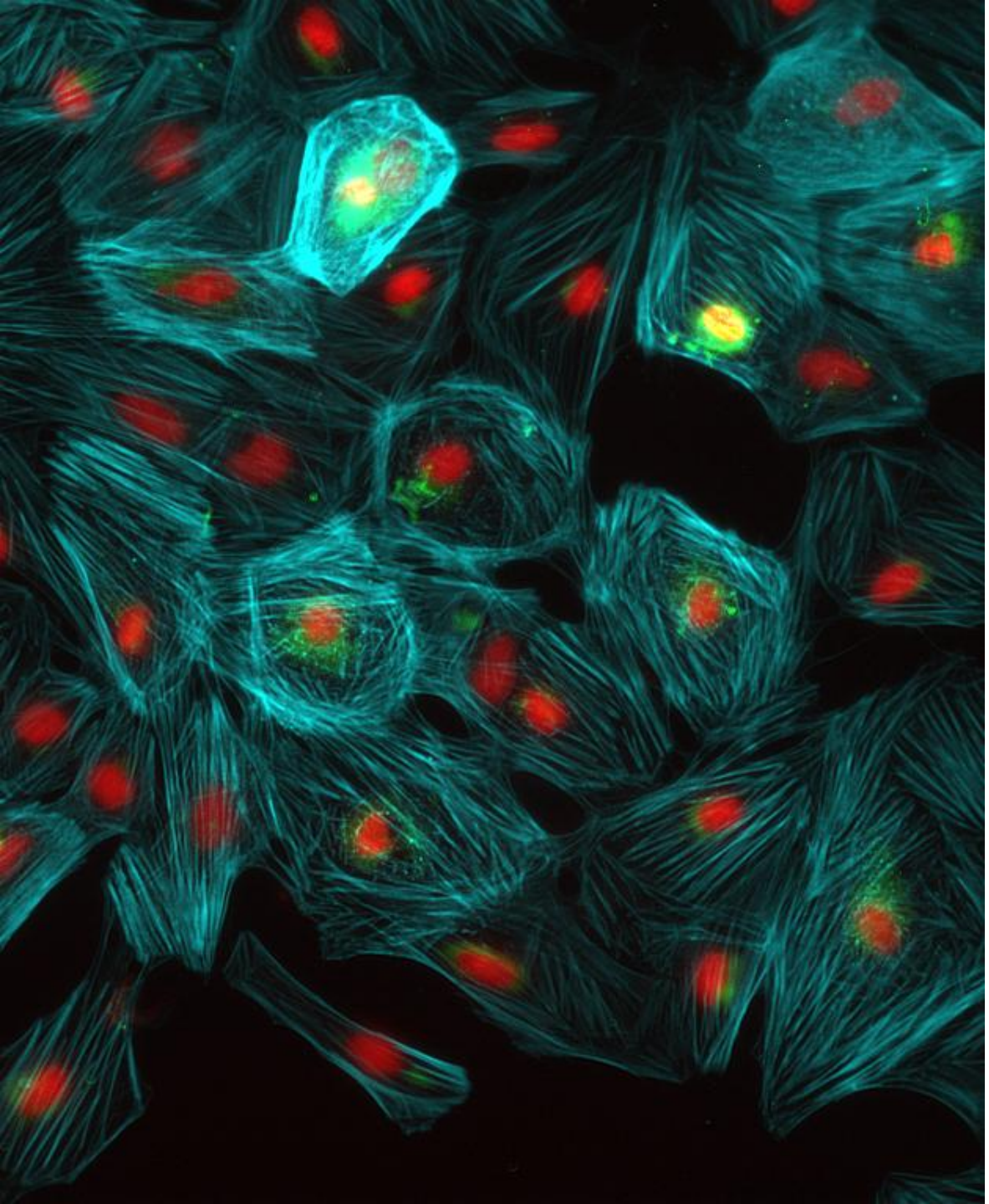


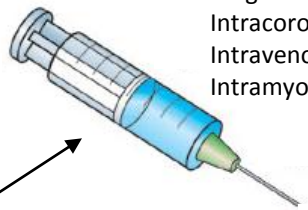
USE OF CARDIAC CELLS FROM HUMAN PLURIPOTENT STEM CELLS TO UNDERSTAND HEART DISEASE

Gábor Földes MD PhD
National Heart and Lung Institute
Imperial College London

Thermo Scientific
UK Summer Symposium 2011

Which stem cells for cardiac repair and modelling?

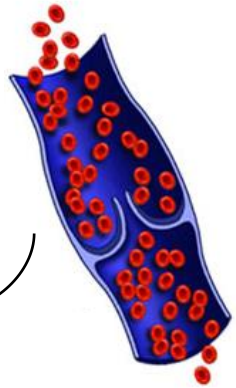
ESCs



Delivery

Surgical
Intracoronary
Intravenous
Intramyocardial

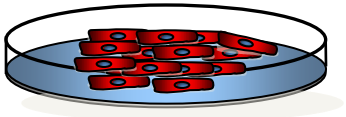
Circulating progenitor cells



iPS generation

Pluripotent cells

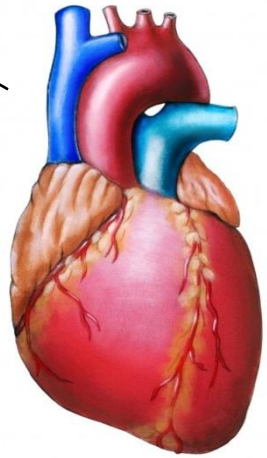
Directed differentiation



Functional
cardiomyocytes
Endothelial cells
Progenitor cells

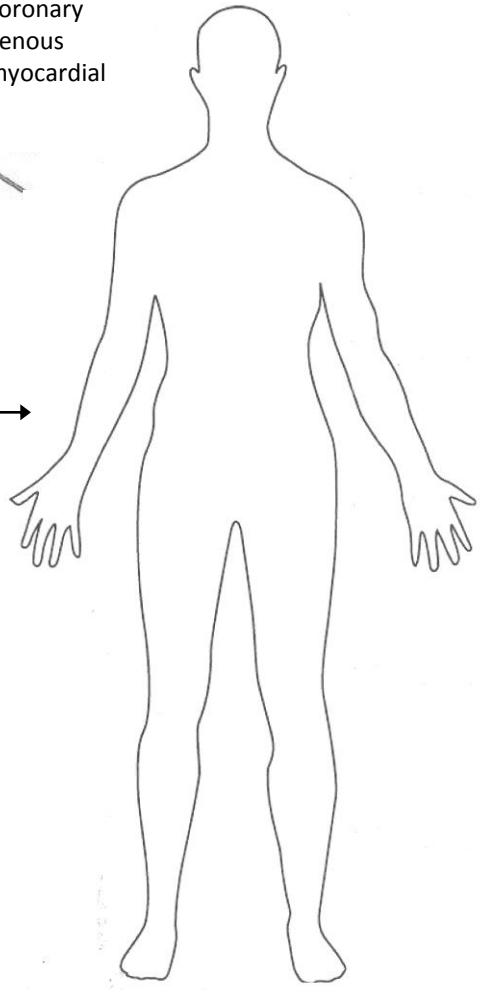
Tissue engineering

Engineered cardiac tissue
Patches, scaffold systems
3D cultures and tissue fabrication



Cardiomyocyte proliferation

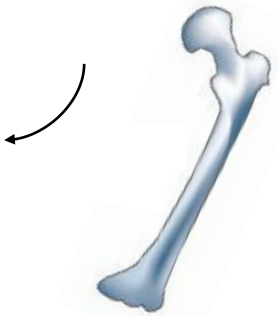
Resident stem cells



Cell isolation

Mobilisation and homing

Transdifferentiation



Bone marrow-derived cells

in Földes et al. Comprehensive Biotechnology 2011

Use of embryonic stem cell derivative models. Cell factory and industrialisation tools for stem cells

1. New *in vitro* model system for cardiomyocyte and endothelial development and pathophysiology

- Long lived hESC-CM in culture – beating clusters maintained for > 1 year
- Human genotype
- Amenable to genetic manipulation
- hESC-CM and hESC-EC have many of the characteristics of adult counterparts
- High throughput measurement screening systems with hESC-CM and hESC-EC can match genomics in rate of discovery

2. Stem cell models for drug toxicity testing

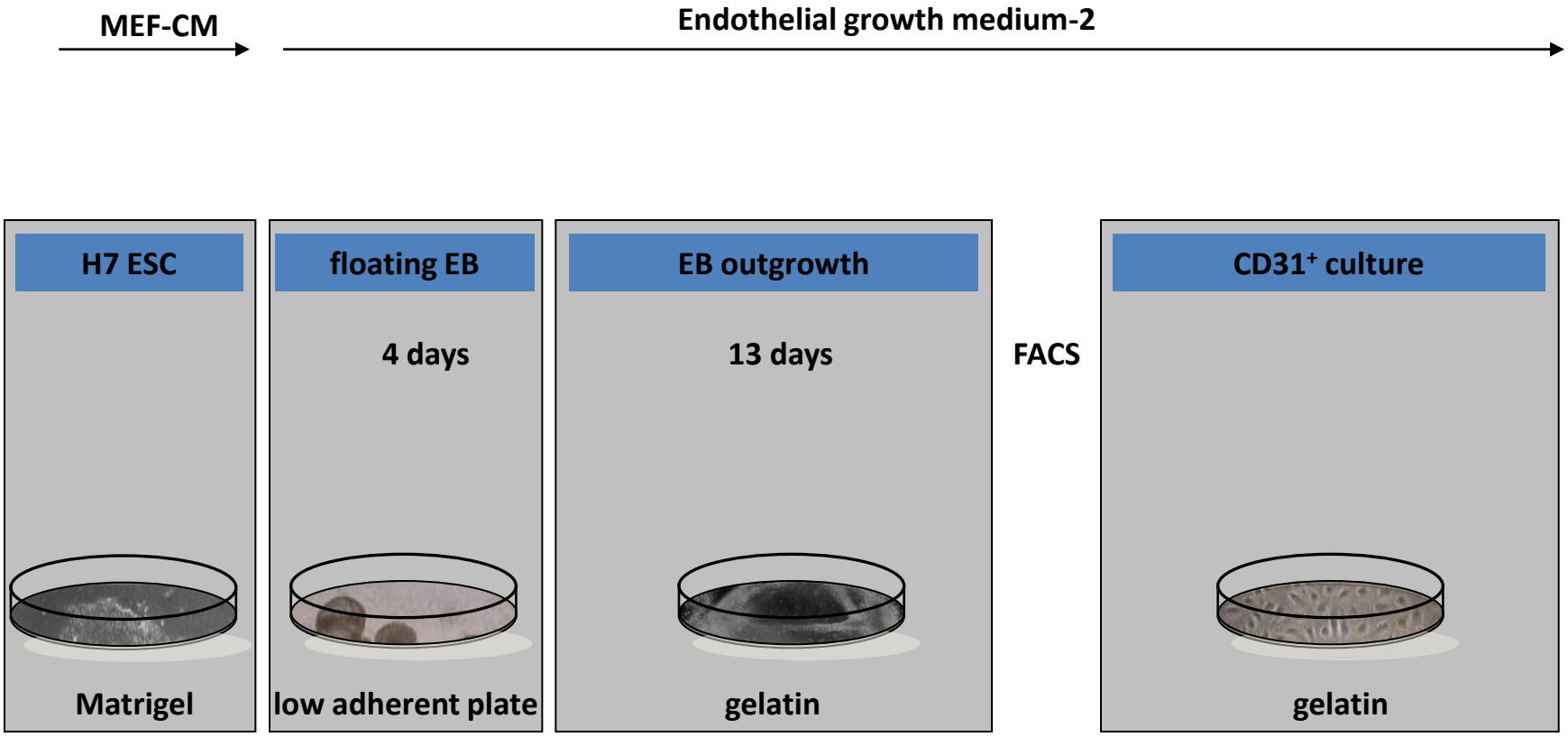
- Pharma seeking in vitro models with improved clinical predictivity
- Government/pharmaceutical partnerships
    - pharma to give compounds which failed in clinical trials
    - aims to reduce attrition of compounds going from animal to clinical studies
    - despite clearance from animal models open protocols for high throughput assays on hESC derivatives
    - validation with respect to current cellular models

3. Therapeutic applications

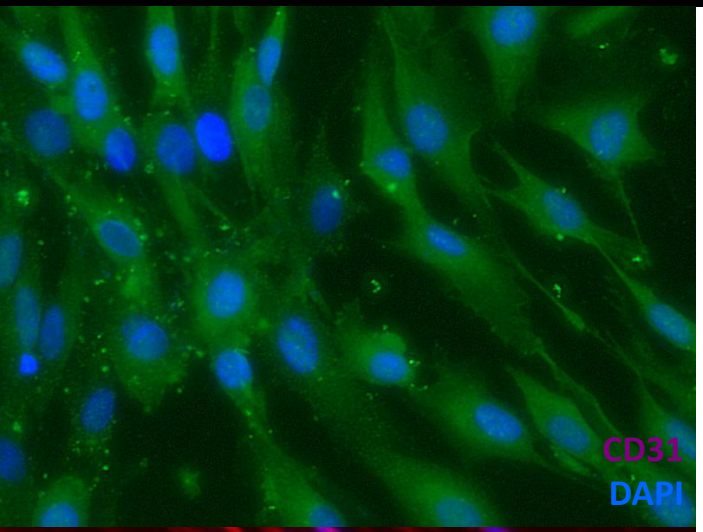
HUMAN PLURIPOTENT STEM CELLS

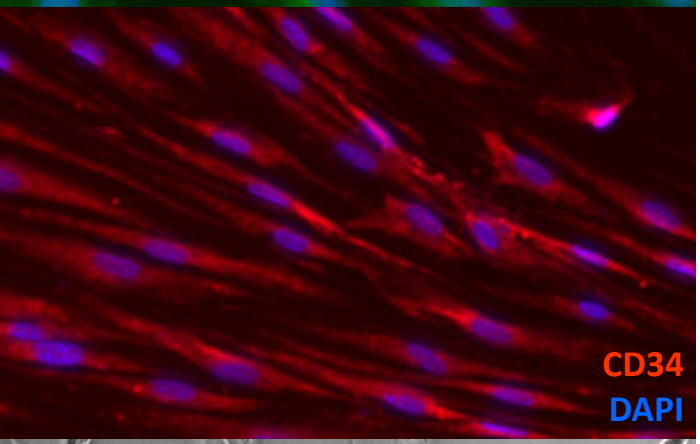
Cardiomyocytes
Endothelial cells
Smooth muscle cells

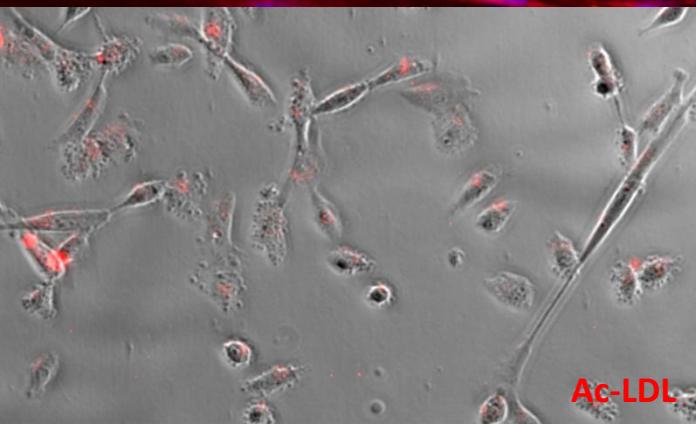
Differentiation of endothelial cells from human embryonic stem cells

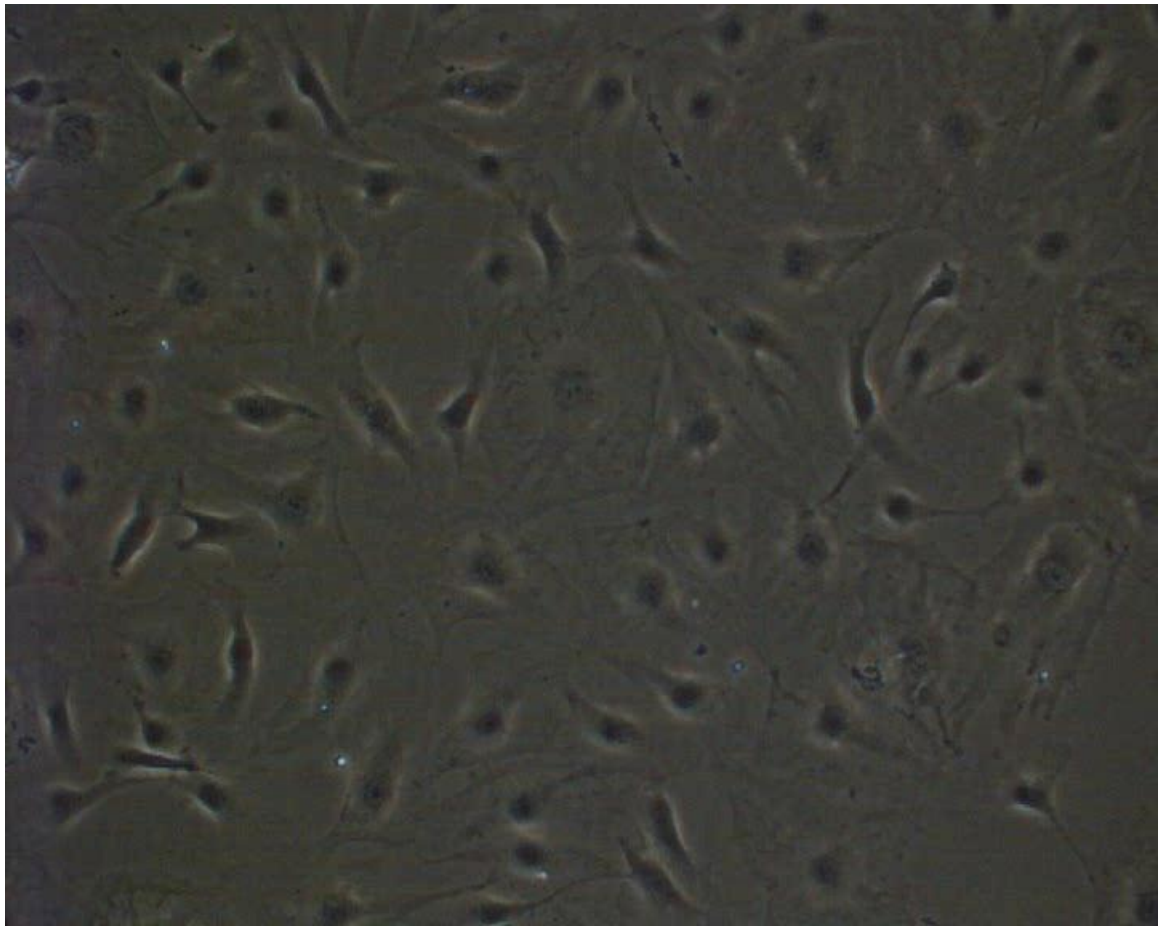


Human embryonic stem cell-derived endothelial cells









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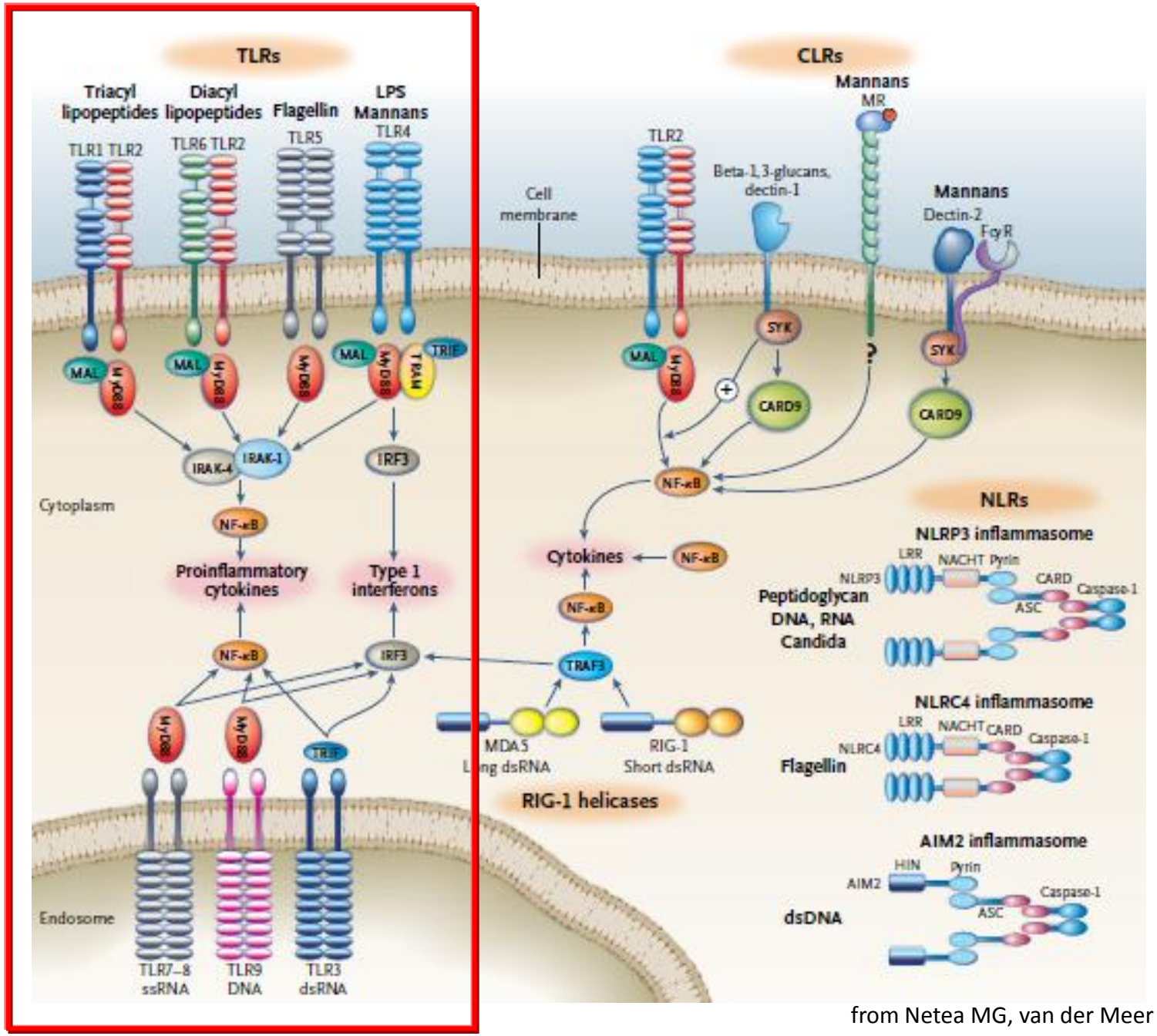
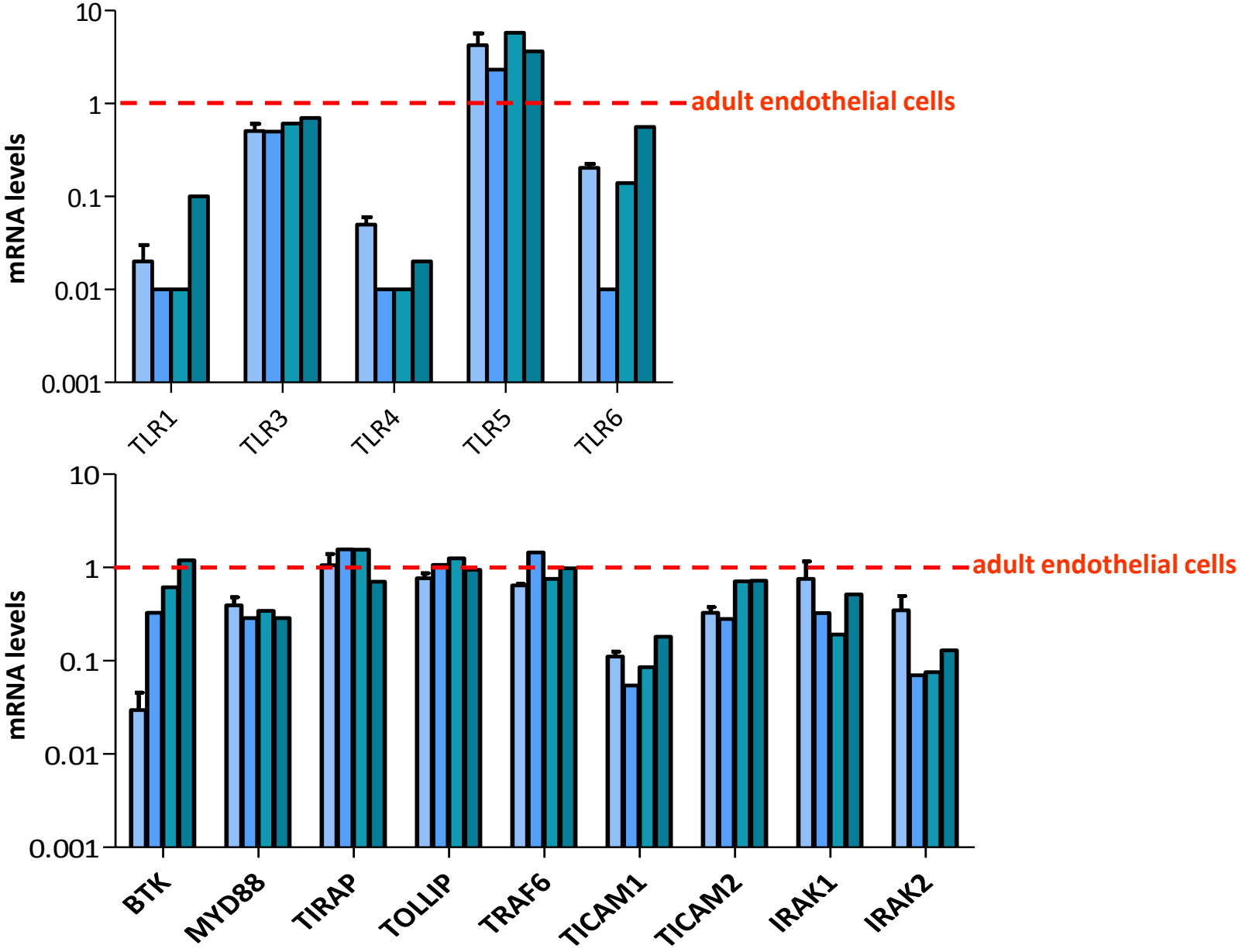
Pattern-recognition receptors: Toll-like receptors

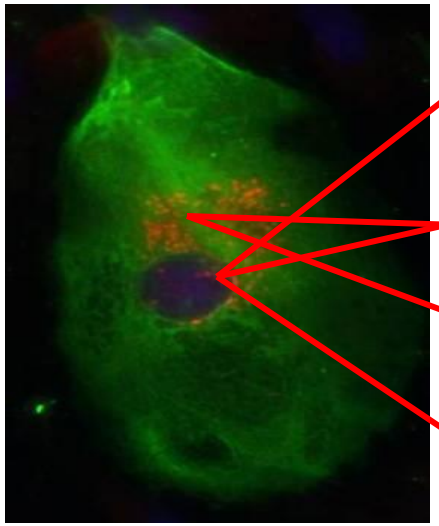


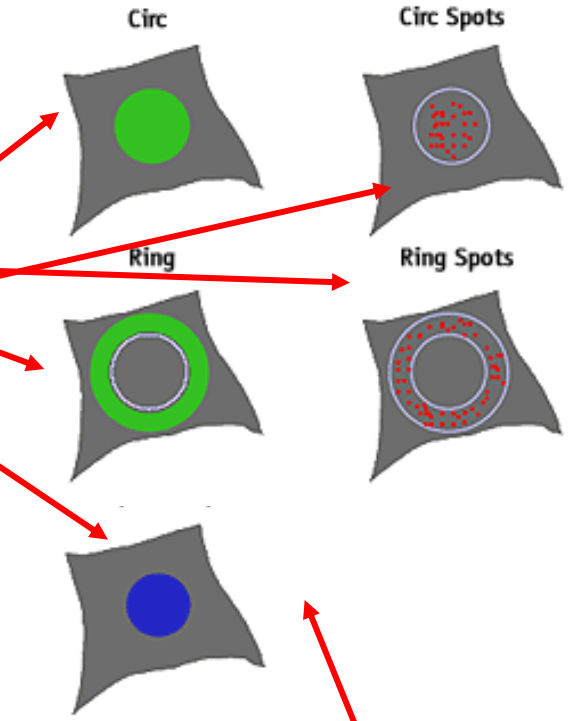
from Netea MG, van der Meer JW. NEJM 2011

Toll-like receptor-related pathways in human embryonic stem cell-derived endothelial cells



Földes et al. Plos ONE. 2010

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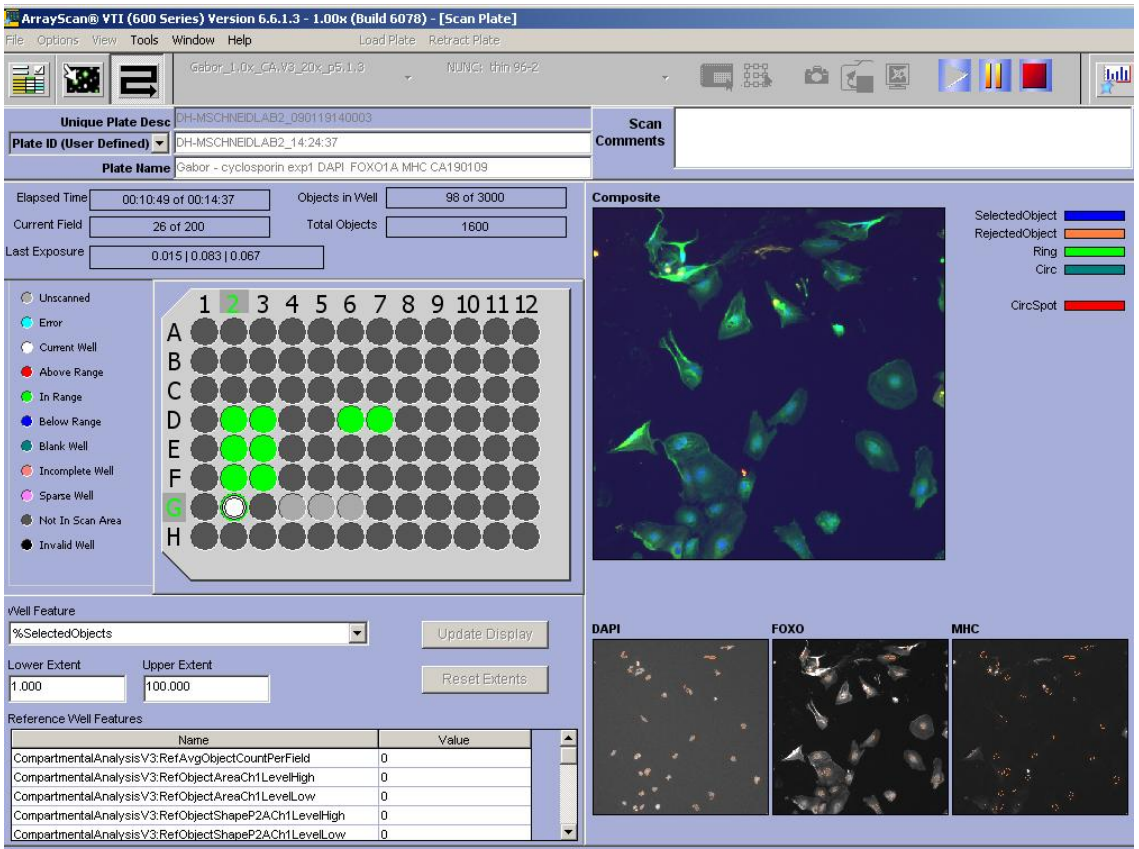
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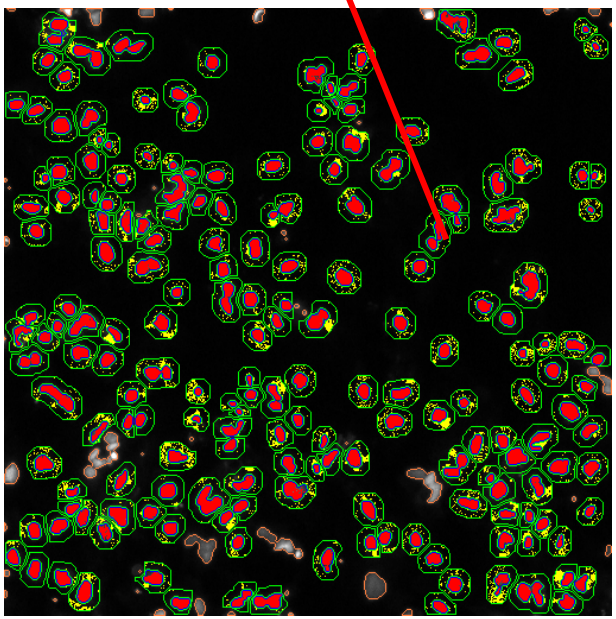
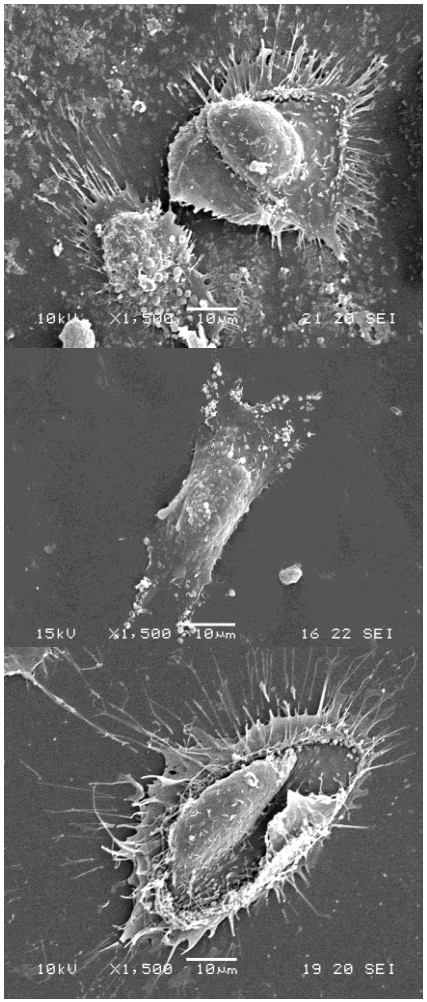
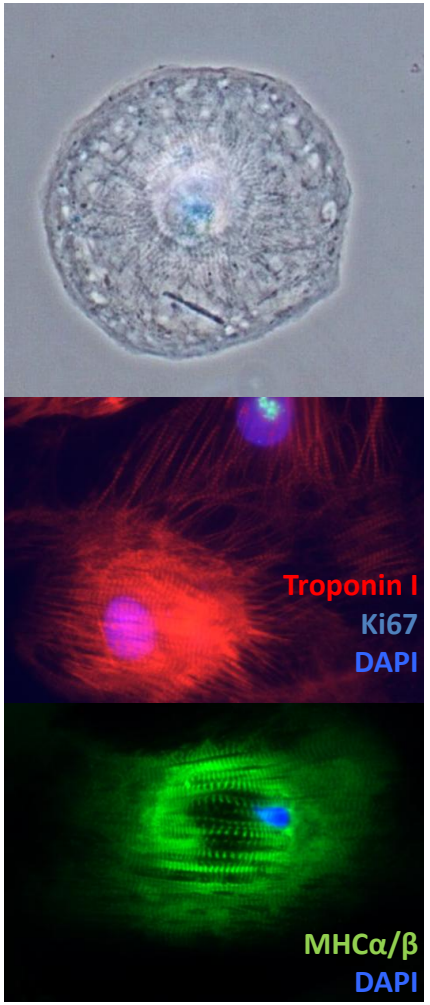


Isolated human embryonic stem cell-derived heart muscle cells





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Use of human embryonic stem cell derivatives

Toxicology for the twenty-first century

Thomas Hartung

The testing of substances for adverse effects on humans and the environment needs a radical overhaul if we are to meet the challenges of ensuring health and safety.

Synthetic chemicals have been components of consumer products for just over a century. A system for identifying which chemicals pose a danger to individuals and the environment was first put in place about 80 years ago. But after several productive decades, in which a patchwork of testing approaches was formed, fewer and fewer of the latest scientific developments were incorporated. The system of regulatory toxicology fell asleep, much like the fairy-tale character Snow White when she bit into the poisonous apple. In the case of toxicology, the poison was international guidelines. This international harmonization was tempting because it allowed manufacturers and suppliers to use fewer resources, and it overcame barriers to trade in global markets. But implementing these guidelines came at a price: the slow and complicated international consensus process hindered self-criticism and modernization of the field of toxicology.

There is almost no other scientific field in which the core experimental protocols have remained nearly unchanged for more than 40 years. Yet consumers continually increase their expectations about the safety of products. One recent effect of this was the instigation of the largest safety assessment of chemicals that has ever been carried out: the European Union introduced the regulation known as Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) by legislation in 2007. Whereas new chemicals have been systematically evaluated in the European Union and the United States for about a quarter of a century, the safety of any chemicals produced before 1981 (which includes 97% of the major chemicals in use, and more than 99% of chemicals produced by volume) has not necessarily been properly addressed. In fact, it is estimated that data for 86% of the chemicals are lacking, and the REACH process seeks to redress this. The regulation affects 27,000 companies, which are required to provide information on the toxic properties and uses of 30,000 chemicals, after a pre-registration phase in 2008. But REACH might turn out to be like the prince whose kiss awoke Snow White after a long sleep, rousing toxicology at last.

Defining the problem

So what is wrong with the current approach to toxicology testing? An ideal study to understand whether an agent is harmful to humans would require an extremely large number of human subjects who are representative of the diversity of humans and who are unknowingly exposed to the agent under realistic conditions. All possible effects should then be assessed. If there is any deviation from these experimental conditions, which are unrealistic and unethical, the study will provide only an approximation of the real situation — it is a model. The crucial question therefore is how useful are the current models, which are mostly animal models, and how incorrect are they? Given that about €10 billion (US$14 billion) is spent on animal experimentation worldwide every year (about €2 billion of which is for toxicological studies), and given that more than 100 million experimental animals are used[1] and that products worth 65.6 trillion are regulated by such testing, the question is certainly appropriate. It encompasses four main issues.

The first issue is the extent to which animal models reflect human responses. It is clear that the use of animals has limitations[2]: we are not 70-kg rats; we take up substances differently; we metabolize them differently; we live longer (allowing certain diseases to develop and prompting evolutionary adaptations to protect against them); and we are exposed to a multitude of environmental factors. However, few studies have systematically measured the accuracy of animal models. In one example, results from animal models were compared with information from poison centres: comparing the dose of a chemical that is lethal to 50% (LD_{50}) of rats tested and the lethal concentration of the same chemical in the blood of humans showed a rather poor correlation (coefficient of correlation of 0.56; unpublished observations from an international validation study[3]). Similarly, in another study, 40% of the chemicals that irritated the skin of rabbits were found not to be irritants in the skin 'patch test' in humans[4].

Given the overall lack of data, this problem can be considered in more general terms by looking at how one species models for another. In several animal species, similar experiments with the same agents have been carried out, and there is no reason to assume that, for example, mice, rats and rabbits predict each other's response to a lesser extent than they predict that of humans. Typical results from such studies show agreement between animal species for 53–60% of chemicals[5,6].

Similar results have also been obtained for pharmaceuticals (as opposed to chemicals) that have been tested in humans. In one study, 43% of toxic effects in humans were correctly predicted by tests in rodents, and 63% by tests when non-rodent animals were also included[7]. It is clear therefore that many adverse effects are not uncovered by such traditional tests. This is also evident in data from the pharmaceutical industry, showing that 20% of the failure of drug candidates occurs as a result of toxicity only after the drugs have been administered to humans in clinical trials[8]. And it is estimated that 6.7% of hospitalized patients experience unexpected adverse reactions to drugs (1 in 20 of which are fatal)[9], showing the limitations of anticipating toxic effects from preclinical animal studies. To improve the toxicity assessment, tests are often carried out in two animal species: usually substances that show no toxic effect in one species are then tested in another species to improve the likelihood of finding any toxic properties. This increases the sensitivity of testing (that is, it increases the proportion of toxic substances that are found) but at the cost of increasing the number of false positives (when non-toxic chemicals seem to be toxic in the tests carried out).

The second key issue facing animal testing relates to the study design, particularly to the highly precautionary (conservative) approach that is taken at present. To limit costs and animal numbers, toxicity testing is typically carried out with the maximum dose of the chemical that can be tolerated, which has previously been determined. Such doses can be more than 1,000-fold higher than the doses intended for humans (in terms of milligrams per kilogram body weight, for example). This strategy yields many false positives and further diminishes the correlation between findings in animal models and humans[10].

with scepticism from experts. Last December, an Indiana physician named Gary Erdy told Illinois officials he had a new photograph of an ivory-billed woodpecker from the same area. They later revoked his search permit.

Meanwhile, experts are dealing with protests by Daniel Rainsong, a landscaper based in Ames, Iowa, who says he recently photographed an ivory-billed woodpecker near the Sabine River in east Texas. Rainsong filed a formal complaint earlier this month alleging ethical and financial misconduct, because biologists he approached would not come with him to the Sabine region to confirm the sighting so that he could collect a $50,000 reward.

Rohrbaugh says the Cornell team will release an analysis of Rainsong's photo in about a week. ■

Rex Dalton

1. Fitzpatrick, J. W. *et al. Science* **308**, 1460–1462 (2005).
2. Dalton, R. *Nature* **437**, 188–190 (2005).
3. Sibley, D. A., Bevier, L. R., Patten, M. A. & Elphick, C. S. *Science* **311**, 1555 (2006).
4. Fitzpatrick, J. W., Lammertink, M., Luneau, M. D. Jr, Gallagher, T. W. & Rosenberg, K. V. *Science* **311**, 1555 (2006).
5. Scott, J. M. *et al. Avian Conserv. Ecol.* **3**, 3 (2008).
6. Roberts, D. L., Elphick, C. S. & Reed, J. M. *Conserv. Biol.* **24**, 189–196 (2010).
7. Elphick, C. S., Roberts, D. L. & Reed, J. M. *Biol. Conserv.* doi:10.1016/j.biocon.2009.11.026 (2009).

CATCHING THE WIND SPEEDS MIGRATION
Moths and butterflies hitch rides on jet streams.
go.nature.com/11M6Qo

Testing time for stem cells

The drug industry is keener on stem-cell technologies than ever before — and not just as a source of new treatments. A wave of new partnerships aims to use stem cells as a way to screen other potential drug candidates.

In the latest such example, Roche last week announced a deal worth some US$20 million with Harvard University in Cambridge, Massachusetts, and Massachusetts General Hospital in Boston. Roche, based in Basel, Switzerland, will use cell lines and protocols developed by academic researchers to screen for drugs to treat cardiovascular disease and other conditions.

Because relevant human cell types are often unavailable, curren
cells from rodents or hum
than the ones researchers
hope is that stem cells cou
the type of cells relevant f

The deal is the latest in
partnerships. Within the
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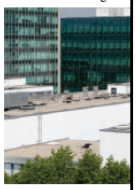


A deal between Roche and t
on stem-cell-based drug scr

moment it's all really early days," says Stephen Minger, who left King's College London last year to head GE Healthcare's efforts to develop drug-screening tests with cells derived from human embryonic stem cells.

"What needs to be demonstrated is the actual application of the technology," adds John Walker, the chief executive of iPierian, a stem-cell company in San Francisco, California. The firm has created motor neurons using induced pluripotent stem (iPS) cells derived from people with and without spinal muscular atrophy, a neurodegenerative disease. Company scientists are investigating whether drug

Burgeoning stem cell product market lures major suppliers

Life sciences supplier Lonza has struck a deal with Axiogenesis of Cologne, Germany, to offer mouse embryonic stem cell–derived cardiomyocytes in its product catalog. The agreement, signed in March, is the latest move of several large reagent and material suppliers to grab a slice of the rapidly expanding market for stem cell products for use in *in vitro* assays and testing kits for predictive toxicology. Life Technologies, which was formed from the merger of Carlsbad, California–based Invitrogen and Foster City, California–based Applied Biosciences, has been aggressively marketing its range of embryonic stem cell (ESC) and induced-pluripotent stem cell (iPSC) reagents of animal-free origin, and GE Healthcare, of Chalfont St. Giles, UK, has forged a two-year partnership with Geron in Menlo Park, California, to scale up production of differentiated cells from human ESCs. Merck's acquisition of US reagent and materials supplier Millipore also signals the growing interest of big pharma in iPSC expertise and capacity—a signal of increasing receptiveness within the pharmaceutical industry to embrace stem cell technology. Indeed, with the political climate in the US now favorable, big pharma is openly pursuing the use of such cells in its preclinical research programs, opening up a sizeable market.

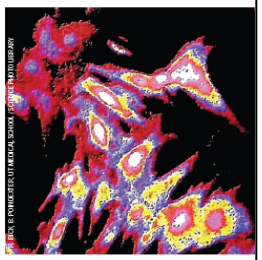
The biopharma industry spends over $4.98 billion a year on R&D for new therapies. The cost of taking a compound through to late-stage development continues to escalate, yet, at the same time, up to 30% of leads fail because of an unacceptable safety profile. Stem cell–derived products are potentially a useful resource for toxicity screens that could identify leads with unacceptable safety profiles. Until now, the scarcity, expense and batch-to-batch variability of differentiated cells derived from donor tissues have hampered the use of such primary cells in preclinical research. With the advent of stem cell–derived products that can potentially create differentiated cells of all the different lineages—endoderm, mesoderm and ectoderm—a plentiful, consistent and competitive source of cells is becoming available for drug screening.

Industry is increasingly recognizing the value of such products for two reasons. First, stem cell products provide a reliable source of primary cells, avoiding the expense, ethical issues and quality control problems associated with deriving such cells from human donor and cadaver tissue. Over the past 20 years, Basel-based Lonza has supplied the research community with primary cells from human donor tissue, says Alex Batchelor, the company's head of marketing-drug discovery. "Unfortunately, some of the more difficult cell types [to obtain from donors] are the most interesting ones for researchers: neural cells, cardiomyocytes, hepatocytes and possibly pancreatic cells." Products differentiated from pluripotent cells can meet that demand for material for testing.

A second impetus for the increased interest in stem cell products is their ability to reduce dependency on human tissue and the number of animals used in drug testing—an issue that is particularly troublesome for the public perception of pharma companies in Europe, according to Mahendra Rao, vice president for research in stem cells & regenerative medicine of Life Technologies in Carlsbad, California. Rao views the recent boost in stem cell interest from pharma companies as a pleasant surprise. The company has not disclosed figures relating to these deals, but Rao points to the flurry of deals including the recent $7.6 billion paid by Darmstadt, Germany–based Merck for Millipore (page 536). Last year, another pharma giant, Paris-based Sanofi-aventis, formed a partnership with the Salk Institute by which the company agreed to provide, among other things, funding to support the Institute's stem cell facility. He attributes this progress—particularly in the US—to both advances in iPSC research and the Obama administration's more receptive policies toward human ESCs.

The move into the marketplace for differentiated products from human pluripotent cells has now become a steady flow (Table 1). In late 2009, Cellular Dynamics International (CDI) in Madison, Wisconsin, began selling iCell Cardiomyocytes (cardiomyocytes derived from human iPSCs), the first such iPSC product to be commercialized. Though CDI originally looked at preclinical toxicology and safety testing as the primary application of cardiomyocytes, the availability of the cells has stimulated new ideas for their use, says chief commercialization officer, Chris Kendrick-Parker. "Already our customers have been able to understand how they can induce a disease state in these cells, which has therefore now moved them into a discovery model," he says. CDI produces and ships billions of cardiomyocytes per day, according to Kendrick-Parker. The company has delivered cardiomyocytes to more than half the top 20 pharma companies, he adds.

GE Healthcare is close behind. In a partnership with Geron, the UK company is scaling up production of differentiated cells from human hESCs at its Cardiff research center; cardiomyocytes will be launched as a commercial product for toxicology testing and drug discovery later this year. According to Stephen Minger, R&D director for cell technologies at GE Healthcare, the initial interest level is huge. After seeing the cells' attributes, potential partners have said, "If you can supply the cells that you just showed us on a routine basis, we will buy a lot of them," Minger adds.



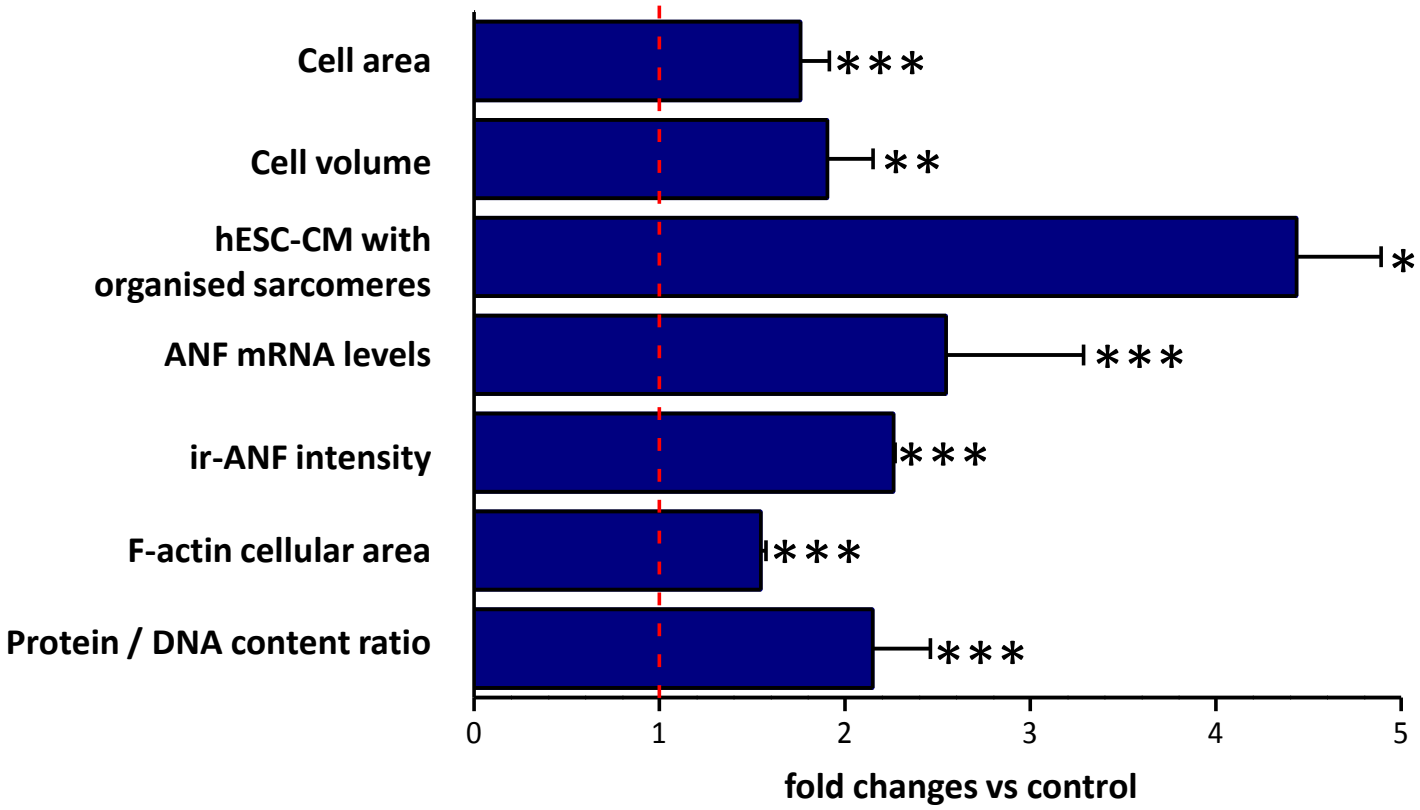
BEN K. POPROCKI/UT MEDICAL SCHOOL, CSHL/PHOTO LIBRARY

Beating heart cells. Firms are using industrialized quantities of stem cell–derived human cardiomyocytes to predict toxicity and screen for efficacy in a dish.

Cardiomyocytes are the initial target of many programs. The differentiation protocols for these cells are robust, and they have a clear visual readout: the cells contract or 'beat' *in vitro*. Hepatocytes, for example, require trickier protocols and several biochemical readouts to determine whether they have differentiated appropriately, Minger says. Both CDI and GE Healthcare are working towards large-scale production of hepatocytes and other differentiated cell types for use in toxicology and drug discovery screening.

One drug company that is embracing the use of such cells in preclinical research is Roche of Basel. The company began a collaboration with CDI in March 2008 to test drug development candidates for their potential to cause toxicity on cardiomyocytes derived from hESCs and iPSC-derived cells. With the Institute for Stem Cell Therapy and Exploration of Monogenic Diseases (I-STEM), for instance, an academic center near Paris, Roche is matching its high-throughput screening expertise with I-STEM's hESC-derived neuronal cells to search for new drug candidates for neurodegenerative and psychiatric disorders. Earlier this year, Roche began working with stem cell researchers at Massachusetts General Hospital in Boston and Harvard University in Cambridge, Massachusetts, to develop cellular models for metabolic and cardiovascular diseases using

Cellular hypertrophy of hESC-CM in response to phenylephrine



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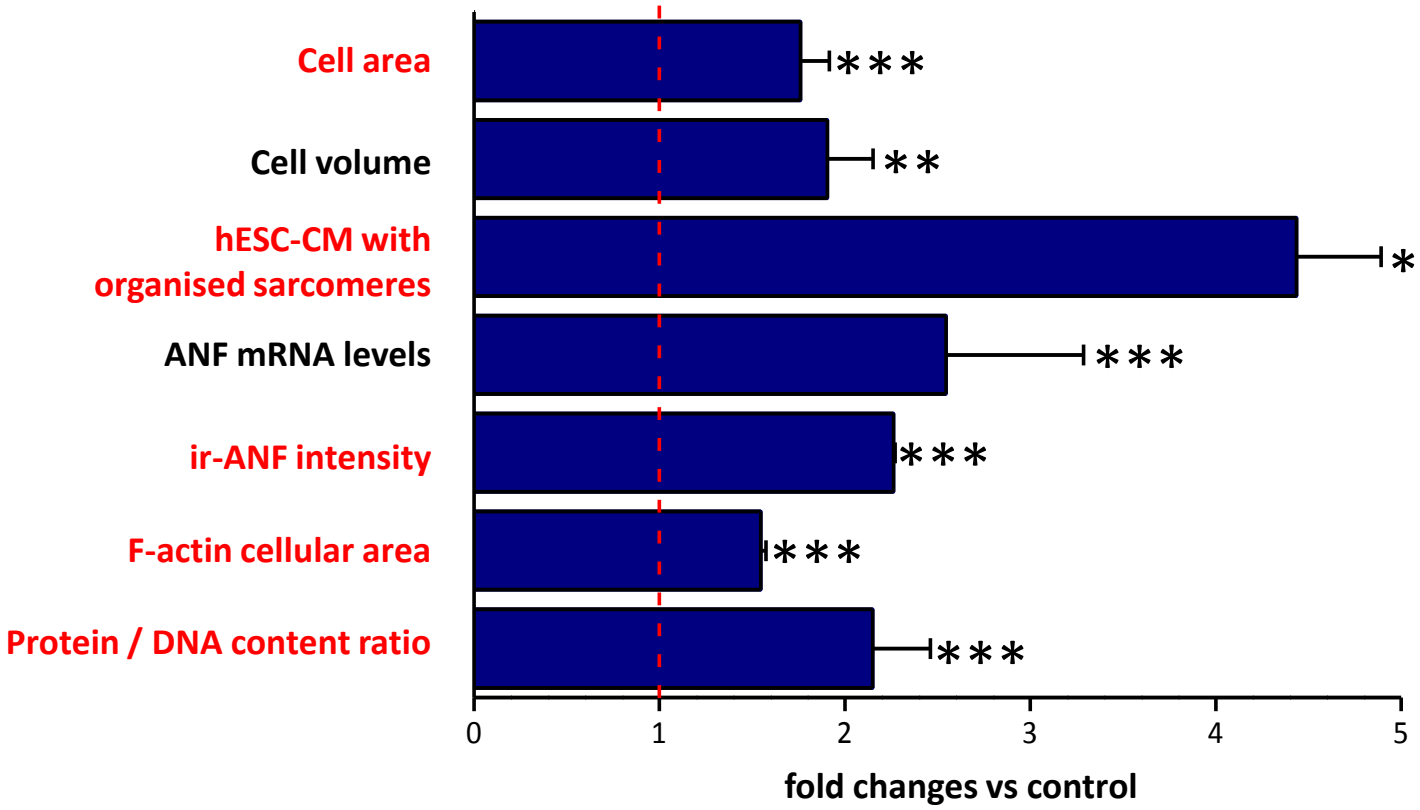
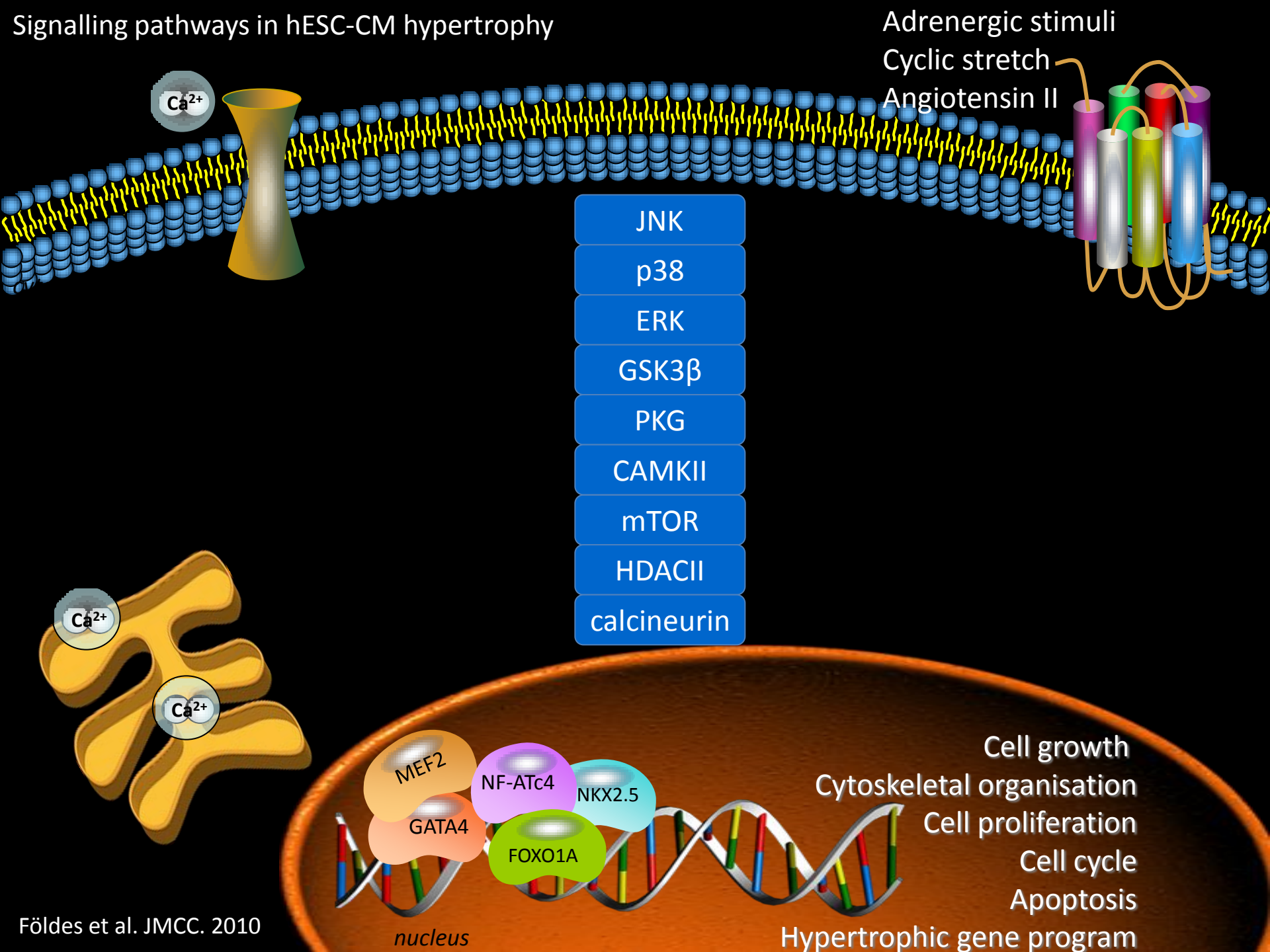
Cellular hypertrophy of hESC-CM in response to phenylephrine

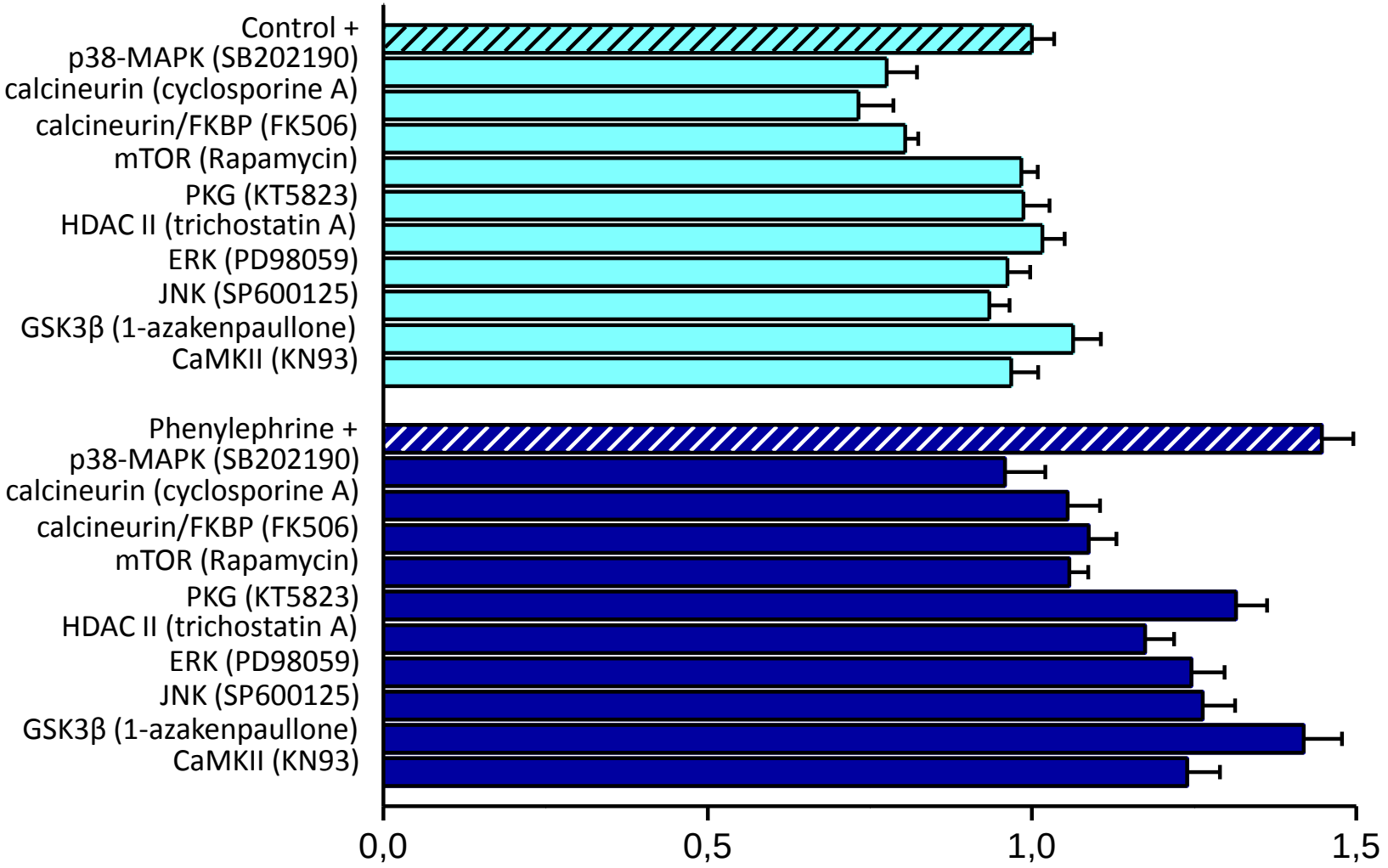


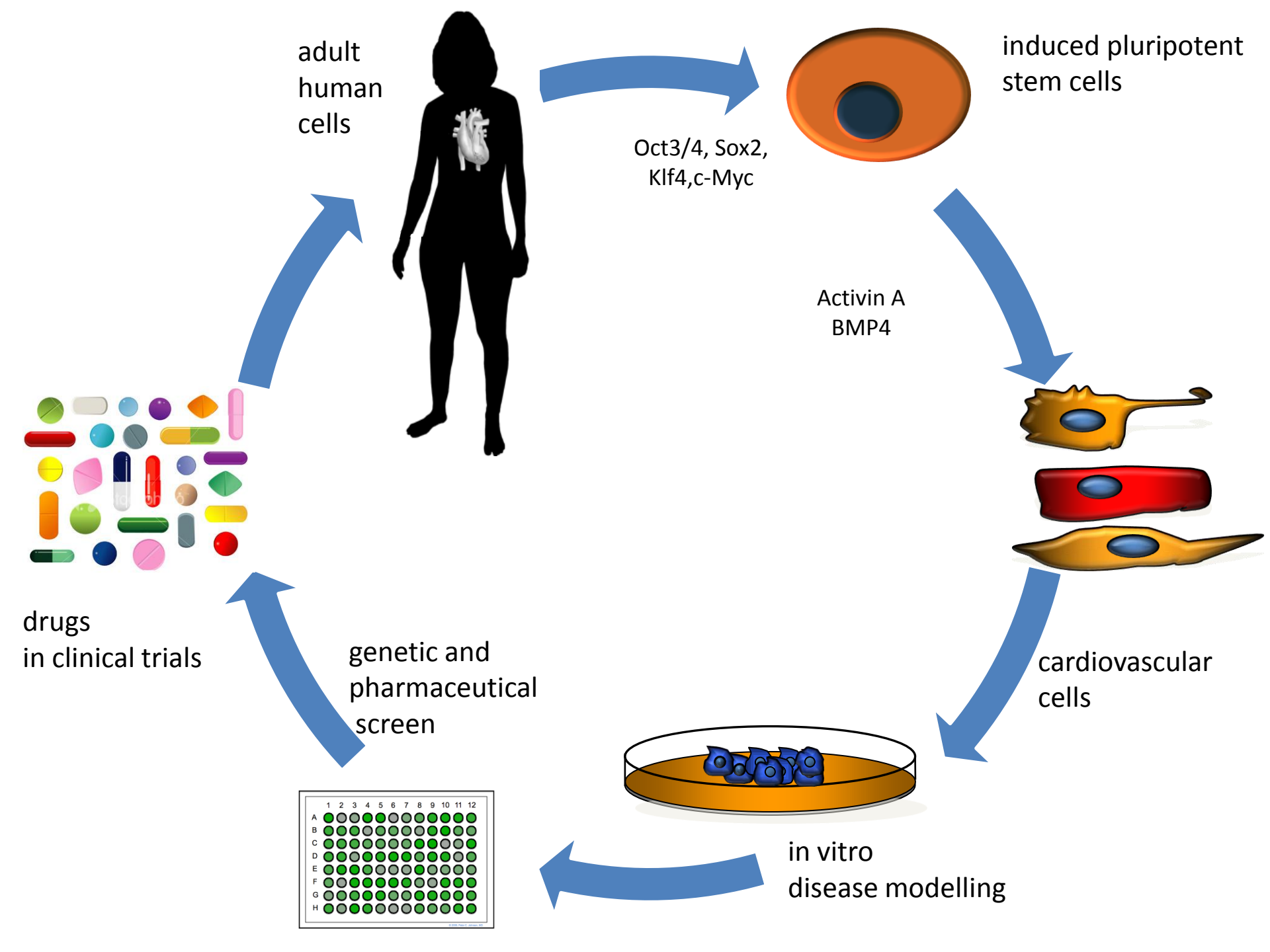
Phenotypic and biochemical assays on hESC-CM by arrayscan:
Structural proteins
Signalling molecules and nuclear translocation processes
Sarcomeric organisation

Földes et al. JMCC. 2010


Signalling pathways in hESC-CM hypertrophy
Adrenergic stimuli
Cyclic stretch
Angiotensin II
Ca2+
JNK
p38
ERK
GSK3β
PKG
CAMKII
mTOR
HDACII
calcineurin
Ca2+
Ca2+
MEF2
NF-ATc4
NKX2.5
GATA4
FOXO1A
nucleus
Cell growth
Cytoskeletal organisation
Cell proliferation
Cell cycle
Apoptosis
Hypertrophic gene program
Földes et al. JMCC. 2010

Small molecule inhibitors of putative hypertrophy pathways in hESC-CM



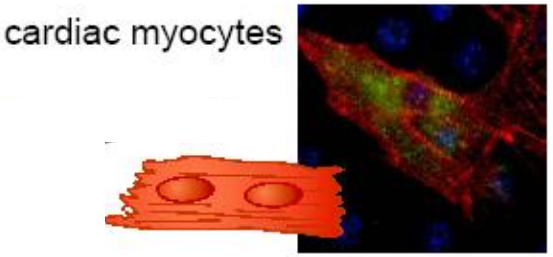

adult
human
cells
induced pluripotent
stem cells
Oct3/4, Sox2,
Klf4,c-Myc
Activin A
BMP4
cardiovascular
cells
in vitro
disease modelling
genetic and
pharmaceutical
screen
drugs
in clinical trials

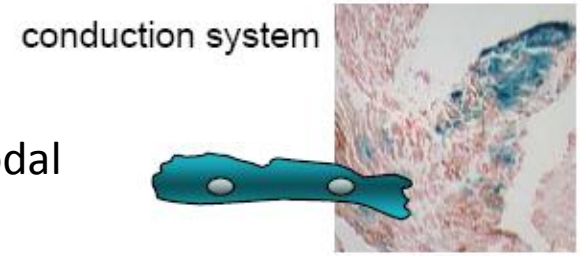
Challenges and limitations of the technology for hESC-CM and hESC-EC use

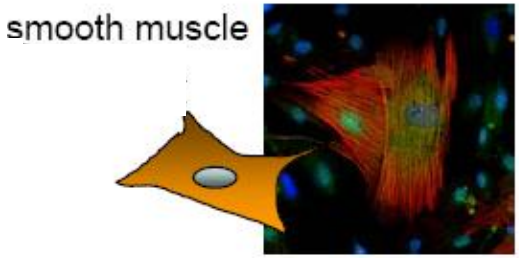
Relative to use of adult/neonatal models challenges remain in terms of

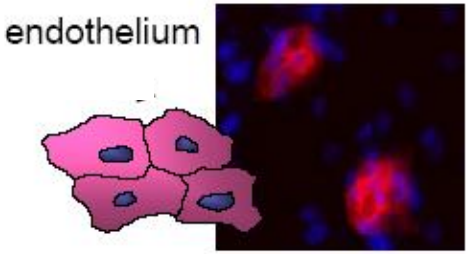
- hESC-CM maturation over long time periods
- Mixed cardiac phenotype: cultures with atrial-ventricular-nodal hESC-CM / arterial-venous-lymphatic hESC-EC
- Mixture with non-cardiovascular cells
- Automated assays and high throughput methodologies

for acute contractile/calcium/electrical effects









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