3** | Current and speculative strategies for developing iPSC models of age-related disease. In order to induce early markers of disease, current protocols induce age in immature hiPSC progeny, however, inducing both maturation and ageing in combination among hiPSC progeny may prove a more effective strategy at capturing the later features of age-related diseases that are more clinically relevant. Solid arrows represent established protocols while the dashed arrows denote the alternative protocols discussed.