# Reprogramming the stem cell for a new generation of cures

By Dr Alex Davenport, Dr Tonya Frolov and Dr Mark Kotter

The development of new and effective treatments with the potential to cure, or at the very least ease patient suffering, has been the driving force in biomedical research. Although tremendous progress has been made over the last few decades, cell therapies today are expensive and not particularly scalable. The development of new drugs has not kept up with the progress in biology. The majority of clinical trials fail because the drugs are either toxic or they do not work in human diseases. Human biology often proves to be distinct and sufficiently different from animal models and associated cell lines used for drug development. Widespread access to reliable human cells is needed to replace suboptimal disease and drug response models the industry currently relies on to enable a new generation of medicine. Human cells derived from induced pluripotent stem cells (iPSCs) provide an excellent platform for disease modelling and a scalable source of starting material for cell therapies. Cellular reprogramming is able to overcome the traditional bottlenecks of their application: lack of consistency, purity and scalability. This synthetic biology paradigm involves activating a new cell type programme directly, skipping the usual intermediate steps that occur during development. As a result, stem cells convert directly into any desired mature cell type. We are now at a dawn of a new age in cellular biology combining *in silico* and *in vitro* techniques to revolutionise drug discovery and the development of novel cell therapies.

Over the past several decades we have made tremendous progress in biomedical research. Scientists are now able to turn individual genes off or on and edit them with CRISPR and assess the expression of many thousand genes, even on a single cell level. Therapies have progressed from small molecules to biologics, with the first CAR-T cell therapies providing a glimpse of a future where cancer could be eradicated. Despite significant scientific breakthroughs and technological advances, today drugs are more likely to fail in clinical development than 40 years ago. Inflation-adjusted Research and Development (R&D) cost per drug has increased nearly 100-fold between 1950 and 2010[1]. This mismatch is concerning. Differences between human biology and the models used for drug discovery are an important contributor to the problem. One of the biggest challenges to R&D productivity remains the lack of valid human screening and disease models.

## Limitations of current models in R&D

The numbers are stark. It costs, on average, $1.78 billion to bring a drug to market over an average time course of 13 years with only a 3.5% probability of success[2]. R&D continues to rely on laboratory animals, cell- and tissue-based systems to identify novel targets and to establish efficacy prior to the design of clinical trials. Yet the majority of clinical trials fail because the drugs turn out to be either toxic or they do not work within human diseases[3].

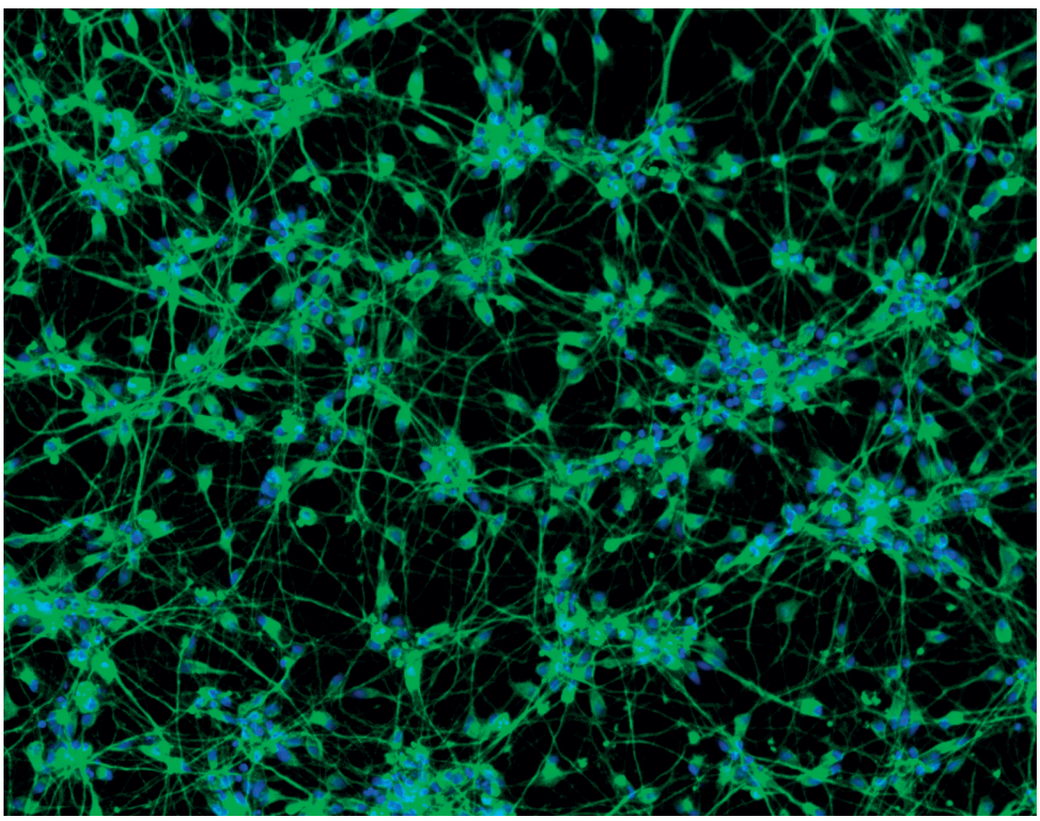
*In vitro* pre-clinical research relies predominantly on animal-derived primary cells and immortalised cell lines. Primary cells, usually sourced from donor tissue, are associated with high variability and a lack of scalability due to often limited expansion capacity. In contrast, cell lines are well adapted for a culture environment, typically highly-proliferative, scalable and easy to transfect. But most cell lines have been immortalised artificially and propagated for decades, diversifying during long-term culture. As a result, they differ substantially from the tissue of origin with regards to genome, phenotype and morphology. Accumulated mutations over time lead to differences in cell characteristics between laboratories and, inevitably, issues of reproducibility. A 2016 survey conducted by *Nature* found that more than two-thirds of respondents have tried and failed to reproduce someone else's experiment, and more than half have failed to reproduce their own data[4]. Continuous propagation of cell lines can result in batch-to-batch variability in the absence of quality control. But given the lack of reliable human cells, academics and pharmaceutical companies continue to depend on the current models due to a lack of viable alternatives.

Animal models still form the backbone for the current drug discovery workflow, despite evidence that their cumulative harm and cost to humans outweigh the benefits[5]. Apart from species differences, many diseases, such as Alzheimer's, are limited to humans. Hence, creating disease models often involves genetic manipulation of animals or the administration of toxins, with the aim of inducing comparable disease phenotypes. Instead of predicting human response to therapies, animal models can lead to misleading biomarkers and safety studies. As a consequence, otherwise effective drugs can be abandoned and resources are diverted away from more reliable models[5]. These issues are further compounded when taking into consideration animal husbandry costs, staff training and months spent writing ethics applications and adhering to good quality regulatory frameworks. Finally, given the limitations with regards to the predictability of animal models, ethical questions have been raised.

## Stem cells – introducing the human element into drug discovery

Human biology often proves to be distinct from animal models and cell lines used for drug development. To bridge the gap, a scalable source of consistent human cells is needed. But primary cells are difficult to source in sufficient quantities, with some cell types proving impossible to obtain. Many in the scientific community have thus sought to take advantage of human stem cells. Unfortunately, to date this has often been fraught with problems, ranging from inconsistency, long and complex protocols that are difficult to reproduce and lack of scalability.

These can be explained by the paradigm that has, for the most time, been applied to the use of stem cells. Traditional 'directed differentiation' approaches seek to replicate developmental processes *in vitro*. Consequently, the time to generate particular mature cell types (often 50-100 days) hinges on the timelines of embryonic development and depends on multiple intermediary steps. Moreover, the cell fate choices that are required to differentiate a pluripotent stem cell to a fully differentiated somatic cell rely on stochastic principles. The accumulation of stochastic events ultimately leads to inconsistent cultures, not only resulting in batch-to-batch but also intra-batch variability.



Human glutamatergic neurons generated by Neurogenin-2-driven reprogramming of stem cells using opti-ox technology. *Image courtesy of Charles River*

## Cellular reprogramming – using synthetic biology to induce cell fate changes

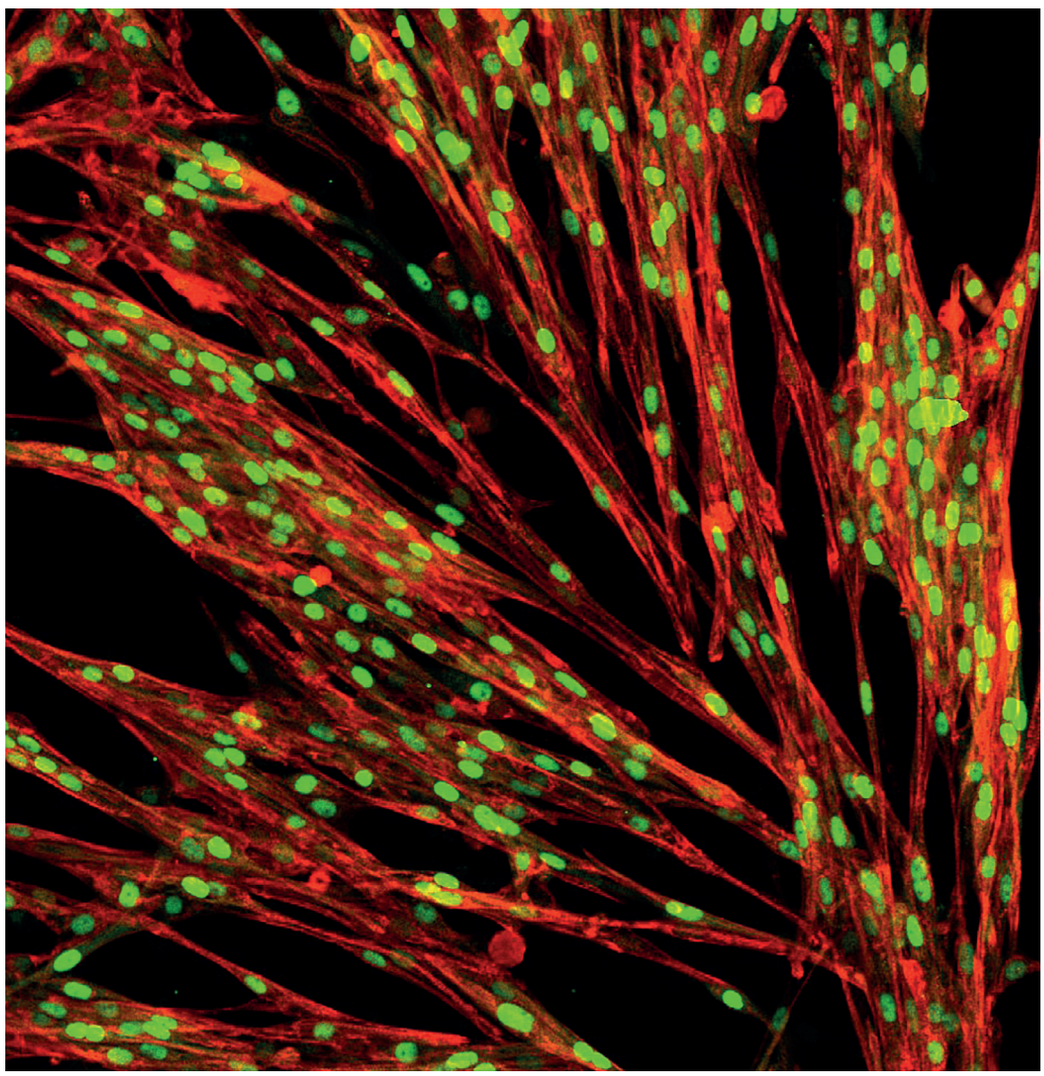
The initial ethical bottleneck with regards to the use of embryonic stem cells was overcome in 2006/07. Japanese researcher Shinya Yamanaka discovered a way to turn adult mouse and human cells into human-induced pluripotent stem cells (hiPSCs), which show striking similarities to embryonic stem cells. This can be achieved by directly activating the pluripotency cell programme by expressing appropriate transcription factors. Known as the Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc), these bypass the need for dissecting human embryos, thus removing the ethical concerns associated with embryonic stem cells and enable a scalable source of progenitor cells. hiPSCs remain in an epigenetically native state in which progenitor cells exhibit an indefinite capacity to proliferate while maintaining the potential to differentiate into any cell type under optimised differentiation protocols. Yamanaka's discovery has opened the possibility to generate any human cell type in unlimited supply and won him and John Gurdon the 2012 Nobel prize for Medicine.

Yamanaka's work built on previous findings from Davis, Weintraub and Lassar, who in 1987 first challenged our understanding of cell types by demonstrating that connective tissue cells (fibroblasts) can be converted into skeletal muscle cells by the single transcription factor MyoD[6]. In 2010 Marius Wernig extended this concept and demonstrated that reprogramming is possible between two different germ layers, by converting fibroblasts into neurons[7]. These discoveries led to a wave of reprogramming research throughout the world that continues unabated to the present day.

Cellular reprogramming is thus not limited to generating induced stem cells from mature cells. An increasing body of literature suggests that it can be applied to the specification of any cell type, be it a liver cell, an immune cell, a neuronal cell or other cell type.

## Next-generation cellular reprogramming

A particularly attractive starting cell for reprogramming is the iPSC: due to rapid proliferation and their unrestricted capability to replicate, iPSCs enable effective scale-up of cell production. However, until recently reprogramming of cells including iPSCs has been hampered by low efficacy and yields. We hypothesised that these are likely



Human skeletal myocytes generated by MYOD1-driven reprogramming of stem cells using opti-ox technology

the result of evolutionary intrinsic protective mechanisms within stem cells which lead to recognition and deactivation of foreign DNA (gene silencing) and thus limit reprogramming efficiency of the transcription factors introduced to stem cells.

By applying a cellular reprogramming approach supported by a uniquely-engineered genetic switch, opti-ox™ (optimised inducible overexpression)[8], we were able to overcome gene silencing and with it the restrictions of inefficient cellular reprogramming. This proprietary technology enables tightly-controlled expression of transcription factors and results in deterministic reprogramming of entire hiPSC cultures. To achieve this, we disassembled a Tet-ON switch into its two components and gene-targeted each of them into two or more distinct genomic safe harbour sites. The result of this is unprecedented homogeneous, controllable expression of inducible transgenes in hiPSCs. When applied to the expression of reprogramming factors this results in rapid, synchronous and highly defined reprogrammed cells.

The first product developed using opti-ox has already been brought to the market: ioNEURONS/glut™ human glutamatergic neurons derived from hiPSCs have successfully been reprogrammed using opti-ox by forced expression of Neurogenin-2 (NGN2). The technology can be applied to generate many other mature cell types too, including human skeletal myocytes, hepatocytes, immune cell types and other CNS cell types.

## Identifying human cell type programmes

These novel stem cell-derived products generated using opti-ox demonstrate that cellular reprogramming is not only possible, but able to achieve consistency and scalability at an affordable cost.

In order to expand the repertoire of cell types, we need to tackle the challenge of identifying the right transcription factor combinations that define a specific cell type. A recent report identified at least 100 major clusters of human cell types[9]. Past classifications were based on different criteria, including morphology, protein expression and function[10], and recent evidence suggests that the actual number of cell types encoded by different transcription factors may be far greater[9]. There are approximately 20,000 genes in our DNA, of which ~3,000 encode DNA binding proteins that theoretically could be involved in coding cellular identity. If we postulate that cell fate is determined by an average of three transcription factor genes, there are 4.5 billion possible combinations to examine!

Advancements in molecular techniques such as single cell RNA sequencing and efforts such as the Human Cell Atlas to develop a 'unique ID card for each cell type' are accelerating the discovery of transcription factor combinations that encode cell types[11]. To deal with the gigantic data sets, scientists are applying machine learning methods to identify relevant patterns.

The use of machine learning will also help to draw connections and maps between transcription factors and DNA binding proteins not yet characterised and thus expand our ability to reprogramme cells. Bioinformatic platforms capable of setting up pipelines to mine the literature and pull out potential candidates are needed along with research projects to break down each organ and cell type into its genetic components. Combining bioinformatic approaches with new molecular gene editing techniques such as CRISPR has increased our understanding of cell type identity and will ultimately enable more accurate targeting of cellular subtypes. A newly-formed consortium between Bit Bio and the London Institute of Mathematical Sciences applies machine learning to large-scale functional transcription factor screens in order to drastically speed up the discovery process with the aim of developing the capability to reprogramme stem cells into any cell type.

## Application of reprogrammed cells

The identification of precise transcription factor combinations driving cell fate and subsequent generation of high-quality human cells has the potential to address the translation gap and thus improve and lower the cost of research and drug discovery. The precision and consistency that this approach offers will result in more predictable models of human disease and, if broadly adopted, help to overcome some of the challenges linked to the 'reproducibility crisis' in science[4]. Stem cell-derived human cells can be used in multi-lineage co-cultures, organoids, organ chips and tissue engineering[12]. Certain human cell types, including neurons and cardiomyocytes, are difficult to isolate and culture *in vitro*; hiPSC-derived cells provide a unique opportunity for investigating pathologies associated with these.

Toxicity screening currently relies on primary hepatocytes that are difficult to culture and display significant variability. Other models used are not always predictive of human response, leading to the potential of clinical failures. A robust and scalable source of stem cell-derived hepatocytes would be the ideal platform to screen candidate compounds for toxicity. Stem cells encompassing common variants or with genetically-engineered mutations would further optimise drug discovery by ensuring that those compounds with successful efficacy are personalised with regards to specific target populations.

## Reprogrammed cells for cell therapy

Recent advances in CRISPR and CAR-Ts technologies spark hopes that a new generation of therapies might finally eradicate cancer. A notable example of the success of Chimeric Antigen Receptor (CAR) technology is in the treatment of childhood Acute Lymphoblastic Leukaemia (ALL) with Kymriah, a patient-specific (autologous) CAR-T cell therapy. This involves isolating T cells from the patient, genetically modifying them to express an anti-cancer antigen receptor (in this case CD19) and then reinfusing them into the patient. Significant efforts are currently under way to develop novel cell therapies. The global stem cell therapy market was valued at $755.4 million in 2018 and is expected to reach $11 billion in 2029[13].

However, cell therapy today is extremely challenging, causing unpredictable responses from the body's immune system, with many patients experiencing neurotoxicity and cytokine release syndrome[14]. Widespread access is limited due to their high price, placing economic burden on patients, insurance companies and governments. The cost of treatment with Kymriah, developed by Novartis, is $475,000. Yescarta, developed by Kite Pharma and approved for large B-cell lymphoma has been priced at $373,000.

Harvesting patient cells and genetically modifying them with a lentivirus or retrovirus brings with it an oncogenic risk[15]. The cost of each batch production is high, and manufacture often fails due to

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poor cell quality, with many patients undergoing rounds of chemotherapy treatment prior to CAR-T cell therapy. A further challenge, common to adoptive T cell therapies is that activation and expansion of T cells from a Naïve state to an effector state increases the chance of activation-induced death of T cells upon transfer[16].

One approach to overcome the challenges of autologous treatments could be to use allogeneic donor T cells, but this comes with the risk of graft-versus-host disease. While this may improve the quality of T cells, as healthy donors do not undergo rounds of chemotherapy, this will not solve the manufacturing complications linked to activating and rapidly expanding T cells.

Forward reprogramming of iPSCs to Naïve T cells in theory could overcome the above challenges by providing a reliable, consistent and scalable platform for the manufacture of cells. The right transcription factor combination would enable the expansion of billions of iPSCs and upon activation of the transcription factor cassette, turn them into the desired T cell phenotype. Additionally, gene engineering the iPSCs to express CARs prior to T cell differentiation could improve therapeutic safety.

With clever engineering we can also mask the cells and avoid the host immune system, thereby increasing the effectiveness of donor-derived therapies, similar to HLA matching/masking seen in bone marrow transplantation.

The use of off-the-shelf T cells will drastically cut the cost and allow for widespread adoption of cell therapies in otherwise difficult-to-treat diseases. Cell therapies, while currently focused on the immune system and cancer, will expand to other indications. Indeed, immune cells are paving the regulatory framework for a much larger market of regenerative medicine, with an almost unlimited number of cell types. It may become possible to use iPSC-derived hepatocytes to regenerate damaged livers that currently require a liver transplant, or to use iPSC-derived cardiomyocytes for treating heart attack patients. Both these cell types are currently difficult to culture from primary samples and fulfil a desperately needed gap in the market. Access to highly-defined, scalable human cells will enable the future of medicine.

## Conclusion

Despite tremendous progress in biomedical research that has resulted in successful treatments for many diseases, we face significant unmet medical needs for individuals and for our societies. Widespread access to human cells will have unprecedented positive implications for academic research, drug development and toxicity testing, and regenerative medicine. Their widespread application has the potential to reduce failure rates in clinical trials and drug development and enable a new generation of cell therapies. DDW

***Dr Alex Davenport*** *holds a PhD in CAR-T cell biology from the University of Melbourne and was a postdoctoral scientist in T cell biology at the University of Cambridge before joining Bit Bio as a Senior Scientist.*

***Dr Tonya Frolov*** *completed her PhD in cancer cell biology at UCL. The lack of relevant disease models for her research led her to join Bit Bio as Product Specialist, with the aim of helping to develop and democratise access to reliable human cell models.*

***Dr Mark Kotter*** *is a stem cell biologist and neurosurgeon at the University of Cambridge. By combining synthetic and stem cell biology, his team has developed a benchmark technology for the efficient and consistent production of human cells for use in research, drug development, and cell therapy. He is Founder & CEO of Bit Bio and co-founder of the cultured meat start-up Meatable.*

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