

# World Journal of *Stem Cells*

*World J Stem Cells* 2019 December 26; 11(12): 1020-1141



**REVIEW**

- 1020** Induced pluripotent stem cells for therapy personalization in pediatric patients: Focus on drug-induced adverse events  
*Genova E, Cavion F, Lucafò M, Leo LD, Pelin M, Stocco G, Decorti G*
- 1045** Influence of olive oil and its components on mesenchymal stem cell biology  
*Casado-Díaz A, Dorado G, Quesada-Gómez JM*
- 1065** Three-dimensional cell culture systems as an *in vitro* platform for cancer and stem cell modeling  
*Chaicharoenaudomrung N, Kunhorm P, Noisa P*
- 1084** Small molecules for mesenchymal stem cell fate determination  
*Cheng YH, Dong JC, Bian Q*
- 1104** Mechanoresponse of stem cells for vascular repair  
*Tian GE, Zhou JT, Liu XJ, Huang YC*

**ORIGINAL ARTICLE****Basic Study**

- 1115** Anti-osteoarthritis effect of a combination treatment with human adipose tissue-derived mesenchymal stem cells and thrombospondin 2 in rabbits  
*Shin K, Cha Y, Ban YH, Seo DW, Choi EK, Park D, Kang SK, Ra JC, Kim YB*
- 1130** MiR-301a promotes embryonic stem cell differentiation to cardiomyocytes  
*Zhen LX, Gu YY, Zhao Q, Zhu HF, Lv JH, Li SJ, Xu Z, Li L, Yu ZR*

**ABOUT COVER**

Editorial Board Member of *World Journal of Stem Cells*, Manabu Akahane, MD, PhD, Associate Professor, Public Health, Health Management and Policy, Nara Medical University School of Medicine, Nara 634-8521, Japan

**AIMS AND SCOPE**

The primary aim of *World Journal of Stem Cells (WJSC, World J Stem Cells)* is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

*WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryoid bodies, embryonal carcinoma stem cells, hemangioblasts, hematopoietic stem cells, lymphoid progenitor cells, myeloid progenitor cells, etc.

**INDEXING/ABSTRACTING**

The *WJSC* is now indexed in PubMed, PubMed Central, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Biological Abstracts, and BIOSIS Previews. The 2019 Edition of Journal Citation Reports cites the 2018 impact factor for *WJSC* as 3.534 (5-year impact factor: N/A), ranking *WJSC* as 16 among 26 journals in Cell and Tissue Engineering (quartile in category Q3), and 94 among 193 journals in Cell Biology (quartile in category Q2).

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Responsible Electronic Editor: Yan-Xia Xing

Proofing Production Department Director: Yun-Xiaojuan Wu

**NAME OF JOURNAL**

*World Journal of Stem Cells*

**ISSN**

ISSN 1948-0210 (online)

**LAUNCH DATE**

December 31, 2009

**FREQUENCY**

Monthly

**EDITORS-IN-CHIEF**

Tong Cao, Shengwen Calvin Li, Carlo Ventura

**EDITORIAL BOARD MEMBERS**

<https://www.wjgnet.com/1948-0210/editorialboard.htm>

**EDITORIAL OFFICE**

Jin-Lei Wang, Director

**PUBLICATION DATE**

December 26, 2019

**COPYRIGHT**

© 2019 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**

<https://www.wjgnet.com/bpg/gerinfo/204>

**GUIDELINES FOR ETHICS DOCUMENTS**

<https://www.wjgnet.com/bpg/GerInfo/287>

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**

<https://www.wjgnet.com/bpg/gerinfo/240>

**PUBLICATION MISCONDUCT**

<https://www.wjgnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## Induced pluripotent stem cells for therapy personalization in pediatric patients: Focus on drug-induced adverse events

Elena Genova, Federica Cavion, Marianna Lucafò, Luigina De Leo, Marco Pelin, Gabriele Stocco, Giuliana Decorti

**ORCID number:** Elena Genova (0000-0002-6692-6573); Federica Cavion (0000-0001-6901-5866); Marianna Lucafò (0000-0003-1355-3782); Luigina De Leo (0000-0003-4013-1630); Marco Pelin (0000-0002-4306-7411); Gabriele Stocco (0000-0003-0964-5879); Giuliana Decorti (0000-0002-9714-6246).

**Author contributions:** The authors contributed equally to this work in all aspects.

**Supported by** Italian Ministry of Health (IRCCS Burlo Garofolo), No. RC 7\_2014, No. RC 10\_2019; progetto fondo di ricerca Ateneo, Università di Trieste, No. FRA2018

**Conflict-of-interest statement:** Authors of this manuscript have no conflicts of interest to disclose.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Received:** March 23, 2019

**Elena Genova**, PhD School in Reproduction and Development Sciences, University of Trieste, Trieste 34127, Italy

**Federica Cavion, Marco Pelin, Gabriele Stocco**, Department of Life Sciences, University of Trieste, Trieste 34127, Italy

**Marianna Lucafò, Luigina De Leo, Giuliana Decorti**, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste 34137, Italy

**Giuliana Decorti**, Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste 34127, Italy

**Corresponding author:** Gabriele Stocco, PhD, Assistant Professor, Department of Life Sciences, University of Trieste, via Fleming 22, Trieste 34127, Italy. [stoccog@units.it](mailto:stoccog@units.it)  
**Telephone:** +39-40-5588634

### Abstract

Adverse drug reactions (ADRs) are major clinical problems, particularly in special populations such as pediatric patients. Indeed, ADRs may be caused by a plethora of different drugs leading, in some cases, to hospitalization, disability or even death. In addition, pediatric patients may respond differently to drugs with respect to adults and may be prone to developing different kinds of ADRs, leading, in some cases, to more severe consequences. To improve the comprehension, and thus the prevention, of ADRs, the set-up of sensitive and personalized assays is urgently needed. Important progress is represented by the possibility of setting up groundbreaking patient-specific assays. This goal has been powerfully achieved using induced pluripotent stem cells (iPSCs). Due to their genetic and physiological species-specific differences and their ability to be differentiated ideally into all tissues of the human body, this model may be accurate in predicting drug toxicity, especially when this toxicity is related to individual genetic differences. This review is an up-to-date summary of the employment of iPSCs as a model to study ADRs, with particular attention to drugs used in the pediatric field. We especially focused on the intestinal, hepatic, pancreatic, renal, cardiac, and neuronal levels, also discussing progress in organoids creation. The latter are three-dimensional *in vitro* culture systems derived from pluripotent or adult stem cells simulating the architecture and functionality of native organs such as the intestine, liver, pancreas, kidney, heart, and brain. Based on the existing knowledge, these models are powerful and promising tools in multiple clinical applications including toxicity screening,

**Peer-review started:** March 26, 2019  
**First decision:** August 1, 2019  
**Revised:** September 5, 2019  
**Accepted:** October 14, 2019  
**Article in press:** October 14, 2019  
**Published online:** December 26, 2019

**P-Reviewer:** Bragança J, Sidhu KS, Song L  
**S-Editor:** Dou Y  
**L-Editor:** Filipodia  
**E-Editor:** Ma YJ



disease modeling, personalized and regenerative medicine.

**Key words:** Induced pluripotent stem cells; Organoids; Adverse drug reactions; Intestinal toxicity; Hepatic toxicity; Pancreatic toxicity; Nephrotoxicity; Cardiotoxicity; Neurotoxicity

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Adverse drug reactions (ADRs) are major clinical problems, especially in pediatric patients, who may respond differently to drugs with respect to adults. This up-to-date review focuses on the employment of patient-derived induced pluripotent stem cells and related systems (*i.e.* stem cell-derived organoids) to study ADRs in adults, and wherever available, in the pediatric field. We especially focused on the intestinal, hepatic, pancreatic, renal, cardiac, and neuronal levels, in which the major ADRs are usually observed. Due to their genetic and physiological species-specific differences, these models may be accurate in predicting drug toxicity.

**Citation:** Genova E, Cavion F, Lucafò M, Leo LD, Pelin M, Stocco G, Decorti G. Induced pluripotent stem cells for therapy personalization in pediatric patients: Focus on drug-induced adverse events. *World J Stem Cells* 2019; 11(12): 1020-1044

**URL:** <https://www.wjnet.com/1948-0210/full/v11/i12/1020.htm>

**DOI:** <https://dx.doi.org/10.4252/wjsc.v11.i12.1020>

## ADVERSE DRUG REACTIONS

Adverse drug reactions (ADRs) are major clinical problems<sup>[1]</sup>, especially for special populations such as pediatric patients<sup>[2,3]</sup>. Indeed, a significant number of drugs can cause adverse effects leading, in some cases, to patients' hospitalization, permanent disability, or even death. ADRs are costly for pharmaceutical industries in terms of drug withdrawal from the market<sup>[4]</sup>, but also in terms of clinical and economical efforts needed to resolve side effects<sup>[1]</sup>. In order to avoid these problems that affect patients' health and greatly increase costs related to treatment, it is necessary to improve clinical trial strategies. In addition, both *in vivo* and *in vitro* models are needed that better assess and evaluate drug safety. Due to newly discovered technologies and progress made in this area, this goal seems achievable<sup>[5-7]</sup>. Indeed, to date, it is possible to establish more sensitive and personalized assays leading to a better comprehension, and thus prevention, of ADRs. One of the most important advances in this field is the possibility to set up groundbreaking patient-specific assays. Indeed, ADRs are related to individual genetic patients' background, leading to a wide range of toxicities of different severities<sup>[8]</sup>. Another important point is that pediatric patients may respond differently to drugs than adults and are susceptible to developing different patterns of ADRs, leading, in some cases, to more severe consequences<sup>[1-3]</sup>. One of the most powerful tools that can be used to individually model drug response and ADR development is represented by induced pluripotent stem cell (iPSC) technology, which was discovered by Takahashi *et al.*<sup>[9]</sup> more than 10 years ago.

iPSCs can be used to evaluate safety during drug preclinical screening, possibly replacing the use of animal models or immortalized human cell lines. Due to genetic and physiological species-specific differences, these models may be accurate in predicting drug toxicity, especially when this toxicity is related to individual genetic differences<sup>[9]</sup>. Indeed, many factors may influence drug pharmacokinetics and pharmacodynamics as well as the development of adverse effects, for instance: (1) Polymorphisms in genes encoding for drug-metabolizing enzymes and transporters, ion channels and receptors, possibly affecting their expression and/or activity<sup>[10]</sup>; (2) Epigenetic alterations such as DNA methylation, histone modification, microRNAs, mRNA instability, and nucleosome positioning<sup>[11]</sup>; and (3) Environmental and nongenetic factors including body mass index and behavioral patterns, concomitant diseases, in particular liver and kidney diseases, and developmental factors, especially those in early life<sup>[12]</sup>.

In this review, we explored the state of the art of iPSCs as a model to study ADRs in adults, and wherever available, in the pediatric field. In particular, we focused on



intestinal, hepatic, pancreatic, renal, cardiac and neuronal levels, also discussing the progress in the creation of organoids (Figure 1).

## ADVERSE DRUG REACTIONS IN PEDIATRIC PATIENTS

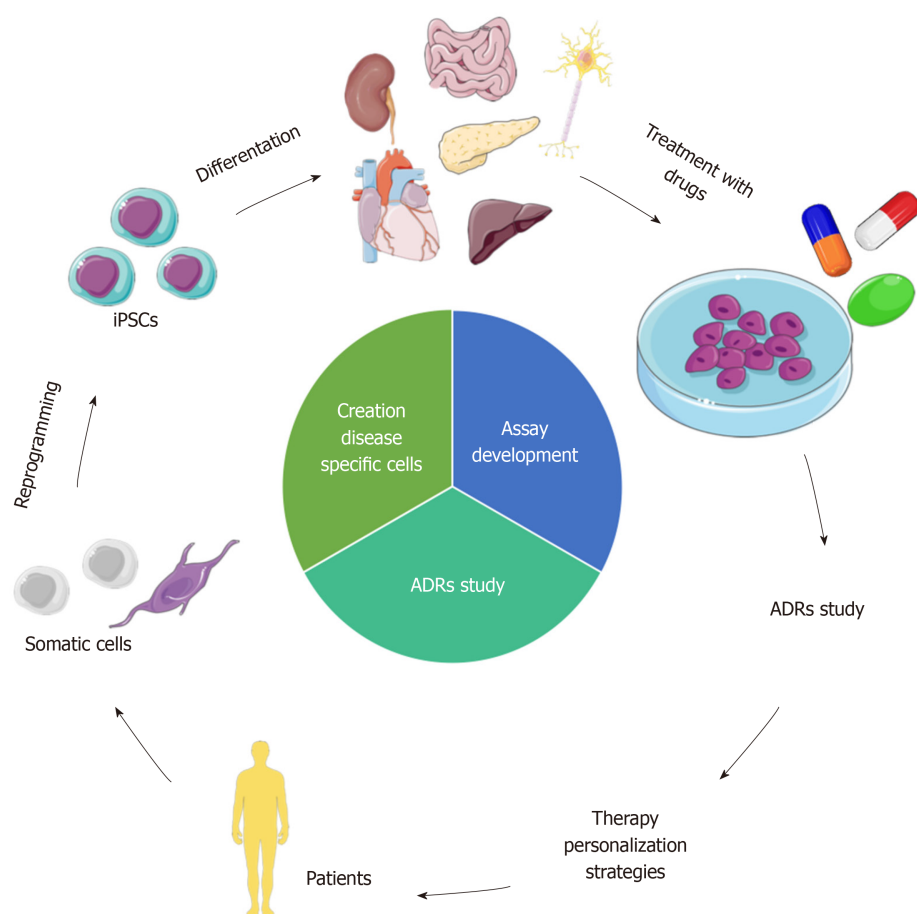
Children can respond differently to many drugs and can present different ADRs than adults<sup>[2,3]</sup>. The reason is mainly the variation in the pharmacokinetic and pharmacodynamic profiles between pediatric and adult patients<sup>[13,14]</sup>. In the era of personalized medicine, it is important to screen and identify genetic variations related to the predisposition to ADR development for certain drugs<sup>[15,16]</sup>. However, in pediatric patients, ADRs have not been studied as thoroughly as in adults. The clinical and research experience on drug safety for children is limited and more works are needed<sup>[17]</sup>. One of the reasons for the lack of these data is that, for ethical reasons, the effects of most drugs have been analyzed in clinical trials only in adults, resulting in limited knowledge on children's responses<sup>[17]</sup>. To date, there is strong interest into therapy personalization for children to further explore, study, and prevent possible new and severe ADRs. In order to avoid the well-known ethical limitation of pediatric trials, to increase the comprehension of drug response in children, and establish safer and personalized treatment, it is possible to use innovative technologies such as iPSCs<sup>[5,18]</sup>. Indeed, several specific pediatric problems could be addressed using iPSCs; for example, this technology is ideal to develop innovative patient-specific models of rare or genetic diseases that often occur in pediatric populations<sup>[13]</sup>. Moreover, several drugs used in pediatric patients lead to adverse effects in organs that are not easily accessible, such as pancreatitis for asparaginases<sup>[18]</sup> for the treatment of acute lymphoblastic leukemia.

## INDUCED PLURIPOTENT STEM CELLS, THERAPY PERSONALIZATION, AND ADRs

Patients' somatic cells can be reprogrammed into iPSCs using the four Yamanaka's factors<sup>[5]</sup>. iPSCs preserve the donor's genetic heritage and are useful for creating patient-specific models<sup>[19]</sup>. For a decade, scientists have been using iPSCs to study complex diseases and to obtain tissues or cells otherwise not simply accessible from patients<sup>[20]</sup>. Moreover, these cells have allowed scientists to set up innovative research to study ADRs in a more personalized way<sup>[18,21-24]</sup>. The focal point of this technology is the possibility to differentiate iPSCs into almost any cell of the human body<sup>[20]</sup>. Progress made in this last decade has allowed the development of groundbreaking tailored models for single individuals, contributing to the modern era of therapy personalization, finding the appropriate treatment for the right patient, and avoiding the development of ADRs<sup>[25]</sup>. To date, a number of studies based on iPSCs for therapy personalization and to study ADRs, especially in the adult field, are available<sup>[18,21-24]</sup>. However, more efforts are needed in the pediatric field, even though some works are already available<sup>[26-28]</sup>. iPSCs may be a great tool to model sensitivity to new, but also old drugs in children, shedding light on the mechanisms of toxicity, resolving in part the problem of the lack of data. Indeed, with a simple blood sample, it is possible to reprogram peripheral blood mononuclear cells into iPSCs that can be subsequently differentiated into somatic cells of interest.

## ORGANOIDS AS GROUNDBREAKING PATIENT-SPECIFIC MODELS FOR PHARMACOLOGICAL STUDIES

Organoids are three-dimensional (3D) *in vitro* culture systems derived from pluripotent stem cells (embryonic stem cells or iPSCs) or adult stem cells, and can simulate the architecture and functionality of native organs<sup>[29]</sup>. Organoids are generated from multiple organs including the intestine<sup>[30,31]</sup>, stomach<sup>[32]</sup>, kidney<sup>[33]</sup>, liver<sup>[34]</sup>, pancreas<sup>[35]</sup>, brain<sup>[36]</sup>, and lung<sup>[37]</sup> and can be used in multiple clinical applications including drug screening, disease modeling, and regenerative medicine. Moreover, the development of human patient-derived organoids may enable personalized medicine. Considering that organoids cultures based on a specific disease and from a specific individual can be expanded in culture maintaining a stable genetic and epigenetic signature<sup>[38]</sup>, they are suitable for biobanking and high-throughput screening. Therefore, organoids represent a powerful tool for drug efficacy and drug toxicity screening. Future analyses may be performed using these



**Figure 1** iPSCs to model ADRs for therapy personalization. iPSCs: Induced pluripotent stem cells; ADRs: Adverse drug reactions. Image adapted from: <https://smart.servier.com/>.

biobanks to identify new drugs, but also to know in advance which patients may benefit from an existing drug treatment. In toxicology screenings, 3D organoids could replace the use of cell lines and animal models. Renal and hepatic toxicities are common in drug administration and hepato-biliary organoids generated together with kidney organoids enable the accurate study of drug metabolism and toxicity. Interestingly, 3D organ-on-a-chip is an experimental system in which tissue architecture and cellular composition are assembled on a fabricated synthetic matrix<sup>[39]</sup>. This technology represents a novel approach to overcome the limitations of conventional model systems, especially to model complex disorders affecting several tissues, and for future pharmacological target identification, safety, and efficacy testing as well as personalized medicine.

## GASTROINTESTINAL TOXICITY

Drug-induced gastrointestinal toxicity is very common and may result in different consequences that range from nausea and dyspepsia to life-threatening events such as the development of complicated gastroduodenal ulcers<sup>[40,41]</sup>. To study and prevent these ADRs, researchers have started to create patient-specific *in vitro* models using iPSCs. These models are based on the differentiation of iPSCs into enterocytes, using innovative protocols, and involve the creation of groundbreaking models based on iPSC-derived human organoids.

### Intestinal ADRs

The small and large intestines are one of the most frequent sites of ADRs. The severity of symptoms can vary from very easy-to-manage to life-threatening illnesses. Well known examples of intestinal ADRs are mucosal damage, hemorrhage, colitis, changes in motility, and malabsorption<sup>[42]</sup>; an important example of frequent gastrointestinal ADR are those caused by nonsteroidal anti-inflammatory drugs

(NSAIDs). To manage and prevent the development of intestinal ADRs, scientists tried to model enterocytes, the most abundant intestinal cells with fundamental functions in nutrient/drug absorption and metabolism. In this regard, iPSCs could be a great tool, as demonstrated by some recent works. In 2014, Iwao *et al.*<sup>[43]</sup> generated for the first time pharmacokinetically functional enterocytes starting from iPSCs, with the aim of building an intestinal pharmacokinetic evaluation system to better study drug absorption and biotransformation. The protocol developed by this group allowed the authors to obtain mature enterocyte-like cells (ELCs) after about 30 days of stimulation using activin A and fibroblast growth factor (FGF)-2 in order to obtain definitive endoderm cells. Then these immature cells were differentiated into ELCs using epidermal growth factor in 2% fetal bovine serum. ELCs displayed increased expression of the specific intestinal markers sucrase-isomaltase, villin 1, intestine-specific homeobox, CDX2, dipeptidyl peptidase 4, and SLC46A1/proton-coupled folate transporter. The authors also noticed that a longer period of differentiation made the process more efficient in terms of the number of mature ELCs obtained.

In a subsequent study, the same group tried to ameliorate the efficiency of the already established protocol using different small molecules<sup>[44]</sup>. In particular, the authors analyzed the effect of GSK-3 inhibitor XV (a highly potent inhibitor of GSK-3), dorsomorphin (a selective bone morphogenetic protein [BMP] inhibitor), PD98059 (a mitogen-activated protein inhibitor), 5-aza-20-deoxycytidine (an inhibitor of DNA methylation), and A-83-01 (a potent and selective transforming growth factor beta [TGF- $\beta$ ] pathway inhibitor). Overall, the differentiated cells expressed intestinal markers, drug transporters and metabolizing enzymes, such as cytochrome P450 1A1/2 (CYP1A1/2), CYP2C9, CYP2C19, CYP2D6, CYP3A4/5, UDP-glucuronosyltransferase (UGT), and sulfotransferase. To analyze the intestinal differentiation of iPSCs after small molecule treatment, the authors considered mRNA expression of sucrase-isomaltase and CYP3A4, which were both markedly increased, while those of intestinal stem cell markers decreased. In particular, CYP3A4 mRNA expression level was mainly induced by the addition of 1 $\alpha$ ,25-dihydroxyvitamin D3. Intestinal drug transporter peptide transporter 1 was also increased by PD98059, 5-aza-29-deoxycytidine, and A-83-01. With this work, the authors created a model of enterocytes that is definitely more useful than primary cells given their poor viability, short life span, limitation of passage number, and difficulty in obtaining human intestinal tissue samples. Moreover, ELCs seem to be a better model with respect to the widely used Caco-2 cells, since the drug transporter expression levels and carrier-mediated drug permeability in Caco-2 are different from those of the human duodenum, and the expression level of CYP3A4 is quite low.

A similar work was performed by Ozawa *et al.*<sup>[45]</sup>, who generated an ELC monolayer characterized by drug absorption and metabolism functions. The authors<sup>[45]</sup> analyzed the barrier formation capacity of the obtained monolayer, measuring the transendothelial electrical resistance (TEER). By this test, the authors found that the ELC monolayers have a weaker barrier function than Caco-2 cell monolayers. Importantly, it is known that TEER values in Caco-2 cell monolayers are higher than those in the small intestine. Therefore, the authors concluded that the ELC monolayers might be a more suitable *in vitro* model for evaluating the absorption of hydrophilic drugs than Caco-2 cell monolayers. Overall, these studies provide a basis for exploiting ELC models to study drug absorption, metabolism, and ADRs.

Regarding our current knowledge, only a very recent work<sup>[46]</sup> applied a 2D ELC model to study ADRs. In particular, Matsunaga's group<sup>[46]</sup> used ELCs, obtained using the protocol already set up and described above<sup>[43,44]</sup>, to study human drug-induced intestinal mucosal damage. In this work<sup>[46]</sup>, mucin 2 (MUC2) mRNA expression as a marker of mucosal damage was analyzed. MUC2 is the main component of intestinal mucus and its expression in the intestine is decreased by several drugs that induce mucosal damage, such as NSAIDs, and is increased by other protective agents including rebamipide. First, the authors compared MUC2 expression in the human intestine, ELCs, and Caco2 cells to validate the ELC model. The results showed that after 26 days of differentiation, ELCs presented MUC2 levels comparable to those of the human intestine, while levels in the Caco2 line were 100 times lower. Authors also examined the effects of NSAIDs and of the mucosal protective agent rebamipide on MUC2 expression in the ELC model, confirming that NSAID exposure reduces MUC2 levels while rebamipide increases it. Given that the fluctuation of MUC2 seemed to be influenced by different cytokines, they also analyzed the mRNA expression levels of cyclooxygenase-2, interleukin (IL)-1 $\beta$ , nuclear factor (NF)- $\kappa$ B, and tumor necrosis factor (TNF)- $\alpha$  after drug treatment. In particular, as reported in other studies<sup>[47-51]</sup>, they found that IL-1 $\beta$ , NF- $\kappa$ B, and TNF- $\alpha$  mRNA expression levels were decreased by indomethacin, but increased by rebamipide. Therefore, taken together, the authors concluded that it seems reasonable that these factors may be involved in MUC2 expression changes in enterocytes.



Regarding the advances for obtaining intestinal models used in drug screening, intestinal organoids also represent a valuable tool<sup>[52]</sup>. Self-renewal of the intestinal epithelium is driven by the proliferation of stem cells and their progenitors located in crypt regions<sup>[30]</sup>. Wingless/integrated 3A (WNT3A), R-spondin, and noggin are factors needed in organoid protocols to promote *in vitro* self-proliferation of intestinal stem cells; moreover, these organoids are composed by different cell types such as enterocytes, Paneth cells, goblet cells, and enteroendocrine cells and manifest many enteric characteristics<sup>[53]</sup>.

Recently, Lu and Rosenbaum<sup>[54]</sup> described the application of crypt organoid cultures from genetically modified mice as a model to evaluate drug metabolism. Irinotecan metabolism and toxicity were studied using crypt organoids generated from both Ugt1<sup>F/F</sup> (control) and Ugt1<sup>ΔIEC</sup> (deletion of the Ugt1 locus) mice. These 3D cultures metabolize the drug to the active topoisomerase inhibitor metabolite SN-38, which is further metabolized by UGT1A1-dependent glucuronidation to form an SN-38 glucuronide. In the absence of Ugt1 gene expression, Ugt1<sup>ΔIEC</sup> crypt cultures exhibit very limited production of SN-38 glucuronide, concordant with increased apoptosis in comparison with Ugt1<sup>F/F</sup> crypt cultures. Glucuronidation is an important phase II pathway responsible for the metabolism of many drugs used also in the pediatric age, such as opioids or acetaminophen<sup>[55]</sup>. These results demonstrate that intestinal organoid cultures can be employed to study drug metabolism, under conditions of altered pharmacogenetics.

Colonic organoids derived from iPSCs have been used to evaluate the toxicity of rapamycin and geneticin; an impairment of cell proliferation was only observed after treatment with rapamycin indicating that this compound can harm the healthy colon<sup>[56]</sup>.

### Hepatic ADRs

Drug-induced liver injury (DILI) is a severe ADR characteristic of more than 1000 drugs<sup>[57]</sup>. The estimation of DILI worldwide is between 1 in 10000 and 1 in 100000 inhabitants, even though some recent studies have reported a higher occurrence<sup>[58]</sup>. In the last several decades, the study and prevention of this ADR have gained increasing interest in the scientific community, given its increasing incidence caused mainly by the number of new drugs on the market<sup>[57]</sup>. Moreover, DILI is the most common cause of drug withdrawal from the market with consequent high costs for industries<sup>[59]</sup>. DILI is divided into acute or chronic conditions including hepatitis and acute liver failure, leading, in the most severe cases, to liver transplant and aggressive treatments<sup>[57-60]</sup>. The mechanism of DILI development can be dose-related or idiosyncratic.

Further efforts are needed to manage and prevent this ADR, given the limitations of current *in vivo* and *in vitro* models<sup>[61,62]</sup>. iPSCs are a great tool to overcome these limitations and to obtain more predictive results. Indeed, several studies have already shown how to differentiate human iPSCs into hepatocyte-like cells (HLCs) to create a source of cells for different purposes such as *in vitro* drug studies. One example is the study performed by Kondo *et al.*<sup>[63]</sup>, where iPSCs were differentiated into HLCs using a three-step protocol, using three growth factors (activin A, hepatocyte growth factor, oncostatin M) and two small molecules (dimethyl sulfoxide, dexamethasone). With this work, the authors established a reproducible and relatively inexpensive method to obtain a greater number of hepatic cells, with respect to other protocols, to perform, for example, pharmacological studies. The differentiated cells expressed the hepatocyte markers hepatocyte nuclear factor 4 alpha (HNF4-α), albumin (ALB), and alpha-fetoprotein (AFP) at similar or higher levels in comparison to primary human hepatocytes (PHH) and HepG2 cells. HLCs were also characterized by the expression of drug metabolizing enzymes such as CYP3A4 and UGT1A1. Authors demonstrated that the mRNA expression levels of CYP3A4 and UGT1A1 were increased by the CYP inducers dexamethasone, rifampicin, and omeprazole. However, the expression levels of drug-metabolizing enzymes were very low compared to those in PHH and mature liver. Two years after Kondo's work, Kang *et al.*<sup>[64]</sup> confirmed the greater similarity of HLCs and PHH, with respect to HepG2 cells, analyzing acetaminophen hepatotoxic effects on all three cell types. In particular, HLCs were more similar to PHH in comparison to HepG2 cells both in terms of cell viability after acetaminophen exposure and CYP450 levels, with similar downregulation of CYP1A2 and CYP3A4 genes by cytotoxic concentration of both agents. In contrast, HepG2 cells showed an increment in CYP levels.

Takayama *et al.*<sup>[21]</sup> investigated whether HLCs, obtained differentiating iPSCs of different donors, could reproduce the interindividual difference in hepatic biotransformation and drug response. In this regard, HLCs were generated from human iPSCs, established by reprogramming donor PHH. iPSCs were generated using a non-integrative method, based on Sendai Virus vectors, a process essential to avoid insertional mutagenesis. After this, they compared the drug metabolism and

drug responsiveness of HLCs to those of their parental PHH. The main purpose of this work was to establish a panel of HLCs that represents the diversity of genetic polymorphisms in humans, in order to use these cells to determine the appropriate drug dosage for the single individual. In particular, they focused on CYP activity levels in the HLCs with respect to the parental PHH. Results showed that CYP activity and drug responsiveness of individual HLCs reflected those of parental cells, suggesting that it might be possible to predict individual CYP activity using HLCs and to perform personalized drug treatment analyzing HLCs of the single patient. Moreover, the presence of a single nucleotide polymorphism (SNP) in genes encoding CYP2D6, related to a different metabolism and drug responsiveness, was successfully reproduced in HLCs. Also, Liu *et al*<sup>[65]</sup> successfully differentiated iPSCs into HLCs with a relatively simple three-step protocol, using the commercial hepatocyte maturation medium HepatoZYME (Life Technologies, Frederick, MD, United States). These authors reprogrammed peripheral blood mononuclear cells (PBMCs) instead of PHH, with obvious advantages, given the greater ease of access of blood with respect to liver biopsies. Similarly, Wilson *et al*<sup>[66]</sup> generated iPSCs and then HLCs from a cohort of individuals affected by alpha-1 anti-trypsin deficiency (AATD), a genetic disorder related to liver cirrhosis and pulmonary emphysema and characterized by low levels of AAT, the main protease inhibitor (PI) in human serum. In particular, the most common deficient allele involved in the development of AATD is the PI homozygous for the Z allele (termed PiZZ by authors). The most common disease variant is caused by an inherited single base pair mutation of the serpin family A member 1 gene, which results in a glutamate to lysine substitution and production of a mutant version of the PI AAT, known as Z AAT. Interestingly, the authors found that the global transcriptomes of iPSCs, carrying PiZZ mutations, diverge from that of the healthy controls (three control individuals without any known disease) only after differentiation to HLCs, when the AAT gene is expressed. Moreover, the obtained HLCs successfully model key features of AAT-associated liver disease, including intracellular accumulation and reduced secretion of AAT protein as well as increased autophagic flux. The authors confirmed an increase in autophagic flux upon treatment with the drug carbamazepine as previously described in mice carrying the mutation. Subsequently, authors tested if the PiZZ mutation can increase the toxicity of different drugs with respect to healthy PHH, and exposed both HLCs and controls to acetaminophen and other drugs known to cause hepatotoxicity. In each case, HLCs carrying the PiZZ mutations were more sensitive to drugs with respect to PHH. However, further studies need to be done to evaluate the cytotoxic mechanisms of drugs in HLCs carrying the PiZZ mutations with respect to healthy HLC controls. The authors concluded that these findings support the utility of iPSCs as tools for drug development or prediction of toxicity. More recently, Kvist *et al*<sup>[67]</sup> deeply analyzed the critical differences in drug metabolic properties of different human hepatic cellular models including PHH, HLCs, and the hepatoma cell lines HepG2 and HepaRG. Surprisingly, these authors showed that HLCs, obtained differentiating iPSCs, should not be used as a model to study drug metabolism, and thus ADRs, since critical differences were detected with respect to human PHH. This conclusion, in contrast with other works<sup>[21,63,64]</sup>, arises from a different analysis performed by Kvist's group analyzing the expression and function of key hepatic proteins important for the metabolic fate of drugs such as CYP enzymes. A principal component analysis to study and compare gene expression of HLCs, PHH, and the hepatoma cell lines HepG2 and HepaRG showed a distance between the two iPSC-derived hepatocytes, as well as HepG2 and HepaRG cells, and the three PHH donors and PHH pool, which were clustered more closely together. This finding was confirmed by another analysis, which clustered HepG2 close to HLCs in terms of gene expression of 91 genes related to the liver function or CYP450. Moreover, HLCs were found to have low activity of several CYPs such as CYP3A and CYP2C9, barely detectable activity of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and a high expression of several extrahepatic P450s such as CYP1A1 and 1B1 that may have significant effects on biotransformation profiles. On the other hand, HepaRG cells showed a CYP profile very similar to PHH, suggesting that this cell line can be a good model in drug metabolism studies and ADRs. The authors concluded that, to date, HLCs derived from patients' iPSCs should not be used as a substitute for PHH in drug toxicity studies. To improve the performance of the HLC model, the authors suggested to culture cells in a 3D rather than the current 2D monolayer, because the 3D model has been shown to improve the performance of PHH<sup>[68]</sup>. In 2018, Smutný *et al*<sup>[69]</sup> used HLCs to study the toxicity of phytochemicals saikosaponin D, triptolide, deoxycalyciphylline B and monocrotaline known to cause DILI, in comparison with hepatoblastoma-derived HepG2 cells and long-term culture of primary human hepatocytes (LTHHs). In order to compare the cytotoxic effects of the tested phytochemicals, the authors analyzed hepatocyte key markers in the HLCs compared to the HepG2 and LTHHs controls. First, they analyzed ALB level, a

specific protein produced only by hepatocytes and hepatoblasts by immunofluorescence staining and real time PCR. Both HLC and HepG2 cells exhibited intense staining of ALB. The mRNA level of ALB in the HLC cells was similar to that of HepG2 cells but was lower than that in reference LTHHs of the two donors. Then, transcription of HNF4 $\alpha$ , a liver-enriched transcription factor associated with the regulation of many liver-specific genes, was confirmed in the HLC model, as well as the expression of CYP3A4, which however was lower with respect to LTHHs. Interestingly, HepG2 cells were negative for the important CYP3A4 drug-metabolizing enzymes. Additionally, the authors analyzed the maturation of HLCs studying three markers: AFP, a typical liver marker expressed in hepatoblasts and fetal hepatocytes, but not in adult hepatocytes; cytokeratin 19 (CK19), a marker of cholangiocytes and hepatic progenitors; and CYP3A7, a CYP450 enzyme expressed mainly in fetal hepatocytes and at a very low level in adult hepatocytes. Overall, the results showed that HLCs resemble to be closer to an immature hepatic phenotype expressing both AFP and CK19 markers. After characterizing HLCs, they analyzed the potential of HLCs, HepG2, and LTHHs to predict DILI using hepatotoxic compounds. Overall, HLCs appeared more sensitive to triptolide and saikosaponin D in comparison to both HepG2 cells and LTHHs. Interestingly, the authors noticed an atypical response of HepG2 cells with less toxicity at higher concentrations of triptolide. This atypical effect could be related to particular resistance mechanisms characteristic of the HepG2 line, such as induction of metabolizing enzymes and/or efflux transporters induced by high doses of this phytochemical. Also, saikosaponin D treatment produced higher cytotoxic effects in HLCs, although LTHHs also showed high sensitivity. However, the HepG2 cells resulted less sensitive to saikosaponin D with effects observed at the highest concentration tested. Regarding monocrotaline, no cytotoxic effect was reported in all lines tested. The authors commented that this observation may be related to targeting by monocrotaline principally of hepatic sinusoidal endothelial cells. However, it would be interesting to further explore the causes of this resistance. Finally, they analyzed deoxycalyciphylline B effects, finding a mild decrease in mitochondrial activity at the maximal tested concentration only in LTHHs cells, while neither HLCs nor HepG2 exhibited any toxic effect. The authors concluded that this study provides a basis for further in-depth studies to confirm HLCs as a competent *in vitro* liver cell model for toxicological assessment; however, further efforts are needed to develop HLCs with a more mature phenotype, expressing typical adult hepatocyte markers such as CYP3A4, HNF4 $\alpha$ , and ALB despite the expression of immature markers typical of fetal hepatocytes and hepatic progenitors such as AFP and CK19. Another recent and interesting work was performed by Yamazaki and Murayama<sup>[70]</sup>, which analyzed CYP450 expression levels of commercial HLCs at different culture times. Authors found a significant increase of CYP450 activities after 3-4 weeks with respect to HLCs cultured for 1 week. After 4 weeks, HLCs reached CYP450 levels similar to those in HepaRG cells. The increase in activity was associated with increasing CYP450 2C9 and 2C19 mRNA levels. This finding can help researchers perform more precise and repeatable studies on HLCs and drugs and is in contrast with the manufacturer's instructions that suggest the use HLCs after 1 week of culture.

In this context, the recent development of liver organoid culture systems derived from iPSCs provides another promising strategy to study drug-induced hepatotoxicity<sup>[71,72]</sup>. Liver organoids closely resemble *in vivo* human liver, preserving their genetic and epigenetic integrity over months in culture<sup>[73]</sup>.

The most recently published method to generate functional hepatobiliary organoids from iPSCs cultured on Matrigel was developed by Wu *et al.*<sup>[74]</sup>. The protocol is based on inclusion at differentiation stages I and II (days 1-15) of 25% mTeSR culture medium into hepatic differentiation medium to induce endodermal and mesodermal commitment; subsequently, at stage III (days 15-45), 10% cholesterol and other small molecules (a Chinese patent pending product called cholesterol\*MIX) were added to the maturation medium to promote the formation and maturation of hepatobiliary organoids by activating the NOTCH2 and TGF- $\beta$  signaling pathways. Concerning drug metabolic functions, the expression of several P450 enzymes was measured: the organoids displayed significantly higher levels of CYP3A4 and CYP2E1 than fetal liver, with comparable expression of CYP2A6, CYP2B6 and CYP2D6. On the contrary, the expression of most P450 enzymes (except for CYP3A4) in liver organoids was significantly lower than that in the adult liver, indicating that these organoids present intermediate maturity between the fetal and adult liver.

Liver organoid culture systems allow hepatotoxicity testing. Recently, Leite *et al.*<sup>[75]</sup> established a method to detect hepatocyte-mediated and drug-induced liver fibrosis based on this platform. After a single dose or repeated exposure for 14 days to the pro-fibrotic compounds allyl alcohol and methotrexate, hepatic organoids displayed fibrotic features such as activation of hepatic stellate cells (HSCs), the major collagen-

producing cells during conditions of sustained hepatic injury. The tested drugs caused significant upregulation of HSC activation-associated mRNAs collagen, type I, alpha 1, collagen, type I, alpha 3, and lysyl oxidase homolog 2 in the organoids. Acetaminophen is another compound identified by these organoids as an inducer of hepatotoxic-mediated HSC activation, which was also confirmed in an *in vivo* model.

### Pancreatic ADRs

Drug induced-pancreatitis is a serious problem for both the patient and the health system. About 0.1%-2% of drugs are related to the development of this ADR and cases can be mainly divided into mild and severe. Severe cases may lead to death, while mild ones lead to patient hospitalization. Recently, our group reviewed the possible application of iPSCs to study and prevent this ADR<sup>[18,76]</sup>. Here, we provide an up-to-date revision of iPSC technology to model patient-specific human pancreatic cells with the purpose to obtain a platform for drug-induced pancreatitis studies. iPSCs can be differentiated into both endocrine and exocrine pancreatic cells applying protocols that provide the addition of specific stimuli to the culture medium. Regarding endocrine differentiation, many protocols are available; however, the main current limitation is the complete maturation of differentiated cells due to its lower hormone release profiles with respect to human islets, thereby resembling more immature fetal endocrine cells. However, to overcome this limitation, some progress has been made in the last several years. The work published by Pagliuca *et al.*<sup>[77]</sup> is an example of the progress made in this field. iPSCs were differentiated into hormone-secreting cells using a six-step protocol and cells were cultured in clusters into spinner flasks. Cells obtained were implanted in mice, resulting, after 3-4 months, in functional  $\beta$ -cells and polyhormonal cells, a particular type of early endocrine cells that appears during pancreas development, able to secrete insulin, glucagon, somatostatin, and pancreatic polypeptide and localized in the walls of pancreatic ducts. These cells can be a great tool for diabetes studies and identification of new therapeutic approaches.

To the best of our knowledge, protocols regarding exocrine differentiation are limited. The most efficient, already described in our previous works<sup>[14,53]</sup>, was developed by Takizawa-Shirasawa *et al.*<sup>[78]</sup>.

Even if the progress made to obtain patient-specific pancreatic cells is increasing, the experience on the application of differentiated cells to create a platform for drug-induced pancreatitis studies is still limited and only two works are available. Indeed, to date, only Hohwieler *et al.*<sup>[79]</sup> developed a model of 3D pancreatic organoids generated from iPSCs of patients with cystic fibrosis. Their work, already reviewed by our group<sup>[18]</sup>, has provided a basis for the development of new research based on this topic. Also, Huang *et al.*<sup>[80]</sup> developed an efficient protocol to obtain exocrine pancreatic organoids starting from human embryonic stem cells. On the other hand, even if works based on iPSCs and the generation of exocrine pancreatic cells are limited, there are several solid works concerning pancreatic organoids created from tumor cells. Therefore, considering these two facts, it is reasonable to believe that more progress can be made in this field to fill the existing lack of pancreatic ADR studies.

## NEPHROTOXICITY

Drug-induced renal injury (DIRI) is a frequent side effect, especially in critical patients undergoing complex pharmacological treatments. DIRI is common in the pediatric field with an incidence of about 25% pediatric patients taking intensive pharmacotherapy<sup>[81,82]</sup>. This high incidence is principally due to the excretory function of the kidney, which is exposed to high concentration of drugs or metabolites. DIRI can lead to severe acute renal failure, which contributes to prolonged hospitalization, and increased costs for healthcare and morbidity. To prevent devastating consequences for patients, it is important to identify markers of this adverse event, taking measures to avoid it. To date, the main clinical indicators of DIRI are serum creatinine levels; however, novel markers are needed to more efficiently prevent its development<sup>[83]</sup>. It is known that this adverse effect can be caused by drugs through different mechanisms that are divided on the basis of the affected kidney component<sup>[84]</sup>. A major problem of DIRI management is the lack of *in vitro* models to test nephrotoxicity of drugs or to find predictive biomarkers of renal drug toxicity<sup>[85,86]</sup>. iPSCs from patients represent a promising model to develop more precise therapies, better studying the mechanisms related to drug-induced nephrotoxicity and creating the possibility to prevent DIRI development.

The differentiation of iPSC to renal cells involves different steps: starting from mesendoderm formation, the intermediate mesoderm can be obtained, from which it is possible to obtain the ureteric bud or metanephric mesenchyme. From the latter, the



differentiation continues with the renal vesicle, from which the podocyte, proximal tubule, or distal tubule can be obtained<sup>[87]</sup>. Taguchi *et al.*<sup>[88]</sup> analyzed a differentiation protocol allowing metanephric nephron progenitors starting from iPSC to be obtained in 14 days. The protocol involved different steps of differentiation: starting from the formation of embryoid bodies (EBs), epiblast, nascent mesoderm, posterior nascent mesoderm, posterior intermediate mesoderm, and finally metanephric mesenchyme. The different steps were obtained with appropriate concentrations and exposure times to stimuli added in the medium: activin-A, and bone morphogenetic protein 4 (BMP-4), CHIR99021, retinoic acid, fibroblast growth factor 2 (FGF-2), FGF-9. Immunohistochemical analysis confirmed the differentiation<sup>[89]</sup>. Xia *et al.*<sup>[89]</sup> derived ureteric bud progenitor-like cells from iPSC in 4 d. The culture medium was supplemented with BMP-4 and FGF-2 for 2 d and then with retinoic acid, activin-A, and BMP-2. The differentiation was evaluated using real-time PCR and immunostaining<sup>[89]</sup>. Musah *et al.*<sup>[90]</sup> developed a differentiation method of iPSC in kidney glomerular podocytes with a feeder-free and serum-free protocol in 21 days. The differentiation procedure was established in three commercial iPSC lines: PGP1, IISH3i-CB6, and IMR-90-1. During differentiation, the cells were cultured on tissue plates coated with lamin-511 E8 fragment and the mesoderm was obtained adding Rho-associated kinase inhibitor Y27632, CHIR99021, and activin-A; the intermediate mesoderm was obtained with CHIR99021 and BMP-7; and finally podocytes were obtained by stimulating the cells with BMP-7, retinoic acid, activin-A, vascular endothelial growth factor (VEGF), and CHIR99021. The differentiation markers analyzed were goosecoid, HAND1, and brachyury in the case of the mesoderm; Pax2 for the nephron progenitor cell markers; WT1 and OSR1 for intermediate mesoderm; and finally, for podocytes WT1, podocin, and nephrin proteins and specific genes such as *MAF*, *PODXL*, *SYNPO*, and *EFNB2*, together with a decrease of progenitor marker genes (*e.g.*, *SALL1* and *PAX2*) and pluripotency genes (such as *SOX2*, *MYC*, *NANOG*, *POU5F1*)<sup>[90,91]</sup>. The authors also created an organ-on-a-chip microfluidic model of glomerular function. Organ-on-a-chip culture models can better reproduce the structure, function, and environment of human organs. The chip was formed by two parallel micro-channels separated by a poly(dimethylsiloxane) membrane: the intermediate mesoderm, subsequently differentiated into podocytes was cultured in the upper part of the channel and in the opposite part the primary human glomerular microvascular endothelial cells were seeded. In this way, it was possible to recreate the podocyte-endothelium interface. Furthermore, two hollow chambers were added on the sides of the central channels and a cyclic suction was applied to mimic the cyclical pulses of the renal blood flow that cause relaxation or motion and dynamic mechanical stretching *in vitro*. The podocytes were obtained by differentiation in the presence or absence of fluid flow or with a combination of fluid flow and mechanical strain; nephrin expression analyses indicated that differentiation may be influenced by mechanical forces. Further analysis showed that with cultures in the presence of flow or the flow-mechanical combination, there was an increase in the number of processes of the podocytes and greater production of VEGF-A (necessary for development of the glomerulus *in vivo*). They analyzed the percentages of retained ALB and inulin filtration in the presence of cyclin mechanical strain and noted that 99% of ALB is retained while 5% of inulin is filtered, suggesting that this represents a good *in vitro* model for glomerular filtration barrier. They also analyzed the production of collagen IV (mainly produced by glomerular podocytes in the mature glomerular basement membrane) and noted that this type of collagen is produced by both cell types present in the chip, even though the greatest production occurs in differentiated podocytes under mechanical strain, demonstrating the greater differentiation efficiency in the presence of mechanical strain. Finally, they analyzed the damage induced by a continuous flow of the adriamycin anti-tumor drug on this organ-on-a-chip. The results revealed an interruption of the podocyte layer and cell detachment in a dose-dependent manner, together with decreased viability and non-selective loss of ALB from vascular channel. This indicated that the tested drug produced lesions and that this model is useful for analyzing glomerular function, therapeutic development, and drug-induced toxicity<sup>[91]</sup>.

Kandasamy *et al.*<sup>[92]</sup> reported an example of proximal tubular-like cell differentiation from iPSCs used to predict drug-induced nephrotoxicity. They used commercial iPSC (foreskin)-4, from WiCell Research Institute and differentiated these cells in human proximal tubular-like cells (HPTC-like) in 8 days, using commercial renal epithelial growth medium supplemented with BMP-2 and BMP-7. They analyzed the expression of markers to evaluate the different steps of differentiation in HPTC-like cells. In summary, the expression of iPSC characteristic genes such as *SOX2*, *NANOG*, *DNMT3D*, and *OCT3/4* decreased starting from day 1 of differentiation, while the characteristic genes of proximal tubular cells (*AQP1*, *GGT*, and *KSP-CAD*) are expressed in HPTC-like cells. The authors also analyzed the expression of 31 different



genes in HPTC-like cells, including genes coding for transporters, epithelial markers and kidney injury markers. Then they analyzed the nephrotoxic effect of two compounds, rifampicin and citrinin, in terms of IL-6 and IL-8 expression and noted an increase in the two ILs after drug treatment. They used the IL-6/IL-8-based assay to test 30 compounds that included substances for which a nephrotoxic effect was known and substances that did not produce such toxicity and treated HPTC-like cells derived from iPSC differentiation and a commercial line of HPTC (American Type Culture Collection, Manassas, VA, United States). To classify the compounds as toxic and non-toxic, an automated classifier was used and the system was trained to recognize the two types of compounds. Finally, they tested how predictive the developed system was and concluded that cells differentiated from iPSCs have higher test accuracy than HPTC. Therefore, HPTC-like cells can be used to predict toxicity using this automatic system. In addition, performance predictions were also analyzed using HPTC derived from nephrectomy samples from two tumor patients and an increased variability in performance was observed. The authors concluded that the use of HPTC-like cells differentiated from iPSCs in this prediction system can avoid inter-donor variability problems and thus could be useful to predict nephrotoxicity of drugs. Finally, authors tested the anti-cancer drug cisplatin using different biomarkers. The treatment produced HPTC-like DNA double-strand breaks and reactive oxygen species (ROS) production. The authors reported that the results obtained are in line with clinical data and animal experiments, and concluded that iPSC-differentiated HPTC-like cells are an effective model for the *in vitro* study of cisplatin-induced toxicity<sup>[92]</sup>.

Although the number of studies on drug-induced nephrotoxicity based on iPSCs is currently limited, such a model represents a good tool to investigate the pathophysiology of many renal diseases and may allow the investigation of more effective therapies. In fact, for many kidney diseases, specific *in vitro* models are not yet available. The use of pluripotent stem cells (including iPSC and embryonic stem cells) have allowed the study of renal diseases due to genetic mutations such as renal cysts, diabetes syndrome, Wolfram syndrome, focal segmental glomerulosclerosis, systemic lupus erythematosus, Wilms tumor, and Alport syndrome<sup>[86]</sup>.

With respect to iPSC-derived proximal tubular cells, 3D kidney organoids are characterized by distinct cell types such as endothelial cells, nephron progenitors, and podocyte-like cells and are therefore promising systems for nephrotoxicity testing<sup>[93]</sup>.

Recent data have shown that treatment of kidney organoids generated from human iPSCs (CRL1502) with nephrotoxic cisplatin induced specific acute apoptosis in mature proximal tubular cells, whereas immature cells did not respond to the drug<sup>[93]</sup>. These results were further confirmed on patient-derived organoids obtained from renal normal tissue of neoplastic patients: a consistent activation of caspase 3 (CASP3), an indicator of apoptosis, after 72h of incubation with cisplatin was detected by different techniques and only tubule cells suffered after drug exposure without affecting organoid architecture<sup>[94]</sup>.

Furthermore, interesting results have been obtained when organoids derived from cultured murine nephron progenitor cells were treated for 24 h with gentamicin at different concentrations; the percentage of CASP3+ cells co-stained with *Lotus tetragonolobus* lectin, a proximal tubule marker, selectively increased up to 80%. In contrast, immunofluorescence analyses of cells stained for podocalyxin, a glomerulus marker, treated with gentamicin showed no cells positive for CASP3, suggesting that the drug caused proximal tubule injury without affecting glomerular structures<sup>[95]</sup>.

The most recently published paper about kidney organoids described the use of glomeruli isolated from iPSC-derived kidney organoids for toxicity screening<sup>[96]</sup>. The method for isolation of intact glomeruli from kidney organoids is based on enzymatic dissociation of mature organoids that generates 3D aggregates of podocytes representing forming glomeruli. In particular, the cultured organoid glomeruli were exposed to increasing concentrations of doxorubicin, and after 48 hours, activation of the pro-apoptotic pathway was evident at the lower doses; reduction in glomerular size following doxorubicin treatment was also detectable.

These preliminary results are encouraging; however, more drugs must be evaluated before kidney organoids can be used as a promising drug testing platform.

## CARDIOTOXICITY

Drug-induced cardiotoxicity may be triggered by several mechanisms of action. In general, drugs can cause different effects at the cardiac level: heart failure due to abrupt decrease of contractile performance, decrease in left ventricular ejection fraction, arrhythmias, and prolonged cardiac repolarization. The latter, associated

with a prolonged QT interval, may increase the risk of serious cardiac arrhythmias. In most cases, the prolonged repolarization phase is caused by drug effects at the ionic channels or pump levels<sup>[97]</sup>. In general, variations in the electrical currents that stimulate the contractions of cardiomyocytes (CMs) are due to alterations in the fluxes of ions such as  $\text{Ca}^{2+}$ ,  $\text{K}^{+}$ , and  $\text{Na}^{+}$  through ionic channels in cardiac cells<sup>[98]</sup>. An example of a potentially lethal arrhythmia is the Torsade de Pointes, often determined by the prolongation of the action potential that affects rapid  $\text{K}^{+}$  current by inhibition of the ether-a-go-go-related gene channel (hERG) in cardiac muscle and is related to prolongation of the QT interval<sup>[99]</sup>. Furthermore, alterations of the ions flux not only have consequences on CM contraction, but suggest oxidative stress condition, a common mechanism of toxicity that may lead to apoptosis<sup>[100]</sup>.

One of the major problems during new drug development in preclinical trials is represented by the potential cardiotoxicity of the therapeutic agent<sup>[9]</sup>. Indeed, during the registration phase of a new drug, 23% of candidates fail for this reason<sup>[101]</sup>. In this view, prolongation of the QT interval has been suggested as the major problem related to the potential cardiotoxicity of drugs by the U.S. Food and Drug Administration (FDA)<sup>[102]</sup>. Hence, in 2005, the International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use created guidelines for cardiotoxicity screening in preclinical<sup>[103]</sup> and clinical studies<sup>[104]</sup>.

Cardiotoxicity is not only a frequent cause of failure of preclinical studies for new drugs, but can also be the cause of drug withdrawal from the pharmaceutical market. Between 1994 and 2006, 45% of all drugs removal from the market were due to cardiotoxicity<sup>[105]</sup>. For instance, some of the most recent withdrawals from the market of drugs due to cardiotoxic effects involve sibutramine and rosiglitazone. The anorectic sibutramine was recalled in 2010 because of increased risk of nonfatal myocardial infarction and nonfatal stroke in patient with pre-existing cardiovascular disease<sup>[106]</sup>. The anti-diabetic rosiglitazone was also recalled in 2010. Although an increase in mortality risk has not been identified, this drug was associated with an increased risk of myocardial infarction and heart failure<sup>[107]</sup>.

Also in this case, a promising approach is the use of iPSCs. iPSCs allow patient-specific studies, since these cells can be easily differentiated into CMs (iPSC-CMs). In addition, iPSC-CMs have the advantage of being cryopreserved to facilitate their use as well as being grown in large numbers and high purity<sup>[108]</sup>. Di Baldassarre *et al.*<sup>[109]</sup> reported three main approaches for the generation of iPSC-CMs: differentiation of the monolayer culture system for which various protocols have been described, co-culture of iPSCs with visceral-endoderm-like cells, and formation of embryonic bodies (3D culture)<sup>[109]</sup>. In general, the protocols provide, as a first step, differentiation towards the mesoderm layer by activation of WNT, activin/NODAL, and/or BMP pathways. For example, the combination of CHIR99021 with differentiation factors such as activin-A and BMP4 are used to activate the WNT pathway<sup>[97]</sup>. The activation set of these pathways, together with the Matrigel sandwich method, has been proven effective<sup>[110]</sup>. However, the obtained iPSC-CMs are qualitatively and quantitatively immature compared to adult CMs. This can significantly impact the validity of *in vitro* studies using these models, in terms of modeling the disease and/or prediction of the drug effects<sup>[110]</sup>. The differences between iPSC-CMs and adult CMs are related to: structural gene expression<sup>[111,112]</sup>, structural features<sup>[113]</sup>, metabolism<sup>[112]</sup>, and contractile function<sup>[113]</sup>. Very recently, Machiraju and Greenway reported successful methods for the maturation of iPSC-CMs, including: biochemical approaches (manipulation of growth conditions through the addition of small molecules or changes in culture medium), environmental manipulation (through various mechanical and electrical forces), and 3D approaches (creation of 3D cultures of CMs, called organoids). According to the authors, the optimal conditions of maturation can be achieved by combining these different approaches<sup>[110]</sup>.

A good example of the use of iPSC derived from patients to perform patient-specific studies to evaluate drug toxicity was reported by Liang *et al.*<sup>[114]</sup>. In this study, iPSC-CMs were obtained from healthy donors and patients with inherited long QT syndrome (LQT), familial hypertrophic cardiomyopathy (HCM) or familial dilated cardiomyopathy. The authors initially characterized iPSC-CMs by immunofluorescence staining (noting that there were phenotypic differences in patients' iPSC-CMs compared to healthy iPSC-CMs due to the associated pathology) and ion channel expression (noting that they are present in all types of cells and therefore potentially able to modulate the electro-physiological responses to drugs). Then, they analyzed divergent aspects such as the morphology of action potentials and the action potential duration in nodal, atrial, and ventricular waveforms in all iPSC-CMs. On these cell models, the authors showed that the iPSC-CMs of patients affected by LQT and HCM treated with cisapride (a gastroprokinetic agent capable of blocking hERG channels) showed a higher susceptibility to arrhythmias, suggesting that the greater sensitivity

to cardiotoxicity induced by this drug could be associated with LQT or HCM mutations. Thus, it is conceivable that iPSC-CMs can be used as good models for evaluation of the patient-specific cardiotoxicity of drugs<sup>[114]</sup>.

On iPSC-CMs, different physiological parameters can be monitored as a measure of possible toxic outcomes induced by drugs at the cardiac level. For instance, continuous real-time monitoring of the beating frequency of CMs is possible thanks to the xCELLigence Real Time Cell Analysis Cardio Instrument, a technique useful for the dynamic monitoring of CM contraction and beating, measured by an electric field differentially modulated by the number of cells covering the electrodes, their morphology and the strength of cell attachment. Nguemo *et al*<sup>[115]</sup> showed that this technique is a useful tool for characterization of the potential cardiotoxicity of drugs. The most recent study using this tool to analyze drug-induced cardiotoxicity on iPSC-CMs was carried out evaluating the cardiotoxicity of etoposide (ETP), a broad-spectrum anti-neoplastic drug. The iPSC-CMs were treated with ETP for 48 hours followed by 2 days of drug washing. An irreversible increase in the beating rate of iPSC-CMs was observed with 30 and 15  $\mu\text{mol}$  of ETP with alterations in the beating profile and arrhythmic beating (measured with XCELLigence). Furthermore, treatment with 10  $\mu\text{mol}$  ETP resulted in initial changes of the beating profile, that however, returned to baseline level after drug washing. A dose-dependent increase in the extracellular level of lactate dehydrogenase after treatment with ETP was observed, indicating membrane damage in iPSC-CMs. The authors also performed gene expression analyses (deregulation of 58 genes and upregulation of 5 miRNAs were found), intracellular calcium handling and mitochondrial membrane potential analyses, immunostaining, and transmission electron microscopy (to confirm the cytoskeletal and mitochondrial damage). Finally, they showed that the apoptosis inhibitor, pifithrin- $\alpha$ , could protect iPSC-CMs from ETP-induced cardiotoxicity<sup>[116]</sup>.

Another useful technique is represented by microelectrode array (MEA), a method that allows measurement of the electric field potential (homogeneous and electrically coupled populations) of cardiac cells<sup>[117]</sup>. The most recent study using this tool to analyze drug-induced cardiotoxicity was carried out on a commercially available iPSC-CM cell line (iCell CMs, Cellular Dynamics International, Madison, WI, United States) testing 25 drugs, with or without serum in the medium, using a MEA system to analyze the field potential duration (FPD) and arrhythmic events. In the case of serum-free medium, only some drugs induced significant changes in FPD, nine with an extension and four with a reduction. In the case of medium with serum, on the other hand, the number of drugs with prolonged FPD was 11. The authors concluded that the presence or absence of serum can affect the results; indeed, components present in the serum such as serum ALB can create optical artifacts during this electrophysiology assays, and therefore, this should be considered during the analysis<sup>[118]</sup>.

Cardiotoxicity is a relevant problem for anti-tumor drugs<sup>[101]</sup>. For example, anthracyclines, especially doxorubicin and its derivative epirubicin, are widely used as anti-cancer drugs in hematological malignancies (*e.g.*, lymphoblastic or aggressive myeloblastic leukemia) and solid tumors (such as breast, endometrial and stomach tumors). The cardiotoxicity induced by doxorubicin can cause congestive heart failure, tachycardia and arrhythmias, asymptomatic reduction of left ventricle ejection fraction, cardiomyopathy, and myocardial infarction<sup>[119]</sup>. The cardiotoxicity induced by this drug acts on several levels: activation of the apoptosis pathway by alteration of mitochondrial functions, generation of ROS, and alteration of gene transcription due to inactivation of topoisomerase II and double-strand breaks<sup>[120]</sup>. One of the most recent studies of cardiotoxicity induced by doxorubicin on patient-specific iPSC-CMs was reported by BurrIDGE *et al*<sup>[119]</sup>. The study was carried out in iPSC-CMs derived by three groups of female patients: healthy controls, breast cancer patients treated with doxorubicin or equivalent who did not experience clinical cardiotoxicity (DOX), and breast cancer patients treated with doxorubicin or equivalent who did experience clinical cardiotoxicity (DOXTOX). Once derived from each of these patients, iPSCs were differentiated into iPSC-CMs that were subsequently exposed to doxorubicin to evaluate the drug-induced cardiotoxicity. iPSC-CMs have been tested for: toxicity of doxorubicin by immunofluorescent imaging to assess the concentration of the sarcomeric disarray, detecting a concentration-dependent increase in DOXTOX cells, but not in DOX cells at 0.1  $\mu\text{mol}$ , as well as an increase in beating rates, more severe in DOXTOX cells; (2) cell viability that was reduced in DOXTOX cells; (3) level of double-stranded DNA damage (by staining for phosphorylated H2A histone family member X), which was higher in DOXTOX patients; and (4) oxidative stress, demonstrating that ROS and  $\text{H}_2\text{O}_2$  production was higher while antioxidant glutathione levels decreased in the DOXTOX patients. Moreover, the effect of doxorubicin on patient-specific gene expression was evaluated by RNA sequencing analysis. An association between homozygous non-synonymous variants in BRCA1

and cardiotoxicity in DOXTOX patients was found. Because of these results, the authors concluded that the study of drug-induced cardiotoxicity using iPSC-CMs allows the evaluation of its molecular mechanisms and genetic bases<sup>[119]</sup>.

Another example of iPSC-CMs used to study the cardiotoxic effects induced by anti-cancer drugs was reported by Sharma *et al.*<sup>[121]</sup>, who investigated the tyrosine kinase inhibitor (TKI)-induced cardiotoxicity. TKIs are anti-cancer agents that act on the tyrosine kinase receptor by inhibiting its phosphorylation. TKIs act in terms of proliferation, migration and survival of the cells. However, reduced left ventricular ejection fraction, heart failure, myocardial infarction, or arrhythmias are common cardiac adverse effects caused by TKI treatment. In this study, patient-specific iPSC-CMs were obtained from the somatic tissues of 11 healthy individuals and 2 cancer patients (patients with kidney cancer and treatment with sunitinib as first-line and axitinib as second-line without significant clinical cardiotoxicity) to evaluate the cardiotoxicity of 21 FDA-approved TKIs. Different endpoints were evaluated such as cytotoxicity, contractility, and the effects on QT intervals. The authors noted the cytotoxic effects of some TKIs (sorafenib, regorafenib, and ponatinib induced the greatest effects) in iPSC-CMs obtained by healthy patients and there were no significant differences when compared to iPSC-CMs obtained by patients treated with sunitinib or axitinib. Regarding the effects on cell contractility, healthy iPSC-CMs were exposed to doses lower than the drugs' median lethal dose, and nilotinib and vandetanib altered the beating rate. The authors concluded that before the death of CMs, they manifested an effect on the beat profile. Finally, regarding the effects on QT interval, prolongation of the contraction time of CMs was observed in healthy iPSC-CMs treated with nilotinib or vandetanib, in addition to a decrease in the beating rate and prolongation of the transitional duration of calcium. In this article, it was also hypothesized that insulin/IGF signaling (that was upregulated after treatment with VEGFR2/PDGFR-inhibiting TKIs) may protect iPSC-CMs from TKI toxicity. The authors concluded that iPSC-CMs can be used to evaluate TKI-induced cardiotoxicity<sup>[121]</sup>.

The response to some drugs is different between adults and children and/or during childhood; for example, in the case of warfarin, in pediatric patient age has a more important effect on pharmacokinetics than polymorphisms (for example in *VKORC1* or *CYP2C9* genes)<sup>[122]</sup>. Hence, of crucial importance is the establishment of patient-specific studies to evaluate the cardiotoxic potential of drugs in pediatric patients. Also in this case, patient-specific iPSCs are a useful tool. For example, Visscher *et al.*<sup>[123]</sup> reported the relationship between the SNP in a panel of genes and anthracycline-induced cardiotoxicity (ACT) in children (independent cohort of 218 patients who included patients treated with anthracyclines who had or not developed cardiotoxicity). Genomic DNA was extracted from blood, saliva, or buccal swabs and 23 SNPs were selected for which an association with ACT was already known. The results showed an association between rs17863783 in *UGT1A6* and ACT, while the association with two SNPs in *SLC28A3* (rs7853758 and rs885004) and one in *SULT2B1* (rs10426377) was close to being significant. They also analyzed the influence of sex and age at the start of treatment: The variant *SULT2B1* rs10426377 was associated with an increased risk of ACT only in males, while the two variants of *ABCB4*, rs4148808, and rs1149222 were only associated with an increased risk in females. Regarding age, an association with ACT was found in the case of the *HNMT* variant rs17583889 in the case of younger children (< 5.3 years)<sup>[123]</sup>. This study highlights the ability of a specific patient study to improve the risk assessment of ACT in children, potentially helping to improve the safety of anti-cancer therapy.

With respect to the traditional 2D cell culture, 3D-engineered human cardiac organoids generated from stem cell-derived CMs provide another functional *in vitro* model for disease modeling and drug screening. Biochemical-inducing factors can be combined with several tissue engineering approaches based on 3D printing and bioscaffold technologies to direct spatial organization of 3D tissue to build human cardiac organoids<sup>[124,125]</sup>. Despite the progress in this area, protocols for the maturation of CMs toward an adult phenotype in defined conditions still need to be further elucidated.

The study published by Voges *et al.*<sup>[126]</sup> demonstrated that human cardiac organoids primarily contain CMs and stromal cells at a ratio that is comparable with the fetal/neonatal heart, and form functional sarcomere units. Moreover, these cellular models are able to completely recover cardiac function following injury, showing many features of regenerative neonatal heart tissue.

Fluid-ejecting 3D human ventricular-like cardiac organoid chambers (hvCOC) can mimic physiologically complex behaviors, such as pressure-volume relationships, and have been used for detecting contractile responses to different pharmacological compounds<sup>[127,128]</sup>. The hvCOC system can accurately identify inotropic effects of pharmacological compounds such as isoproterenol and levosimendan, with increased



sensitivity with respect to human ventricular-like cardiac tissues strips.

The utility of these structures has been additionally demonstrated for environmental contaminants screening, for example heavy metal and pesticide, confirming the accurate responses to external stimuli<sup>[129]</sup>.

These preliminary results demonstrate that in the future, these platforms could provide patient-specific models for personalized drug screening to achieve optimal therapeutic applications.

## NEUROTOXICITY

Neurotoxicity can be caused by physical, chemical, or biological agents exhibiting adverse effects at the central or peripheral nervous system level, altering its function or structure. Neurotoxicity can affect attention, executive functions, decision making, and memory, based on the severity and location of the injury, compromising the quality of life. Brain functions can be altered by drug activation of different neurotransmitter systems, including dopamine and glutamate<sup>[130]</sup>.

Drug-induced neurotoxicity is divided, according to the damaged region, in: myelinopathy, induced by drugs such as amiodarone, which causes damage to Schwann cells<sup>[131]</sup>; axonopathy, induced by drugs such as vinca alkaloids (*i.e.* vincristine and paclitaxel), inducing microtubule-dependent axons damage<sup>[132]</sup>; and neuronopathy, induced by platinum-based compounds (cisplatin and oxaliplatin) due to oxidative or mitochondrial stress resulting in death of the dorsal root of ganglion neurons<sup>[132]</sup>.

An example of ADR is represented by seizure, a serious neurological complication that is commonly associated with treatment with antibiotics<sup>[133]</sup>. Seizures involve an abnormal and transient discharge of neurons at the brain level. To test drug-induced seizure-liability many models are currently available, for example acute slice assay carried out on surgical slides obtained from any part of the brain (especially hippocampus), organotypic slice cultures, primary central nervous system cultures, iPSC-derived cultures. The latter are useful to study drug-induced neurotoxicity using different *in vitro* techniques, such as calcium imaging and MEA. This method allows the measurement of the electrophysiological activities of the neural networks in a non-invasive way<sup>[134]</sup>. For instance, Odawara *et al.*<sup>[135]</sup> used this tool to evaluate the efficiency of response to two convulsant agents (pentylenetetrazole, a GABA blocker, and 4-aminopyridine, a K<sup>+</sup>-channel blocker) in an *in vitro* system constituted by co-cultures of commercial neurons and astrocytes derived from iPSCs. The results obtained indicated that the synchronized bursts firings, indicative of functional maturation in synaptic transmission, and the analysis of the peaks in the synchronized bursts firings allows the neurotoxic effects of these two drugs to be distinguished. Moreover, the co-culture improves the spontaneous activity of neuronal networks<sup>[135]</sup>. The latter result was obtained by Ishii *et al.*<sup>[136]</sup>, who analyzed the response to drugs (gabazine and kaliotoxin) on synchronized burst and spontaneous firing, demonstrating that the co-culture system is more efficient as an *in vitro* model than the individual cultures of commercial astrocytes or iPSC-derived neurons<sup>[136]</sup>.

One of the protocols reported in the literature for the differentiation of human iPSCs into a mix of neurons and glia cells takes about 28 days. The first step is the generation of embryonic bodies, followed by the generation of neuroepithelial aggregates (rosettes) that requires 5 days, and then the dissociation of the rosettes and the neuronal differentiation (20 days) in a mix of neurons and glial cells. This protocol allows heterogeneous cultures of glutamatergic, dopaminergic, and GABAergic neuronal cells to be obtained, together with glial cells<sup>[137]</sup>. Mukherjee *et al.*<sup>[138]</sup> reported that the main method of differentiation of iPSCs involves the mimics of development signals using culture medium added with morphogens, small molecules, and/or growth factors. Differentiation can occur basically using two different methods: culture in suspension in a single cell or in adhesion with the subsequent formation of aggregates of embryonic bodies and the cultivation in medium, allowing the formation of the definitive neuroectoderm and neural rosettes; and inhibition of one important protein of the small mothers against decapentaplegic family in iPSCs cultured as a monolayer and the formation of rosettes by inhibition of BMP. The main differences between the two methods are the efficiency of differentiation and the duration of culture, while the involved pathways are similar (BMP/TGF- $\beta$ /Wnt). The cortical GABAergic neurons are obtained by adding, before the phase of terminal differentiation, inhibitors of sonic hedgehog (SHH) and/or Wnt, for the dopaminergic neurons of the caudal midbrain (mesencephalon) with the FGF-8, SHH, and Wnt agonist, while using FGF-2 and insulin it is possible to obtain Purkinje cells<sup>[138]</sup>.

Also, for drug-induced neurotoxicity, as for other forms of toxicity, there is a



correlation between patient-specific gene variability and the development of adverse drug-induced effects. In line with this observation, also in this case, iPSCs offer the advantage of specific patient studies to better understand the correlation between gene expression and drug toxicity.

An example was described by Ohara *et al*<sup>[139]</sup>. In this study, iPSCs were obtained from PBMCs or T-lymphocytes of two patients with Charcot-Marie-Tooth disease (CMT), with a mutation of a gene encoding a mitochondrial protein (mitofusin-2), and two healthy individuals. iPSCs were differentiated to motor neurons, noting that the mutation also remains in the differentiated neurons, and analysis at the mitochondria level indicated that the neurons derived from patients affected by CMT present mitochondrial dysfunctions. The results showed that two drugs (vincristine and paclitaxel) cause mitochondrial aggregation (a parameter of mitochondrial abnormalities to evaluate the neurotoxic effects) in healthy and CMT-derived neurons, but the greatest effect was observed in patients. The authors concluded that the effect of the tested drugs is different between patients and healthy donors and that the analysis of mitochondria is a good parameter to study neurotoxicity<sup>[139]</sup>.

Permanent peripheral neuropathy is the most common non-hematologic toxicity of anti-cancer chemotherapy, with an incidence of around 20%-40%<sup>[140]</sup>. It has been hypothesized that calcium signaling, oxidative stress, or mitochondrial changes are effects caused by anti-cancer drugs and that these effects may induce neuropathy<sup>[132]</sup>. Several studies are available on commercial cells to study neurotoxicity. Wheeler *et al*<sup>[140]</sup> studied the neurotoxic effects of three anti-cancer drugs (paclitaxel, vincristine, and cisplatin) on neurons derived from commercial human iPSCs (from Cellular Dynamics International). They analyzed the neurite outgrowth phenotype and observed a dose-dependent decrease in neurite processes in the case of treatment with the drugs (the greatest effect was observed for vincristine). Using the Caspase-Glo 3/7 assay, drug-induced apoptosis was evaluated, noting that the greatest effect of increased caspase activity was present in the case of treatment with cisplatin, followed by treatment with vincristine, while paclitaxel had no effect<sup>[140]</sup>. The effect of paclitaxel on neurite outgrowth without affecting caspase 3/7 activation was also observed by Wing *et al*<sup>[141]</sup>. In this study, neurotoxicity of other anti-cancer drugs was evaluated on two commercial human iPSC-derived neuronal cell lines: iCell® Neurons (Cellular Dynamics International) and Peri.4U (Axiogenesis, Cologne, Germany), demonstrating a differential sensitivity of neurons to different chemotherapeutics<sup>[141]</sup>. Another study carried out on Peri.4U neurons (from Axiogenesis) is reported by Rana *et al*<sup>[142]</sup>, who studied the effect of 16 chemotherapeutic agents. One of the most important findings was that some drugs such as epothilone, taxane, and vinca alkaloid chemotherapeutics did not produce cytotoxicity even though a reduction in the length of the neurite was observed<sup>[142]</sup>.

Snyder *et al*<sup>[143]</sup> evaluated the neurotoxic effects of different classes of chemotherapeutics on various commercial neurons derived from iPSC: Peripheral iPSC-neurons (Axiogenesis), iCell® Neurons (Cellular Dynamics International), ReproNeuro glutamatergic neurons (ReproCell, Glasgow, United Kingdom), and human cerebral cortical neurons (Axol Bioscience, Cambridge, United Kingdom). Initially, they evaluated the expression of the characteristic markers of each type of iPSC neurons by gene expression and protein quantification, while the neurotoxic effect of the compounds was evaluated as neurite dynamics and apoptosis<sup>[143]</sup>.

Another example was reported by Yamada *et al*<sup>[144]</sup>, who evaluated the neurotoxicity and the influence on neuronal development of neuronal cells derived from commercial iPSCs of 5-fluorouracil. These cells have been used as an *in vitro* model of human fetal stage. Real-time PCR results showed that this drug has inhibitory effects on the early neural differentiation of iPSCs. All of these studies have shown that commercial neurons derived from iPSC may be useful models for the study of drug-induced neurotoxicity, especially chemotherapeutics.

Considering the different physiological characteristics between children and adults, particular care should be taken in the investigation of ADRs in children, also in the case of drug-induced neurotoxicity. An example is represented by anesthetics, as children seem to be more vulnerable to the adverse effects of these drugs. The problem arises by the fact that many of the anesthetic protocols used in children were developed because of those used in adults<sup>[145]</sup>. Numerous studies have indicated that neuronal damage due to anesthetic depends on many factors such as duration of exposure, developmental phase at the time of exposure, activated receptor subtype, and dose and route of administration. These agents can damage immature neurons, inducing morphological changes and functional impairment of mitochondria, releasing ROS that can further damage the tissue<sup>[146]</sup>.

Neurotoxicity induced by chemotherapy is another problem in children as it may compromise the quality of life and longevity. For example, vincristine is used for ALL, which is the most common childhood cancer. However, this drug causes motor and

sensory dysfunction and neuropathic pain. Diouf *et al*<sup>[147]</sup> performed genotyping analysis on two cohorts of pediatric patients with ALL treated with vincristine and noted that the rs924607 polymorphism (*CEP72* gene) could be associated with the development of vincristine-induced neuropathy. Analysis of the neurite development such as outgrowth, number of processes and number of branches on commercial iCell neurons derived from iPSC (Cellular Dynamics International) showed a relationship between *CEP7* gene expression and vincristine-induced neurotoxicity; a reduced expression of this gene was associated with a greater sensitivity to the drug. This may suggest that patient-specific therapy would be required for a safer use of vincristine<sup>[147]</sup>.

Considering the extreme complexity of the 3D structure of the brain, the study of its development and modeling of disease processes in 2D cultures could have several limitations. Therefore, the use of human iPSCs for the generation of 3D *in vitro* organoids has revolutionized the research in this field by recapitulating the organization of different regions of the nervous system such as retina, cerebral cortex, and choroid plexus<sup>[148-150]</sup>. The protocol described by Lancaster and Knoblich<sup>[148]</sup> to develop cerebral organoids begins with the generation of embryoid bodies from iPSCs. Embryoid bodies are subjected to neural induction in a culture medium that favors only the neuroectoderm to expand and are then transferred into droplet of Matrigel, which allows outgrowth of neuroepithelial buds. In the end, the tissues are moved to a spinning bioreactor, or in an orbital shaking plate, which contributes to increase oxygen and nutrient interchange and further development into defined brain regions.

Human brain organoids provide insights into the pathogenesis of neurological disorders and may offer new approaches for investigating the mechanism of the neurotoxicity of drugs. For instance, Liu and collaborators<sup>[151]</sup> used this *in vitro* model to study the neurotoxic effects of vincristine treatment. In particular, human iPSC (SC101A-1) were cultured to generate cerebral organoids and subsequently were treated with different concentrations of vincristine for 48 hours; the treatment resulted in a dose-dependent growth inhibition evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test and caused the enhanced expression of cleaved caspase 3, indicating the induction of apoptosis<sup>[151]</sup>. In the same paper, several cellular markers were analyzed in cerebral organoids exposed to the drug, highlighting the inhibition of the microtubule-associated protein tubulin, fibronectin, and the downregulation of matrix metalloproteinases 10.

Another recent report published by Huang *et al*<sup>[152]</sup> describes the cerebral organoids as an innovative tool to investigate the toxicity of drugs. The authors showed that tranylcypromine, which is used to treat refractory depression, caused neurotoxicity on brain organoids generated from human iPSCs (SC101A-1), leading to a decreased proliferation activity and apoptosis induction<sup>[152]</sup>. These data were confirmed by measuring the expressions of the neuron-specific markers TUJ1 and GFAP, which stained for neurons and astrocytes respectively: tranylcypromine treatment impaired cell density and arrangement affecting both cell types. Moreover, tranylcypromine treatment abolished the transcriptional activity of BHC110/LSD1-targeted genes and increased the expression of histone di-methylated K4.

### Limits of iPSCs

One of the first problems that can be highlighted regarding iPSCs technology is the low efficiency in reprogramming. The most important problem is related to the use of viral vectors such as retrovirus and lentivirus, which deliver the reprogramming transcription factors by integration in the host cell genome, leading to chromosomal instability and tumorigenesis from insertional mutagenesis.

However, during the past several years, new safer and non-integrative reprogramming methods have been developed, reaching better efficiency and avoiding the already mentioned issues. Another interesting point to discuss regards epigenetic memory and clonal variability. iPSCs can present an epigenetic memory of the parent somatic cells that can influence the differentiation propensity and therefore the outcomes of studies applied to this technology. This peculiarity of iPSCs, not characteristic of ESCs, may predispose them to differentiate more readily into their parental cells than others and can be useful for example in cell replacement therapy.

Kim *et al*<sup>[153]</sup> observed in iPSCs obtained by transcription factor-based reprogramming, a residual DNA methylation pattern characteristic of the parent cells, which increase their propensity to differentiate along lineages related to the donor tissue, while restricting alternative cell fates. The authors tried to reset the epigenome of the parent tissue by differentiation and serial reprogramming, or by treatment of iPSCs with chromatin-modifying drugs. These approaches could help researchers in increasing the differentiation efficiency of iPSCs, limiting the problem related to epigenetic memory. However, other aspects could affect epigenetic memory of iPSCs,

such as the number of passages performed. For example, Polo *et al*<sup>[154]</sup> demonstrated that increasing the number of passages there is a decrement in differentially methylated regions with respect to the parent cells. One of the reasons that can explain the spontaneous loss of epigenetic memory was postulated by the authors; probably, the loss of parent cells pattern is based on a slow replication-dependent process. However, further studies must be performed to consolidate this hypothesis as indicated by authors and, to date, the origins of epigenetic loss remain to be determined.

Another important point related to iPSCs limits is the intra-variability differences of clones of the same patient. Thatava *et al*<sup>[155]</sup> addressed this problem analyzing the variation in terms of pancreatic beta-cells differentiation of three iPSCs clone lines of three patients suffering from type 1 diabetes. Interestingly, a notable intra-patient variation, comparable to interpatient one, was identified by authors leading to conclude the necessity for a comprehensive fingerprinting of multiple patient-specific clones to obtain a representative pool of cells useable for biomedical applications such as ADRs studies. In addition to Thatava study, also Yokobayashi *et al*<sup>[156]</sup> found that the efficiency in iPSCs clones differentiation capability into primordial germ-cell was different; in particular, they observed that the XX clones were less efficient in germ-cell differentiation. However, interindividual variability is more important to consider than intraindividual variation for complex genetic disorders<sup>[157]</sup>. Deneault *et al*<sup>[158]</sup> analyzed both types of variabilities in iPSCs-derived neuron cells of patients with autism spectrum disorder, a heterogeneous and complex genetic condition, finding significant intra-variability only on few lines, corroborating what has just been said. Concluding, given the contrasts still present on this aspect, the intra-variability between different iPSCs clones should be investigated and it is advisable to analyze the genetic and epigenetic features of the clones generated. To study ADRs, the clonal variability is a key point to keep in mind in order to set up useful standardized tools. Therefore, before developing a model useful to predict patients' sensitivity, different clones should be first genetically checked to exclude for instance chromosomic aberrations, alterations in differentiation efficiency and variability in DNA methylation profiles. Also, the sensitivity to the drugs of interest should be analyzed in the different clones from the same patients, to exclude a variability in the response.

Besides iPSCs, the direct differentiation of somatic cells could be used to obtain the cells of interest. This approach has both advantages and limitations with respect to iPSCs technology regarding the study of ADRs (Table 1). A great advantage of direct differentiation is the short period of time necessary to obtain the cells of interest, avoiding the iPSCs step. Moreover, using this direct method, all DNA instability problems in generating and expanding iPSCs, such as point mutations and karyotypic abnormalities, can be avoided. In addition, to study ADRs affecting aged populations, it is desirable to obtain cells reflecting the disease-related DNA mutations together with the age of the patient. However, there is still an open debate if the best method to recapitulate the age of the patient is the iPSCs technique or the somatic direct differentiation<sup>[159]</sup>. The direct method has also several limitations; iPSCs have a higher proliferation capacity and they can self-replicate in culture differently from parental primary cell lines used in the direct method that can be cultured for a very short time with fast accumulation of epigenetic changes. Regarding ADR studies, it is important to generate a great number of differentiated cells of interest and this purpose can be more easily achieved with iPSCs rather than with the direct method based on the great expandability of iPSCs, as discussed above. Heterogenicity of the differentiated cells is another critical point for both techniques. This point is particularly critical for cell therapy and there are several works trying to ameliorate this aspect. Some limitations have to be underlined also for organoid technology: the complex culture medium contains a cocktail of growth factors and molecular inhibitors which might affect the drug responses of organoids; the extracellular matrix Matrigel, used to grow the organoids, might hamper drug penetration and limit their potential in drug screening; genetic and epigenetic aberrations might occur during long-term culture making organoids unsuitable for transplantation/regenerative medicine. Therefore, further efforts are needed to overcome these limits and improve this promising technology for clinical applications.

## CONCLUSION

ADRs are one of the major clinical problems, especially in populations at risk, such as pediatric patients. One of the most important tools to improve the comprehension, and thus the prevention, of ADRs, is represented by the possibility to set up groundbreaking patient-specific assays. This goal has been powerfully achieved using

**Table 1 Main differences of differentiation techniques based on iPSCs and direct somatic differentiation technologies**

	iPSCs	Direct somatic differentiation
Reprogramming time	Long	Not necessary
Differentiation time	Long	Brief
DNA instability	Occasional	Rare
Proliferation of pre-differentiated cells	High (iPSCs)	Low ( <i>e.g.</i> , fibroblasts)
Self-renewal of pre-differentiated cells	Yes (iPSCs)	No ( <i>e.g.</i> , fibroblasts)
Heterogeneity of the differentiated population	Critical	Critical

iPSCs: Induced pluripotent stem cells.

iPSCs, being characterized by genetic and physiological patient-specific differences and being able to be differentiated ideally into all the tissues of human body. The knowledge gained so far has demonstrated that iPSCs can be fruitfully used in multiple clinical applications, including not only toxicity screening in the frame of ADRs characterization, but also disease modelling, personalized and regenerative medicines. In addition, the recent development of related models, such as 3D organoids derived from pluripotent or adult stem cells, resembling the 3D architecture of different organs in a more physiological way, has paved the way to better characterize ADRs in a personalized way.

It should be considered that the number of studies on this topic is still limited, especially concerning the pediatric field. In this case, particular care should be taken, considering that children are a population at risk for ADRs and because of the difficulties to obtain biological materials necessary to establish iPSCs models or stem cells-based organoids. However, based also on studies carried out on these models derived from adult patients or by commercial suppliers, a major advancement has been made to develop and establish differentiation procedures to obtain more efficient differentiation protocols and toxicity screening methods. It is therefore conceivable that in the near future, thanks to this progress, also the number of pediatric studies will increase, enabling the possibility to efficiently study and predict ADRs also in pediatric patients.

## ACKNOWLEDGEMENTS

Figures were modified by smart server medical art.

## REFERENCES

- 1 **Sultana J**, Cutroneo P, Trifirò G. Clinical and economic burden of adverse drug reactions. *J Pharmacol Pharmacother* 2013; **4**: S73-S77 [PMID: [24347988](#) DOI: [10.4103/0976-500X.120957](#)]
- 2 **Priyadharsini R**, Surendiran A, Adithan C, Sreenivasan S, Sahoo FK. A study of adverse drug reactions in pediatric patients. *J Pharmacol Pharmacother* 2011; **2**: 277-280 [PMID: [22025857](#) DOI: [10.4103/0976-500X.85957](#)]
- 3 **Chien JY**, Ho RJ. Drug delivery trends in clinical trials and translational medicine: evaluation of pharmacokinetic properties in special populations. *J Pharm Sci* 2011; **100**: 53-58 [PMID: [20589750](#) DOI: [10.1002/jps.22253](#)]
- 4 **Kola I**, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov* 2004; **3**: 711-715 [PMID: [15286737](#) DOI: [10.1038/nrd1470](#)]
- 5 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: [16904174](#) DOI: [10.1016/j.cell.2006.07.024](#)]
- 6 **Raja K**, Patrick M, Elder JT, Tsoi LC. Machine learning workflow to enhance predictions of Adverse Drug Reactions (ADRs) through drug-gene interactions: application to drugs for cutaneous diseases. *Sci Rep* 2017; **7**: 3690 [PMID: [28623363](#) DOI: [10.1038/s41598-017-03914-3](#)]
- 7 **McCauley HA**, Wells JM. Pluripotent stem cell-derived organoids: using principles of developmental biology to grow human tissues in a dish. *Development* 2017; **144**: 958-962 [PMID: [28292841](#) DOI: [10.1242/dev.140731](#)]
- 8 **Wilke RA**, Lin DW, Roden DM, Watkins PB, Flockhart D, Zineh I, Giacomini KM, Krauss RM. Identifying genetic risk factors for serious adverse drug reactions: current progress and challenges. *Nat Rev Drug Discov* 2007; **6**: 904-916 [PMID: [17971785](#) DOI: [10.1038/nrd2423](#)]
- 9 **Patel P**, Mital S. Stem cells in pediatric cardiology. *Eur J Pediatr* 2013; **172**: 1287-1292 [PMID: [23292032](#) DOI: [10.1007/s00431-012-1920-4](#)]
- 10 **Evans WE**, McLeod HL. Pharmacogenomics--drug disposition, drug targets, and side effects. *N Engl J Med* 2003; **348**: 538-549 [PMID: [12571262](#) DOI: [10.1056/NEJMra020526](#)]



- 11 **Loscalzo J**, Handy DE. Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series). *Pulm Circ* 2014; **4**: 169-174 [PMID: [25006435](#) DOI: [10.1086/675979](#)]
- 12 **Handy DE**, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. *Circulation* 2011; **123**: 2145-2156 [PMID: [21576679](#) DOI: [10.1161/CIRCULATIONAHA.110.956839](#)]
- 13 **Stephenson T**. How children's responses to drugs differ from adults. *Br J Clin Pharmacol* 2005; **59**: 670-673 [PMID: [15948930](#) DOI: [10.1111/j.1365-2125.2005.02445.x](#)]
- 14 **Fernandez E**, Perez R, Hernandez A, Tejada P, Arteta M, Ramos JT. Factors and Mechanisms for Pharmacokinetic Differences between Pediatric Population and Adults. *Pharmaceutics* 2011; **3**: 53-72 [PMID: [24310425](#) DOI: [10.3390/pharmaceutics3010053](#)]
- 15 **Dean L**, Pratt V, McLeod H, Rubinstein W, Dean L, Kattman B. Warfarin Therapy and VKORC1 and CYP Genotype. Pratt V, McLeod H, Rubinstein W, Dean L, Kattman B. *SourceMedical Genetics Summaries*. Bethesda (MD): National Center for Biotechnology Information (US) 2012;
- 16 **Daly AK**. Pharmacogenomics of adverse drug reactions. *Genome Med* 2013; **5**: 5 [PMID: [23360680](#) DOI: [10.1186/gm409](#)]
- 17 **Joseph PD**, Craig JC, Caldwell PH. Clinical trials in children. *Br J Clin Pharmacol* 2015; **79**: 357-369 [PMID: [24325152](#) DOI: [10.1111/bcp.12305](#)]
- 18 **Stocco G**, Lanzi G, Yue F, Giliani S, Sasaki K, Tommasini A, Pelin M, Martelossi S, Ventura A, Decorti G. Patients' Induced Pluripotent Stem Cells to Model Drug Induced Adverse Events: A Role in Predicting Thiopurine Induced Pancreatitis? *Curr Drug Metab* 2015; **17**: 91-98 [PMID: [26526832](#) DOI: [10.2174/1389200216666151103120220](#)]
- 19 **Kilpinen H**, Goncalves A, Leha A, Afzal V, Alasoo K, Ashford S, Bala S, Bensaddek D, Casale FP, Culley OJ, Danecek P, Faulconbridge A, Harrison PW, Kathuria A, McCarthy D, McCarthy SA, Meleckyte R, Memari Y, Moens N, Soares F, Mann A, Streeter I, Agu CA, Alderton A, Nelson R, Harper S, Patel M, White A, Patel SR, Clarke L, Halai R, Kirton CM, Kolb-Kokocinski A, Beales P, Birney E, Danovi D, Lamond AI, Ouwehand WH, Vallier L, Watt FM, Durbin R, Stegle O, Gaffney DJ. Common genetic variation drives molecular heterogeneity in human iPSCs. *Nature* 2017; **546**: 370-375 [PMID: [28489815](#) DOI: [10.1038/nature22403](#)]
- 20 **Sayed N**, Liu C, Wu JC. Translation of Human-Induced Pluripotent Stem Cells: From Clinical Trial in a Dish to Precision Medicine. *J Am Coll Cardiol* 2016; **67**: 2161-2176 [PMID: [27151349](#) DOI: [10.1016/j.jacc.2016.01.083](#)]
- 21 **Takayama K**, Morisaki Y, Kuno S, Nagamoto Y, Harada K, Furukawa N, Ohtaka M, Nishimura K, Imagawa K, Sakurai F, Tachibana M, Sumazaki R, Noguchi E, Nakanishi M, Hirata K, Kawabata K, Mizuguchi H. Prediction of interindividual differences in hepatic functions and drug sensitivity by using human iPS-derived hepatocytes. *Proc Natl Acad Sci U S A* 2014; **111**: 16772-16777 [PMID: [25385620](#) DOI: [10.1073/pnas.1413481111](#)]
- 22 **Krueger W**, Boelsterli UA, Rasmussen TP. Stem Cell Strategies to Evaluate Idiosyncratic Drug-induced Liver Injury. *J Clin Transl Hepatol* 2014; **2**: 143-152 [PMID: [26355943](#) DOI: [10.14218/JCTH.2014.00012](#)]
- 23 **Takeda M**, Miyagawa S, Fukushima S, Saito A, Ito E, Harada A, Matsuura R, Iseoka H, Sougawa N, Mochizuki-Oda N, Matsusaki M, Akashi M, Sawa Y. Development of In Vitro Drug-Induced Cardiotoxicity Assay by Using Three-Dimensional Cardiac Tissues Derived from Human Induced Pluripotent Stem Cells. *Tissue Eng Part C Methods* 2018; **24**: 56-67 [PMID: [28967302](#) DOI: [10.1089/ten.TEC.2017.0247](#)]
- 24 **Pei Y**, Peng J, Behl M, Sipes NS, Shockley KR, Rao MS, Tice RR, Zeng X. Comparative neurotoxicity screening in human iPSC-derived neural stem cells, neurons and astrocytes. *Brain Res* 2016; **1638**: 57-73 [PMID: [26254731](#) DOI: [10.1016/j.brainres.2015.07.048](#)]
- 25 **Agrawal S**, Khan F. Human genetic variation and personalized medicine. *Indian J Physiol Pharmacol* 2007; **51**: 7-28 [PMID: [17877289](#)]
- 26 **Simkin D**, Kiskinis E. Modeling Pediatric Epilepsy Through iPSC-Based Technologies. *Epilepsy Curr* 2018; **18**: 240-245 [PMID: [30254520](#) DOI: [10.5698/1535-7597.18.4.240](#)]
- 27 **Durbin MD**, Cadar AG, Chun YW, Hong CC. Investigating pediatric disorders with induced pluripotent stem cells. *Pediatr Res* 2018; **84**: 499-508 [PMID: [30065271](#) DOI: [10.1038/s41390-018-0064-2](#)]
- 28 **Jang J**, Quan Z, Yum YJ, Song HS, Paek S, Kang HC. Induced pluripotent stem cells for modeling of pediatric neurological disorders. *Biotechnol J* 2014; **9**: 871-881 [PMID: [24838856](#) DOI: [10.1002/biot.201400010](#)]
- 29 **Dutta D**, Heo I, Clevers H. Disease Modeling in Stem Cell-Derived 3D Organoid Systems. *Trends Mol Med* 2017; **23**: 393-410 [PMID: [28341301](#) DOI: [10.1016/j.molmed.2017.02.007](#)]
- 30 **Sato T**, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009; **459**: 262-265 [PMID: [19329995](#) DOI: [10.1038/nature07935](#)]
- 31 **Spence JR**, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM, Shroyer NF, Wells JM. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature* 2011; **470**: 105-109 [PMID: [21151107](#) DOI: [10.1038/nature09691](#)]
- 32 **Barker N**, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M, Danenberg E, van den Brink S, Korving J, Abo A, Peters PJ, Wright N, Poulsom R, Clevers H. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* 2010; **6**: 25-36 [PMID: [20085740](#) DOI: [10.1016/j.stem.2009.11.013](#)]
- 33 **Takasato M**, Er PX, Chiu HS, Little MH. Generation of kidney organoids from human pluripotent stem cells. *Nat Protoc* 2016; **11**: 1681-1692 [PMID: [27560173](#) DOI: [10.1038/nprot.2016.098](#)]
- 34 **Huch M**, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, Sato T, Hamer K, Sasaki N, Finegold MJ, Haft A, Vries RG, Grompe M, Clevers H. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature* 2013; **494**: 247-250 [PMID: [23354049](#) DOI: [10.1038/nature11826](#)]
- 35 **Huch M**, Bonfanti P, Boj SF, Sato T, Loomans CJ, van de Wetering M, Sojoodi M, Li VS, Schuijers J, Gracanin A, Ringnald F, Begthel H, Hamer K, Mulder J, van Es JH, de Koning E, Vries RG, Heimberg H, Clevers H. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J* 2013; **32**: 2708-2721 [PMID: [24045232](#) DOI: [10.1038/emboj.2013.204](#)]
- 36 **Lancaster MA**, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, Homfray T, Penninger JM, Jackson AP, Knoblich JA. Cerebral organoids model human brain development and microcephaly. *Nature* 2013; **501**: 373-379 [PMID: [23995685](#) DOI: [10.1038/nature12517](#)]
- 37 **Huang SX**, Islam MN, O'Neill J, Hu Z, Yang YG, Chen YW, Mumau M, Green MD, Vunjak-Novakovic



- G, Bhattacharya J, Snoeck HW. Efficient generation of lung and airway epithelial cells from human pluripotent stem cells. *Nat Biotechnol* 2014; **32**: 84-91 [PMID: [24291815](#) DOI: [10.1038/nbt.2754](#)]
- 38 **Kraiczy J**, Nayak KM, Howell KJ, Ross A, Forbester J, Salvestrini C, Mustata R, Perkins S, Andersson-Rolf A, Leenen E, Liebert A, Vallier L, Rosenstiel PC, Stegle O, Dougan G, Heuschkel R, Koo BK, Zilbauer M. DNA methylation defines regional identity of human intestinal epithelial organoids and undergoes dynamic changes during development. *Gut* 2019; **68**: 49-61 [PMID: [29141958](#) DOI: [10.1136/gutjnl-2017-314817](#)]
- 39 **Huh D**, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. *Trends Cell Biol* 2011; **21**: 745-754 [PMID: [22033488](#) DOI: [10.1016/j.tcb.2011.09.005](#)]
- 40 **Leong RW**, Chan FK. Drug-induced side effects affecting the gastrointestinal tract. *Expert Opin Drug Saf* 2006; **5**: 585-592 [PMID: [16774495](#) DOI: [10.1517/14740338.5.4.585](#)]
- 41 **Suk KT**, Kim DJ. Drug-induced liver injury: present and future. *Clin Mol Hepatol* 2012; **18**: 249-257 [PMID: [23091804](#) DOI: [10.3350/cmh.2012.18.3.249](#)]
- 42 **Zeino Z**, Sisson G, Bjarnason I. Adverse effects of drugs on small intestine and colon. *Best Pract Res Clin Gastroenterol* 2010; **24**: 133-141 [PMID: [20227027](#) DOI: [10.1016/j.bpg.2010.02.008](#)]
- 43 **Iwao T**, Toyota M, Miyagawa Y, Okita H, Kiyokawa N, Akutsu H, Umezawa A, Nagata K, Matsunaga T. Differentiation of human induced pluripotent stem cells into functional enterocyte-like cells using a simple method. *Drug Metab Pharmacokinet* 2014; **29**: 44-51 [PMID: [23822979](#) DOI: [10.2133/dmpk.DMPK-13-RG-005](#)]
- 44 **Iwao T**, Kodama N, Kondo Y, Kabeya T, Nakamura K, Horikawa T, Niwa T, Kurose K, Matsunaga T. Generation of enterocyte-like cells with pharmacokinetic functions from human induced pluripotent stem cells using small-molecule compounds. *Drug Metab Dispos* 2015; **43**: 603-610 [PMID: [25650381](#) DOI: [10.1124/dmd.114.062604](#)]
- 45 **Ozawa T**, Takayama K, Okamoto R, Negoro R, Sakurai F, Tachibana M, Kawabata K, Mizuguchi H. Generation of enterocyte-like cells from human induced pluripotent stem cells for drug absorption and metabolism studies in human small intestine. *Sci Rep* 2015; **5**: 16479 [PMID: [26559489](#) DOI: [10.1038/srep16479](#)]
- 46 **Kondo S**, Mizuno S, Hashita T, Iwao T, Matsunaga T. Using human iPS cell-derived enterocytes as novel in vitro model for the evaluation of human intestinal mucosal damage. *Inflamm Res* 2018; **67**: 975-984 [PMID: [30317465](#) DOI: [10.1007/s00011-018-1193-0](#)]
- 47 **Kim YD**, Jeon JY, Woo HJ, Lee JC, Chung JH, Song SY, Yoon SK, Baek SH. Interleukin-1 $\beta$  induces MUC2 gene expression and mucin secretion via activation of PKC-MEK/ERK, and PI3K in human airway epithelial cells. *J Korean Med Sci* 2002; **17**: 765-771 [PMID: [12482999](#) DOI: [10.3346/jkms.2002.17.6.765](#)]
- 48 **Enss ML**, Cornberg M, Wagner S, Gebert A, Henrichs M, Eisenblätter R, Beil W, Kownatzki R, Hedrich HJ. Proinflammatory cytokines trigger MUC gene expression and mucin release in the intestinal cancer cell line LS180. *Inflamm Res* 2000; **49**: 162-169 [PMID: [10858016](#) DOI: [10.1007/s000110050576](#)]
- 49 **Van Seuningen I**, Pigny P, Perrais M, Porchet N, Aubert JP. Transcriptional regulation of the 11p15 mucin genes. Towards new biological tools in human therapy, in inflammatory diseases and cancer? *Front Biosci* 2001; **6**: D1216-D1234 [PMID: [11578973](#) DOI: [10.2741/A675](#)]
- 50 **Mejias-Luque R**, Lindén SK, Garrido M, Tye H, Najdovska M, Jenkins BJ, Iglesias M, Ernst M, de Bolós C. Inflammation modulates the expression of the intestinal mucins MUC2 and MUC4 in gastric tumors. *Oncogene* 2010; **29**: 1753-1762 [PMID: [20062084](#) DOI: [10.1038/onc.2009.467](#)]
- 51 **Fischer BM**, Rochelle LG, Voynow JA, Akley NJ, Adler KB. Tumor necrosis factor- $\alpha$  stimulates mucin secretion and cyclic GMP production by guinea pig tracheal epithelial cells in vitro. *Am J Respir Cell Mol Biol* 1999; **20**: 413-422 [PMID: [10030839](#) DOI: [10.1165/ajrcmb.20.3.3393](#)]
- 52 **Li M**, Izpisua Belmonte JC. Organoids - Preclinical Models of Human Disease. *N Engl J Med* 2019; **380**: 569-579 [PMID: [30726695](#) DOI: [10.1056/NEJMr1806175](#)]
- 53 **Fujii M**, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, Sugimoto S, Sato T. Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition. *Cell Stem Cell* 2018; **23**: 787-793.e6 [PMID: [30526881](#) DOI: [10.1016/j.stem.2018.11.016](#)]
- 54 **Lu W**, Rettenmeier E, Paszek M, Yueh MF, Tukey RH, Trotter J, Barbier O, Chen S. Crypt Organoid Culture as an in Vitro Model in Drug Metabolism and Cytotoxicity Studies. *Drug Metab Dispos* 2017; **45**: 748-754 [PMID: [28468837](#) DOI: [10.1124/dmd.117.075945](#)]
- 55 **Lu H**, Rosenbaum S. Developmental pharmacokinetics in pediatric populations. *J Pediatr Pharmacol Ther* 2014; **19**: 262-276 [PMID: [25762871](#) DOI: [10.5863/1551-6776-19.4.262](#)]
- 56 **Crespo M**, Vilar E, Tsai SY, Chang K, Amin S, Srinivasan T, Zhang T, Pipalia NH, Chen HJ, Witherspoon M, Gordillo M, Xiang JZ, Maxfield FR, Lipkin S, Evans T, Chen S. Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. *Nat Med* 2017; **23**: 878-884 [PMID: [28628110](#) DOI: [10.1038/nm.4355](#)]
- 57 **Alempijevic T**, Zec S, Milosavljevic T. Drug-induced liver injury: Do we know everything? *World J Hepatol* 2017; **9**: 491-502 [PMID: [28443154](#) DOI: [10.4254/wjh.v9.i10.491](#)]
- 58 **Licata A**, Minissale MG, Calvaruso V, Craxi A. A focus on epidemiology of drug-induced liver injury: analysis of a prospective cohort. *Eur Rev Med Pharmacol Sci* 2017; **21**: 112-121 [PMID: [28379588](#)]
- 59 **Lasser KE**, Allen PD, Woolhandler SJ, Himmelstein DU, Wolfe SM, Bor DH. Timing of new black box warnings and withdrawals for prescription medications. *JAMA* 2002; **287**: 2215-2220 [PMID: [11980521](#) DOI: [10.1001/jama.287.17.2215](#)]
- 60 **Vinken M**, Maes M, Vanhaecke T, Rogiers V. Drug-induced liver injury: mechanisms, types and biomarkers. *Curr Med Chem* 2013; **20**: 3011-3021 [PMID: [23746274](#) DOI: [10.2174/0929867311320240006](#)]
- 61 **Ghallab A**. In vitro test systems and their limitations. *EXCLI J* 2013; **12**: 1024-1026 [PMID: [27034642](#)]
- 62 **Saeidnia S**, Manayi A, Abdollahi M. From in vitro Experiments to in vivo and Clinical Studies; Pros and Cons. *Curr Drug Discov Technol* 2015; **12**: 218-224 [PMID: [26778084](#) DOI: [10.2174/1570163813666160114093140](#)]
- 63 **Kondo Y**, Iwao T, Nakamura K, Sasaki T, Takahashi S, Kamada N, Matsubara T, Gonzalez FJ, Akutsu H, Miyagawa Y, Okita H, Kiyokawa N, Toyoda M, Umezawa A, Nagata K, Matsunaga T, Ohmori S. An efficient method for differentiation of human induced pluripotent stem cells into hepatocyte-like cells retaining drug metabolizing activity. *Drug Metab Pharmacokinet* 2014; **29**: 237-243 [PMID: [24334537](#) DOI: [10.2133/dmpk.DMPK-13-RG-104](#)]
- 64 **Kang SJ**, Lee HM, Park YI, Yi H, Lee H, So B, Song JY, Kang HG. Chemically induced hepatotoxicity in human stem cell-induced hepatocytes compared with primary hepatocytes and HepG2. *Cell Biol Toxicol*

- 2016; **32**: 403-417 [PMID: 27287938 DOI: 10.1007/s10565-016-9342-0]
- 65 **Liu J**, Brzeszczynska J, Samuel K, Black J, Palakkan A, Anderson RA, Gallagher R, Ross JA. Efficient episomal reprogramming of blood mononuclear cells and differentiation to hepatocytes with functional drug metabolism. *Exp Cell Res* 2015; **338**: 203-213 [PMID: 26256888 DOI: 10.1016/j.yexcr.2015.08.004]
- 66 **Wilson AA**, Ying L, Liesa M, Segeritz CP, Mills JA, Shen SS, Jean J, Lonza GC, Liberti DC, Lang AH, Nazaire J, Gower AC, Müller FJ, Mehta P, Ordóñez A, Lomas DA, Vallier L, Murphy GJ, Mostoslavsky G, Spira A, Shirihaï OS, Ramirez MI, Gadue P, Kotton DN. Emergence of a stage-dependent human liver disease signature with directed differentiation of alpha-1 antitrypsin-deficient iPSC cells. *Stem Cell Reports* 2015; **4**: 873-885 [PMID: 25843048 DOI: 10.1016/j.stemcr.2015.02.021]
- 67 **Kvist AJ**, Kanebratt KP, Walentinsson A, Palmgren H, O'Hara M, Björkbohm A, Andersson LC, Ahlqvist M, Andersson TB. Critical differences in drug metabolic properties of human hepatic cellular models, including primary human hepatocytes, stem cell derived hepatocytes, and hepatoma cell lines. *Biochem Pharmacol* 2018; **155**: 124-140 [PMID: 29953844 DOI: 10.1016/j.bcp.2018.06.026]
- 68 **Bell CC**, Hendriks DF, Moro SM, Ellis E, Walsh J, Renblom A, Fredriksson Puigvert L, Dankers AC, Jacobs F, Snoeys J, Sison-Young RL, Jenkins RE, Nordling Å, Mkrichian S, Park BK, Kitteringham NR, Goldring CE, Lauschke VM, Ingelman-Sundberg M. Characterization of primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease. *Sci Rep* 2016; **6**: 25187 [PMID: 27143246 DOI: 10.1038/srep25187]
- 69 **Smutný T**, Harjumäki R, Kanninen L, Yliperttula M, Pávek P, Lou YR. A feasibility study of the toxic responses of human induced pluripotent stem cell-derived hepatocytes to phytochemicals. *Toxicol In Vitro* 2018; **52**: 94-105 [PMID: 29902661 DOI: 10.1016/j.tiv.2018.06.012]
- 70 **Murayama N**, Yamazaki H. Cytochrome P450-dependent drug oxidation activities in commercially available hepatocytes derived from human induced pluripotent stem cells cultured for 3 weeks. *J Toxicol Sci* 2018; **43**: 241-245 [PMID: 29618712 DOI: 10.2131/jts.43.241]
- 71 **Hu H**, Gehart H, Artegiani B, López-Iglesias C, Dekkers F, Basak O, van Es J, Chuva de Sousa Lopes SM, Begthel H, Korving J, van den Born M, Zou C, Quirk C, Chiriboga L, Rice CM, Ma S, Rios A, Peters PJ, de Jong YP, Clevers H. Long-Term Expansion of Functional Mouse and Human Hepatocytes as 3D Organoids. *Cell* 2018; **175**: 1591-1606.e19 [PMID: 30500538 DOI: 10.1016/j.cell.2018.11.013]
- 72 **Takebe T**, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang RR, Ueno Y, Zheng YW, Koike N, Aoyama S, Adachi Y, Taniguchi H. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 2013; **499**: 481-484 [PMID: 23823721 DOI: 10.1038/nature12271]
- 73 **Huch M**, Gehart H, van Bockel R, Hamer K, Blokzijl F, Verstegen MM, Ellis E, van Wenum M, Fuchs SA, de Ligt J, van de Wetering M, Sasaki N, Boers SJ, Kemperman H, de Jonge J, Ijzermans JN, Nieuwenhuis EE, Hoekstra R, Strom S, Vries RR, van der Laan LJ, Cuppen E, Clevers H. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 2015; **160**: 299-312 [PMID: 25533785 DOI: 10.1016/j.cell.2014.11.050]
- 74 **Wu F**, Wu D, Ren Y, Huang Y, Feng B, Zhao N, Zhang T, Chen X, Chen S, Xu A. Generation of hepatobiliary organoids from human induced pluripotent stem cells. *J Hepatol* 2019; **70**: 1145-1158 [PMID: 30630011 DOI: 10.1016/j.jhep.2018.12.028]
- 75 **Leite SB**, Roosens T, El Taghdouini A, Mannaerts I, Smout AJ, Najimi M, Sokal E, Noor F, Chesne C, van Grunsven LA. Novel human hepatic organoid model enables testing of drug-induced liver fibrosis in vitro. *Biomaterials* 2016; **78**: 1-10 [PMID: 26618472 DOI: 10.1016/j.biomaterials.2015.11.026]
- 76 **Genova E**, Pelin M, Sasaki K, Yue F, Lanzi G, Masneri S, Ventura A, Stocco G, Decorti G. Induced Pluripotent Stem Cells as a Model for Therapy Personalization of Pediatric Patients: Disease Modeling and Drug Adverse Effects Prevention. *Curr Med Chem* 2018; **25**: 2826-2839 [PMID: 28782468 DOI: 10.2174/0929867324666170804150131]
- 77 **Pagliuca FW**, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, Peterson QP, Greiner D, Melton DA. Generation of functional human pancreatic  $\beta$  cells in vitro. *Cell* 2014; **159**: 428-439 [PMID: 25303535 DOI: 10.1016/j.cell.2014.09.040]
- 78 **Takizawa-Shirasawa S**, Yoshie S, Yue F, Mogi A, Yokoyama T, Tomotsune D, Sasaki K. FGF7 and cell density are required for final differentiation of pancreatic amylase-positive cells from human ES cells. *Cell Tissue Res* 2013; **354**: 751-759 [PMID: 23996199 DOI: 10.1007/s00441-013-1695-6]
- 79 **Hohwieler M**, Illing A, Hermann PC, Mayer T, Stockmann M, Perkhofer L, Eiseler T, Antony JS, Müller M, Renz S, Kuo CC, Lin Q, Sessler M, Breunig M, Kleiderman SM, Lechel A, Zenker M, Leichsenring M, Rosendahl J, Zenke M, Sainz B, Mayerle J, Costa IG, Seufferlein T, Kormann M, Wagner M, Liebau S, Kleger A. Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modelling. *Gut* 2017; **66**: 473-486 [PMID: 27633923 DOI: 10.1136/gutjnl-2016-312423]
- 80 **Huang L**, Holtzinger A, Jagan I, BeGora M, Lohse I, Ngai N, Nostro C, Wang R, Muthuswamy LB, Crawford HC, Arrowsmith C, Kalloger SE, Renouf DJ, Connor AA, Cleary S, Schaeffer DF, Roehrl M, Tsao MS, Gallinger S, Keller G, Muthuswamy SK. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat Med* 2015; **21**: 1364-1371 [PMID: 26501191 DOI: 10.1038/nm.3973]
- 81 **Faught LN**, Greff MJ, Rieder MJ, Koren G. Drug-induced acute kidney injury in children. *Br J Clin Pharmacol* 2015; **80**: 901-909 [PMID: 25395343 DOI: 10.1111/bcp.12554]
- 82 **Ghane Shahrabaf F**, Assadi F. Drug-induced renal disorders. *J Renal Inj Prev* 2015; **4**: 57-60 [PMID: 26468475 DOI: 10.12861/jrip.2015.12]
- 83 **Córdova-Sánchez BM**, Herrera-Gómez Á, Namendys-Silva SA. Acute Kidney Injury Classified by Serum Creatinine and Urine Output in Critically Ill Cancer Patients. *Biomed Res Int* 2016; **2016**: 6805169 [PMID: 27803928 DOI: 10.1155/2016/6805169]
- 84 **Nolin TD**, Himmelfarb J. Mechanisms of drug-induced nephrotoxicity. *Handb Exp Pharmacol* 2010; **111**: 130 [PMID: 20020261 DOI: 10.1007/978-3-642-00663-0\_5]
- 85 **Tiong HY**, Huang P, Xiong S, Li Y, Vathsala A, Zink D. Drug-induced nephrotoxicity: clinical impact and preclinical in vitro models. *Mol Pharm* 2014; **11**: 1933-1948 [PMID: 24502545 DOI: 10.1021/mp400720w]
- 86 **Freedman BS**. Modeling Kidney Disease with iPSCs. *Biomark Insights* 2015; **10**: 153-169 [PMID: 26740740 DOI: 10.4137/BMI.S20054]
- 87 **Lam AQ**, Freedman BS, Bonventre JV. Directed differentiation of pluripotent stem cells to kidney cells. *Semin Nephrol* 2014; **34**: 445-461 [PMID: 25217273 DOI: 10.1016/j.semnephrol.2014.06.011]
- 88 **Taguchi A**, Kaku Y, Ohmori T, Sharmin S, Ogawa M, Sasaki H, Nishinakamura R. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from

- pluripotent stem cells. *Cell Stem Cell* 2014; **14**: 53-67 [PMID: [24332837](#) DOI: [10.1016/j.stem.2013.11.010](#)]
- 89 **Xia Y**, Nivet E, Sancho-Martinez I, Gallegos T, Suzuki K, Okamura D, Wu MZ, Dubova I, Esteban CR, Montserrat N, Campistol JM, Izpisua Belmonte JC. Directed differentiation of human pluripotent cells to ureteric bud kidney progenitor-like cells. *Nat Cell Biol* 2013; **15**: 1507-1515 [PMID: [24240476](#) DOI: [10.1038/ncb2872](#)]
  - 90 **Musah S**, Dimitrakakis N, Camacho DM, Church GM, Ingber DE. Directed differentiation of human induced pluripotent stem cells into mature kidney podocytes and establishment of a Glomerulus Chip. *Nat Protoc* 2018; **13**: 1662-1685 [PMID: [29995874](#) DOI: [10.1038/s41596-018-0007-8](#)]
  - 91 **Musah S**, Mammoto A, Ferrante TC, Jeanty SSF, Hirano-Kobayashi M, Mammoto T, Roberts K, Chung S, Novak R, Ingram M, Fatanat-Didar T, Koshy S, Weaver JC, Church GM, Ingber DE. Mature induced-pluripotent-stem-cell-derived human podocytes reconstitute kidney glomerular-capillary-wall function on a chip. *Nat Biomed Eng* 2017; **1** [PMID: [29038743](#) DOI: [10.1038/s41551-017-0069](#)]
  - 92 **Kandasamy K**, Chuah JK, Su R, Huang P, Eng KG, Xiong S, Li Y, Chia CS, Loo LH, Zink D. Prediction of drug-induced nephrotoxicity and injury mechanisms with human induced pluripotent stem cell-derived cells and machine learning methods. *Sci Rep* 2015; **5**: 12337 [PMID: [26212763](#) DOI: [10.1038/srep12337](#)]
  - 93 **Takasato M**, Er PX, Chiu HS, Maier B, Baillie GJ, Ferguson C, Parton RG, Wolvetang EJ, Roost MS, Lopes SM, Little MH. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 2016; **536**: 238 [PMID: [27120161](#) DOI: [10.1038/nature17982](#)]
  - 94 **Grassi L**, Alfonsi R, Francescangeli F, Signore M, De Angelis ML, Addario A, Costantini M, Flex E, Ciolfi A, Pizzi S, Bruselles A, Pallocca M, Simone G, Haoui M, Falchi M, Milella M, Sentinelli S, Di Matteo P, Stellacci E, Gallucci M, Muto G, Tartaglia M, De Maria R, Bonci D. Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases. *Cell Death Dis* 2019; **10**: 201 [PMID: [30814510](#) DOI: [10.1038/s41419-019-1453-0](#)]
  - 95 **Li Z**, Azaoka T, Wu J, Liao HK, Li M, Lazo M, Zhou B, Sui Y, Wu MZ, Tamura I, Xia Y, Beyret E, Matsusaka T, Pastan I, Rodriguez Esteban C, Guillen I, Guillen P, Campistol JM, Izpisua Belmonte JC. 3D Culture Supports Long-Term Expansion of Mouse and Human Nephrogenic Progenitors. *Cell Stem Cell* 2016; **19**: 516-529 [PMID: [27570066](#) DOI: [10.1016/j.stem.2016.07.016](#)]
  - 96 **Hale LJ**, Howden SE, Phipson B, Lonsdale A, Er PX, Ghoobrial I, Hosawi S, Wilson S, Lawlor KT, Khan S, Oshlack A, Quinlan C, Lennon R, Little MH. 3D organoid-derived human glomeruli for personalised podocyte disease modelling and drug screening. *Nat Commun* 2018; **9**: 5167 [PMID: [30514835](#) DOI: [10.1038/s41467-018-07594-z](#)]
  - 97 **Magdy T**, Schuldt AJT, Wu JC, Bernstein D, Burrridge PW. Human Induced Pluripotent Stem Cell (hiPSC)-Derived Cells to Assess Drug Cardiotoxicity: Opportunities and Problems. *Annu Rev Pharmacol Toxicol* 2018; **58**: 83-103 [PMID: [28992430](#) DOI: [10.1146/annurev-pharmtox-010617-053110](#)]
  - 98 **Anson BD**, Kolaja KL, Kamp TJ. Opportunities for use of human iPS cells in predictive toxicology. *Clin Pharmacol Ther* 2011; **89**: 754-758 [PMID: [21430658](#) DOI: [10.1038/clpt.2011.9](#)]
  - 99 **Moss AJ**, Kass RS. Long QT syndrome: from channels to cardiac arrhythmias. *J Clin Invest* 2005; **115**: 2018-2024 [PMID: [16075042](#) DOI: [10.1172/JCI25537](#)]
  - 100 **Deavall DG**, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. *J Toxicol* 2012; **2012**: 645460 [PMID: [22919381](#) DOI: [10.1155/2012/645460](#)]
  - 101 **Ferri N**, Siegl P, Corsini A, Herrmann J, Lerman A, Benghozi R. Drug attrition during pre-clinical and clinical development: understanding and managing drug-induced cardiotoxicity. *Pharmacol Ther* 2013; **138**: 470-484 [PMID: [23507039](#) DOI: [10.1016/j.pharmthera.2013.03.005](#)]
  - 102 **Braam SR**, Tertoolen L, van de Stolpe A, Meyer T, Passier R, Mummery CL. Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes. *Stem Cell Res* 2010; **4**: 107-116 [PMID: [20034863](#) DOI: [10.1016/j.scr.2009.11.004](#)]
  - 103 **International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use.** ICH Harmonised Tripartite. The non-clinical evaluation of the potential for delayed ventricular repolarization (QT Interval Prolongation) by human pharmaceuticals S7B. 2005; Available from: [https://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Safety/S7B/Step4/S7B\\_Guideline.pdf](https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S7B/Step4/S7B_Guideline.pdf)
  - 104 **International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use.** ICH Harmonised Tripartite. The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs E14. 2005; Available from: [https://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Efficacy/E14/E14\\_Guideline.pdf](https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Guideline.pdf)
  - 105 **Varga ZV**, Ferdinandy P, Liaudet L, Pacher P. Drug-induced mitochondrial dysfunction and cardiotoxicity. *Am J Physiol Heart Circ Physiol* 2015; **309**: H1453-H1467 [PMID: [26386112](#) DOI: [10.1152/ajpheart.00554.2015](#)]
  - 106 **James WP**, Caterson ID, Coutinho W, Finer N, Van Gaal LF, Maggioni AP, Torp-Pedersen C, Sharma AM, Shepherd GM, Rode RA, Renz CL; SCOUT Investigators. Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects. *N Engl J Med* 2010; **363**: 905-917 [PMID: [20818901](#) DOI: [10.1056/NEJMoa1003114](#)]
  - 107 **Singh S**, Loke YK, Furberg CD. Long-term risk of cardiovascular events with rosiglitazone: a meta-analysis. *JAMA* 2007; **298**: 1189-1195 [PMID: [17848653](#) DOI: [10.1001/jama.298.10.1189](#)]
  - 108 **Ma J**, Guo L, Fiene SJ, Anson BD, Thomson JA, Kamp TJ, Kolaja KL, Swanson BJ, January CT. High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. *Am J Physiol Heart Circ Physiol* 2011; **301**: H2006-H2017 [PMID: [21890694](#) DOI: [10.1152/ajpheart.00694.2011](#)]
  - 109 **Di Baldassarre A**, Cimetta E, Bollini S, Gaggi G, Ghinassi B. Human-Induced Pluripotent Stem Cell Technology and Cardiomyocyte Generation: Progress and Clinical Applications. *Cells* 2018; **7** [PMID: [29799480](#) DOI: [10.3390/cells7060048](#)]
  - 110 **Machiraju P**, Greenway SC. Current methods for the maturation of induced pluripotent stem cell-derived cardiomyocytes. *World J Stem Cells* 2019; **11**: 33-43 [PMID: [30705713](#) DOI: [10.4252/wjsc.v11.i1.33](#)]
  - 111 **van den Berg CW**, Okawa S, Chuva de Sousa Lopes SM, van Iperen L, Passier R, Braam SR, Tertoolen LG, del Sol A, Davis RP, Mummery CL. Transcriptome of human foetal heart compared with cardiomyocytes from pluripotent stem cells. *Development* 2015; **142**: 3231-3238 [PMID: [26209647](#) DOI: [10.1242/dev.123810](#)]
  - 112 **Zhou Y**, Wang L, Liu Z, Alimohamadi S, Yin C, Liu J, Qian L. Comparative Gene Expression Analyses

- Reveal Distinct Molecular Signatures between Differentially Reprogrammed Cardiomyocytes. *Cell Rep* 2017; **20**: 3014-3024 [PMID: 28954220 DOI: 10.1016/j.celrep.2017.09.005]
- 113 **Denning C**, Borgdorff V, Crutchley J, Firth KS, George V, Kalra S, Kondrashov A, Hoang MD, Mosqueira D, Patel A, Prodanov L, Rajamohan D, Skarnes WC, Smith JG, Young LE. Cardiomyocytes from human pluripotent stem cells: From laboratory curiosity to industrial biomedical platform. *Biochim Biophys Acta* 2016; **1863**: 1728-1748 [PMID: 26524115 DOI: 10.1016/j.bbamcr.2015.10.014]
  - 114 **Liang P**, Lan F, Lee AS, Gong T, Sanchez-Freire V, Wang Y, Diecke S, Sallam K, Knowles JW, Wang PJ, Nguyen PK, Bers DM, Robbins RC, Wu JC. Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation* 2013; **127**: 1677-1691 [PMID: 23519760 DOI: 10.1161/CIRCULATIONAHA.113.001883]
  - 115 **Nguemo F**, Šarić T, Pfannkuche K, Watzele M, Reppel M, Hescheler J. In vitro model for assessing arrhythmogenic properties of drugs based on high-resolution impedance measurements. *Cell Physiol Biochem* 2012; **29**: 819-832 [PMID: 22613982 DOI: 10.1159/000188069]
  - 116 **Nemade H**, Chaudhari U, Acharya A, Hescheler J, Hengstler JG, Papadopoulos S, Sachinidis A. Cell death mechanisms of the anti-cancer drug etoposide on human cardiomyocytes isolated from pluripotent stem cells. *Arch Toxicol* 2018; **92**: 1507-1524 [PMID: 29397400 DOI: 10.1007/s00204-018-2170-7]
  - 117 **Clements M**. Multielectrode Array (MEA) Assay for Profiling Electrophysiological Drug Effects in Human Stem Cell-Derived Cardiomyocytes. *Curr Protoc Toxicol* 2016; **68**: 22.4.1-22.4.32 [PMID: 27145112 DOI: 10.1002/cptx.2]
  - 118 **Schocken D**, Stohman J, Vicente J, Chan D, Patel D, Matta MK, Patel V, Brock M, Millard D, Ross J, Strauss DG, Blinova K. Comparative analysis of media effects on human induced pluripotent stem cell-derived cardiomyocytes in proarrhythmia risk assessment. *J Pharmacol Toxicol Methods* 2018; **90**: 39-47 [PMID: 29155283 DOI: 10.1016/j.vascn.2017.11.002]
  - 119 **Burridge PW**, Li YF, Matsa E, Wu H, Ong SG, Sharma A, Holmström A, Chang AC, Coronado MJ, Ebert AD, Knowles JW, Telli ML, Witteles RM, Blau HM, Bernstein D, Altman RB, Wu JC. Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nat Med* 2016; **22**: 547-556 [PMID: 27089514 DOI: 10.1038/nm.4087]
  - 120 **Nebigil CG**, Désaubry L. Updates in Anthracycline-Mediated Cardiotoxicity. *Front Pharmacol* 2018; **9**: 1262 [PMID: 30483123 DOI: 10.3389/fphar.2018.01262]
  - 121 **Sharma A**, Burridge PW, McKeithan WL, Serrano R, Shukla P, Sayed N, Churko JM, Kitani T, Wu H, Holmström A, Matsa E, Zhang Y, Kumar A, Fan AC, Del Álamo JC, Wu SM, Moslehi JJ, Mercola M, Wu JC. High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells. *Sci Transl Med* 2017; **9** [PMID: 28202772 DOI: 10.1126/scitranslmed.aaf2584]
  - 122 **Visscher H**, Amstutz U, Sistonen J, Ross CJ, Hayden MR, Carleton BC. Pharmacogenomics of cardiovascular drugs and adverse effects in pediatrics. *J Cardiovasc Pharmacol* 2011; **58**: 228-239 [PMID: 21386709 DOI: 10.1097/FJC.0b013e3182163b82]
  - 123 **Visscher H**, Ross CJ, Rassekh SR, Sandor GS, Caron HN, van Dalen EC, Kremer LC, van der Pal HJ, Rogers PC, Rieder MJ, Carleton BC, Hayden MR; CPNDS Consortium. Validation of variants in SLC28A3 and UGT1A6 as genetic markers predictive of anthracycline-induced cardiotoxicity in children. *Pediatr Blood Cancer* 2013; **60**: 1375-1381 [PMID: 23441093 DOI: 10.1002/pbc.24505]
  - 124 **Tiburcy M**, Hudson JE, Balfanz P, Schlick S, Meyer T, Chang Liao ML, Levent E, Raad F, Zeidler S, Wingender E, Riegler J, Wang M, Gold JD, Kehat I, Wettwer E, Ravens U, Dierickx P, van Laake LW, Goumans MJ, Khadjeh S, Toischer K, Hasenfuss G, Couture LA, Unger A, Linke WA, Araki T, Neel B, Keller G, Gepstein L, Wu JC, Zimmermann WH. Defined Engineered Human Myocardium With Advanced Maturation for Applications in Heart Failure Modeling and Repair. *Circulation* 2017; **135**: 1832-1847 [PMID: 28167635 DOI: 10.1161/CIRCULATIONAHA.116.024145]
  - 125 **Hoang P**, Wang J, Conklin BR, Healy KE, Ma Z. Generation of spatial-patterned early-developing cardiac organoids using human pluripotent stem cells. *Nat Protoc* 2018; **13**: 723-737 [PMID: 29543795 DOI: 10.1038/nprot.2018.006]
  - 126 **Voges HK**, Mills RJ, Elliott DA, Parton RG, Porrello ER, Hudson JE. Development of a human cardiac organoid injury model reveals innate regenerative potential. *Development* 2017; **144**: 1118-1127 [PMID: 28174241 DOI: 10.1242/dev.143966]
  - 127 **Li RA**, Keung W, Cashman TJ, Backeris PC, Johnson BV, Bardot ES, Wong AOT, Chan PKW, Chan CWY, Costa KD. Bioengineering an electro-mechanically functional miniature ventricular heart chamber from human pluripotent stem cells. *Biomaterials* 2018; **163**: 116-128 [PMID: 29459321 DOI: 10.1016/j.biomaterials.2018.02.024]
  - 128 **Keung W**, Chan PKW, Backeris PC, Lee EK, Wong N, Wong AOT, Wong GKY, Chan CWY, Fermini B, Costa KD, Li RA. Human Cardiac Ventricular-Like Organoid Chambers and Tissue Strips From Pluripotent Stem Cells as a Two-Tiered Assay for Inotropic Responses. *Clin Pharmacol Ther* 2019; **106**: 402-414 [PMID: 30723889 DOI: 10.1002/cpt.1385]
  - 129 **Forsythe SD**, Devarasetty M, Shupe T, Bishop C, Atala A, Soker S, Skardal A. Environmental Toxin Screening Using Human-Derived 3D Bioengineered Liver and Cardiac Organoids. *Front Public Health* 2018; **6**: 103 [PMID: 29755963 DOI: 10.3389/fpubh.2018.00103]
  - 130 **Mohammad Ahmadi Soleimani S**, Ekhtiari H, Cadet JL. Drug-induced neurotoxicity in addiction medicine: From prevention to harm reduction. *Prog Brain Res* 2016; **223**: 19-41 [PMID: 26806769 DOI: 10.1016/bs.pbr.2015.07.004]
  - 131 **Niimi N**, Yako H, Tsukamoto M, Takaku S, Yamauchi J, Kawakami E, Yanagisawa H, Watabe K, Utsunomiya K, Sango K. Involvement of oxidative stress and impaired lysosomal degradation in amiodarone-induced schwannopathy. *Eur J Neurosci* 2016; **44**: 1723-1733 [PMID: 27152884 DOI: 10.1111/ejn.13268]
  - 132 **Carozzi VA**, Canta A, Chiorazzi A. Chemotherapy-induced peripheral neuropathy: What do we know about mechanisms? *Neurosci Lett* 2015; **596**: 90-107 [PMID: 25459280 DOI: 10.1016/j.neulet.2014.10.014]
  - 133 **Sutter R**, Rüegg S, Tschudin-Sutter S. Seizures as adverse events of antibiotic drugs: A systematic review. *Neurology* 2015; **85**: 1332-1341 [PMID: 26400582 DOI: 10.1212/WNL.0000000000002023]
  - 134 **Grainger AI**, King MC, Nagel DA, Parri HR, Coleman MD, Hill EJ. *In vitro* Models for Seizure-Liability Testing Using Induced Pluripotent Stem Cells. *Front Neurosci* 2018; **12**: 590 [PMID: 30233290 DOI: 10.3389/fnins.2018.00590]
  - 135 **Odawara A**, Matsuda N, Ishibashi Y, Yokoi R, Suzuki I. Toxicological evaluation of convulsant and anticonvulsant drugs in human induced pluripotent stem cell-derived cortical neuronal networks using an



- MEA system. *Sci Rep* 2018; **8**: 10416 [PMID: 29991696 DOI: 10.1038/s41598-018-28835-7]
- 136 **Ishii MN**, Yamamoto K, Shoji M, Asami A, Kawamata Y. Human induced pluripotent stem cell (hiPSC)-derived neurons respond to convulsant drugs when co-cultured with hiPSC-derived astrocytes. *Toxicology* 2017; **389**: 130-138 [PMID: 28666936 DOI: 10.1016/j.tox.2017.06.010]
- 137 **Pistollato F**, Canovas-Jorda D, Zagoura D, Price A. Protocol for the Differentiation of Human Induced Pluripotent Stem Cells into Mixed Cultures of Neurons and Glia for Neurotoxicity Testing. *J Vis Exp* 2017 [PMID: 28654077 DOI: 10.3791/55702]
- 138 **Mukherjee O**, Acharya S, Rao M. Making NSC and Neurons from Patient-Derived Tissue Samples. *Methods Mol Biol* 2019; **1919**: 9-24 [PMID: 30656618 DOI: 10.1007/978-1-4939-9007-8\_2]
- 139 **Ohara R**, Imamura K, Morii F, Egawa N, Tsukita K, Enami T, Shibukawa R, Mizuno T, Nakagawa M, Inoue H. Modeling Drug-Induced Neuropathy Using Human iPSCs for Predictive Toxicology. *Clin Pharmacol Ther* 2017; **101**: 754-762 [PMID: 27859025 DOI: 10.1002/cpt.562]
- 140 **Wheeler HE**, Wing C, Delaney SM, Komatsu M, Dolan ME. Modeling chemotherapeutic neurotoxicity with human induced pluripotent stem cell-derived neuronal cells. *PLoS One* 2015; **10**: e0118020 [PMID: 25689802 DOI: 10.1371/journal.pone.0118020]
- 141 **Wing C**, Komatsu M, Delaney SM, Krause M, Wheeler HE, Dolan ME. Application of stem cell derived neuronal cells to evaluate neurotoxic chemotherapy. *Stem Cell Res* 2017; **22**: 79-88 [PMID: 28645005 DOI: 10.1016/j.scr.2017.06.006]
- 142 **Rana P**, Luerman G, Hess D, Rubitski E, Adkins K, Soms C. Utilization of iPSC-derived human neurons for high-throughput drug-induced peripheral neuropathy screening. *Toxicol In Vitro* 2017; **45**: 111-118 [PMID: 28843493 DOI: 10.1016/j.tiv.2017.08.014]
- 143 **Snyder C**, Yu L, Ngo T, Sheinson D, Zhu Y, Tseng M, Misner D, Staflin K. In vitro assessment of chemotherapy-induced neuronal toxicity. *Toxicol In Vitro* 2018; **50**: 109-123 [PMID: 29427706 DOI: 10.1016/j.tiv.2018.02.004]
- 144 **Yamada S**, Yamazaki D, Kanda Y. 5-Fluorouracil inhibits neural differentiation via Mfn1/2 reduction in human induced pluripotent stem cells. *J Toxicol Sci* 2018; **43**: 727-734 [PMID: 30518710 DOI: 10.2131/jts.43.727]
- 145 **Anderson BJ**. Pharmacology in the very young: anaesthetic implications. *Eur J Anaesthesiol* 2012; **29**: 261-270 [PMID: 22543573 DOI: 10.1097/EJA.0b013e3283542329]
- 146 **Wang C**, Liu F, Patterson TA, Paule MG, Slikker W Jr. Anesthetic Drug-Induced Neurotoxicity and Compromised Neural Stem Cell Proliferation. *Ashdin J Drug and Alcohol Res* 2015; **4**: 8 [DOI: 10.4303/jdar/235905]
- 147 **Diouf B**, Crews KR, Lew G, Pei D, Cheng C, Bao J, Zheng JJ, Yang W, Fan Y, Wheeler HE, Wing C, Delaney SM, Komatsu M, Paugh SW, McCorkle JR, Lu X, Winick NJ, Carroll WL, Loh ML, Hunger SP, Devidas M, Pui CH, Dolan ME, Relling MV, Evans WE. Association of an inherited genetic variant with vincristine-related peripheral neuropathy in children with acute lymphoblastic leukemia. *JAMA* 2015; **313**: 815-823 [PMID: 25710658 DOI: 10.1001/jama.2015.0894]
- 148 **Lancaster MA**, Knoblich JA. Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc* 2014; **9**: 2329-2340 [PMID: 25188634 DOI: 10.1038/nprot.2014.158]
- 149 **Monzel AS**, Smits LM, Hemmer K, Hachi S, Moreno EL, van Wuelten T, Jarazo J, Walter J, Brüggemann I, Boussaad I, Berger E, Fleming RMT, Bolognin S, Schwamborn JC. Derivation of Human Midbrain-Specific Organoids from Neuroepithelial Stem Cells. *Stem Cell Reports* 2017; **8**: 1144-1154 [PMID: 28416282 DOI: 10.1016/j.stemcr.2017.03.010]
- 150 **Muguruma K**, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep* 2015; **10**: 537-550 [PMID: 25640179 DOI: 10.1016/j.celrep.2014.12.051]
- 151 **Liu F**, Huang J, Liu Z. Vincristine Impairs Microtubules and Causes Neurotoxicity in Cerebral Organoids. *Neuroscience* 2019; **404**: 530-540 [PMID: 30599272 DOI: 10.1016/j.neuroscience.2018.12.047]
- 152 **Huang J**, Liu F, Tang H, Wu H, Li L, Wu R, Zhao J, Wu Y, Liu Z, Chen J. Tranylcypromine Causes Neurotoxicity and Represses BHC110/LSD1 in Human-Induced Pluripotent Stem Cell-Derived Cerebral Organoids Model. *Front Neurol* 2017; **8**: 626 [PMID: 29270148 DOI: 10.3389/fneur.2017.00626]
- 153 **Kim K**, Doi A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Aryee MJ, Ji H, Ehrlich LI, Yabuuchi A, Takeuchi A, Cunniff KC, Hongguang H, McKinney-Freeman S, Naveiras O, Yoon TJ, Irizarry RA, Jung N, Seita J, Hanna J, Murakami P, Jaenisch R, Weissleder R, Orkin SH, Weissman IL, Feinberg AP, Daley GQ. Epigenetic memory in induced pluripotent stem cells. *Nature* 2010; **467**: 285-290 [PMID: 20644535 DOI: 10.1038/nature09342]
- 154 **Polo JM**, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, Apostolou E, Stadtfeld M, Li Y, Shioda T, Natesan S, Wagers AJ, Melnick A, Evans T, Hochedlinger K. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol* 2010; **28**: 848-855 [PMID: 20644536 DOI: 10.1038/nbt.1667]
- 155 **Thatava T**, Kudva YC, Edukulla R, Squillace K, De Lamo JG, Khan YK, Sakuma T, Ohmine S, Terzic A, Ikeda Y. Inpatient variations in type 1 diabetes-specific iPS cell differentiation into insulin-producing cells. *Mol Ther* 2013; **21**: 228-239 [PMID: 23183535 DOI: 10.1038/mt.2012.245]
- 156 **Yokobayashi S**, Okita K, Nakagawa M, Nakamura T, Yabuta Y, Yamamoto T, Saitou M. Clonal variation of human induced pluripotent stem cells for induction into the germ cell fate. *Biol Reprod* 2017; **96**: 1154-1166 [PMID: 28453617 DOI: 10.1093/biolre/iox038]
- 157 **Hoffman GE**, Schrodte N, Flaherty E, Brennand KJ. New considerations for hiPSC-based models of neuropsychiatric disorders. *Mol Psychiatry* 2019; **24**: 49-66 [PMID: 29483625 DOI: 10.1038/s41380-018-0029-1]
- 158 **Deneault E**, Faheem M, White SH, Rodrigues DC, Sun S, Wei W, Piekna A, Thompson T, Howe JL, Chalil L, Kwan V, Walker S, Pasceri P, Roth FP, Yuen RK, Singh KK, Ellis J, Scherer SW. CNTN5-/- or EHMT2-/- human iPSC-derived neurons from individuals with autism develop hyperactive neuronal networks. *Elife* 2019; **8** [PMID: 30747104 DOI: 10.7554/eLife.40092]
- 159 **Tanabe K**, Haag D, Wernig M. Direct somatic lineage conversion. *Philos Trans R Soc Lond B Biol Sci* 2015; **370**: 20140368 [PMID: 26416679 DOI: 10.1098/rstb.2014.0368]



Published By Baishideng Publishing Group Inc  
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA  
Telephone: +1-925-2238242  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <https://www.f6publishing.com/helpdesk>  
<https://www.wjgnet.com>

