Patients’ induced pluripotent stem cells to model drug induced adverse events: a role in predicting thiopurine induced pancreatitis?

Running title: iPSC to study drug induced pancreatitis

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Abstract: Induced pluripotent stem cells (iPSC) can be produced from adult cells by transfecting them with a definite set of pluripotency-associated genes. Under adequate growth conditions and stimulation iPSC can differentiate to almost every somatic lineage in the body. Patients' derived iPSC are an innovative model to study mechanisms of adverse drug reactions in individual patients and in cell types that cannot be easily obtained from human subjects. Proof-of concept studies with known toxicants have been performed for liver, cardiovascular and central nervous system cells: neurons obtained from iPSC have been used to elucidate the mechanism of chemotherapy-induced peripheral neuropathy by evaluating the effects of neurotoxic drugs such as vincristine. However, no study has been performed yet on pancreatic tissue and drug induced pancreatitis. Thiopurines (azathioprine and mercaptopurine) are immunosuppressive antimetabolite drugs, commonly used to treat Crohn's disease. About 5% of Crohn's disease patients treated with thiopurines develop pancreatitis, a severe idiosyncratic adverse event; these patients have to stop thiopurine administration and may require medical treatment, with significant personal and social costs. Molecular mechanism of thiopurine induced pancreatitis (TIP) is currently unknown and no fully validated biomarker is available to assist clinicians in preventing this adverse event. Hence, in this review we have reflected upon the probable research applications of exocrine pancreatic cells generated from patient specific iPS cells. Such pancreatic cells can provide excellent insights into the molecular mechanism of TIP. In particular three hypotheses on the mechanism of TIP could be explored: drug biotransformation, innate immunity and adaptative immunity.

Key-words: induced pluripotent stem cells, adverse drug reactions, pancreatitis, inflammatory bowel disease, thiopurines
Adverse drug reactions and drug induced pancreatitis

Adverse drug reactions are an important issue for patients, national health services and drug companies [1]. Indeed, development of an adverse drug reaction may be life threatening or cause permanent disabilities [2,3]; social costs to treat adverse drug reactions are high [4], as the costs associated with failure in new drug development because of severe adverse reactions [1]. Drug attrition rates have raised in past years, determining increased costs for the pharmaceutical industry and patients; the reasons for this comprise the paucity of in vitro models that properly predict clinical efficacy and toxicity [5]. Among the adverse drug reactions, idiosyncratic reactions are the most severe, given their dose independence, rapid onset and usually requirement for permanent drug discontinuation [6].

Drug induced pancreatitis is a particularly severe form of idiosyncratic adverse drug reaction; the incidence of this adverse event has been estimated as 0.1-2% by earlier reports [3,7-9], while present day studies describe an incidence higher than 5% [10] and limited data suggests that the incidence is increasing [11]. Drugs are the third most common determinant of pancreatitis after biliary stones and alcohol [12]. Pancreatitis occurs as a consequence of injury of the acinar cells and/or pancreatic duct that causes undue accumulation and activation of proenzymes within the pancreas. The activated pancreatic enzymes damage the cellular and tissue components of the pancreas, leading to an inflammatory response, which augments the vascular permeability and may determine haemorrhage, edema, ischemia, and necrosis [13]. In severe pancreatitis, a systemic inflammatory response syndrome can be triggered and patients may develop sepsis and multiple organ failure. Treatment of patients with severe pancreatitis can require extended
hospital stays associated with high health care costs: indeed about one fourth of patients who develop pancreatitis will have to receive intensive care treatment [14]. A retrospective study reported that patients with acute pancreatitis who required intensive care therapy had an average intensive care unit stay of 9 days and an average total hospital stay of 39 days, and the average overall hospital cost was approximately 100,000$ [15]. Recovery after acute pancreatitis is typically complete and patients can generally return to their job and other normal activities [14,15]. However, around one out of ten pancreatitis cases evolves to chronic pancreatitis [13].

Over 500 drugs have been associated with pancreatitis in clinical case studies and adverse drug reactions databases [12,16]. Pancreatitis is associated with the use of several commonly used medications such as HMG-CoA reductase inhibitors (simvastatin) [3], oral contraceptives [17], highly active antiretroviral therapy (HAART) for HIV [17] and especially thiopurine antimetabolites (azathioprine and mercaptopurine) [3,8,12,17,18]. Drug that induce pancreatitis are classified (class I-IV) based on the number of cases reported, demonstration of a consistent latency period (time from initiation of drug to development of pancreatitis), and recurrence with rechallenge [16]. Class I and II drugs have the greatest potential for causing acute pancreatitis, representing medications in which at least one case study has reported acute pancreatitis’ recurrence subsequent to a rechallenge and with a consistent latency in 75% or more of the cases described [16]. According to this classification, azathioprine and mercaptopurine belong to Class I. Molecular and cellular mechanisms underpinning drug induced pancreatitis are mainly unexplored [12]; however, a number of different mechanisms have been proposed including immunologic reactions, direct toxic effect and accumulation of a toxic metabolite [11]. Drug-induced pancreatitis has limited peculiar clinical features; therefore careful
drug history and a high index of suspicion are essential for making the diagnosis. The interval of time necessary to develop pancreatitis depends on the medication involved: pancreatitis may indeed develop within a few weeks since the start of a drug associated with an immunologically mediated adverse effect; on the other hand, pancreatitis due to the accumulation of harmful metabolites generally occurs after several months of drug use. Proving the association of a pancreatitis episode with a particular medication may be difficult and patients restarted on a suspected drug should be carefully followed up and the medication promptly interrupted if symptoms reappear.

**Thiopurines in the treatment of IBD and manifestation of adverse drug reactions as pancreatitis**

Thiopurine antimetabolites (azathioprine and mercaptopurine) are active and useful for the therapy of inflammatory bowel disease (IBD), a chronic, relapsing severe inflammation of the gastrointestinal tract [19,20]. The major forms of IBD are Crohn’s disease and ulcerative colitis [21,22]. Despite introduction in therapy of biological drugs, such as TNF-α inhibitors, thiopurines are still extensively employed to treat patients with active, steroid-refractory and steroid dependent IBD, and have been proven to be particularly effective for maintaining remission of Crohn’s disease [23]. However, these medications are related to the development of adverse drug effects in up to 40% of patients [24-26]. The most common adverse drug reaction associated with thiopurines is dose dependent bone marrow suppression. However, thiopurines are among the medications most strongly associated with the development of pancreatitis as a severe idiosyncratic adverse drug reaction: a review of the literature indicates that these medications are implicated in many reported cases of acute pancreatitis, with several documented cases following re-exposure.
Frequency of TIP has been reported to be 5% in Crohn’s disease, while it is less frequent (less than 1.5%) in other conditions in which thiopurines are used as immunosuppressants, such as autoimmune hepatitis or after renal or heart transplantation [27], suggesting that molecular mechanisms involved in Crohn’s disease, such as innate immunity, may also contribute to TIP pathogenesis. Indeed the major zymogen glycoprotein 2 (MZGP2) is the primary autoantigen of pancreatic autoantibodies and anti-MZGP2 are highly specific for Crohn’s disease and are also associated with disease severity phenotypes [28]. Development of TIP is a severe adverse event for patients: it can be life threatening, impedes the patient from continuing thiopurine therapy and forces clinicians to use of other medications, which may be less active or more expensive: prevention of TIP would be therefore highly useful [29].

**Personalized medicine approaches to prevent adverse drug reactions and tailor therapy**

The aim of personalized medicine is to provide the most appropriate cure to the right patient, at the right dose and at the right time [30,31]. Application of personalized medicine should streamline clinical decision-making by distinguishing in advance those patients most likely to benefit from a given treatment from those who will suffer side effects and incur increased costs without gaining significant benefit [30,32,33]. A potential evolution of the personalized medicine concept is that of precision medicine, indicating cure strategies that comprehensively consider individual variability, now feasible thanks to large-scale biologic databases (e.g., human genome sequence), powerful approaches for evaluating patients (e.g., genomics, proteomics, cellular test), powerful informatics systems for processing large data sets [34]. Stratification based on biomarkers can be thought of as a core element of personalized/precision medicine. Pharmacogenomics, i.e.
the analysis of DNA and RNA variants associated with drug response, is a critically
important component of personalized medicine where significant and consolidated
progress has recently been made [35].

Thiopurines are pro-drugs that require bioactivation to thioguanine nucleotides (TGN),
through enzymes of the salvage pathway for nucleotides synthesis. Genetic
polymorphisms of enzymes involved in azathioprine’s biotransformation influence
treatment efficacy and toxicity: reduced enzymatic activity of thiopurine-
methyltransferase (TPMT), due to inheritance of inactive variant genotypes, was
associated with increased risk for adverse reactions during treatment with thiopurines
[36]. These variants are however associated mainly to dose dependent toxicity (e.g., bone
marrow suppression) and not to idiosyncratic adverse drug reactions like pancreatitis
[37,38].

Besides genetic biomarkers, in vitro assays performed on biological samples collected from
patients can be useful to predict patients’ response and can be applied to tailor therapy
intensity in order increase efficacy or decrease drug induced adverse drug reactions
[39,40]: sensitivity of leukemia cells to chemotherapeutic agents at diagnosis is
significantly associated with treatment outcome [41]. In vitro assays on patient’s tissue
samples are important for drug companies during the development of new medications, in
order to identify compounds with an increased risk of toxicity in particular tissues and
therefore with higher risk of failure at later stages of clinical trial [5]. However so far the
approach of testing in vitro drug sensitivity on tissue samples taken from patients can be
performed only for tissues that are easily collected, such as blood or bone marrow, and
cannot be implemented in tissues that are not readily accessible, as the pancreas. Tissues
obtained from patients’ iPSC could become a valuable tool for *in vitro* assay to evaluate drug sensitivity [42-44].

**Genetic markers for thiopurine-induced pancreatitis in inflammatory bowel disease patients**

Enzymes involved in thiopurine pharmacokinetics (e.g., TPMT) and pharmacodynamics (e.g., Rac1) may influence thiopurine clinical effects and particularly the incidence of adverse drug reactions. For TIP, several studies have considered a candidate gene approach: most of these studies, as already mentioned in this paper, did not identify a significant association of TPMT genetically determined activity with increased incidence of TIP. A recent study performed in Brazil, however, reported an increased incidence of patients with *TPMT* variants among those developing pancreatitis while on azathioprine [45]. Our group previously examined variants in *TPMT* and glutathione-S-transferase (*GST*) as potential candidate determinants of azathioprine induced adverse events, including pancreatitis. We did not identify an increased incidence of pancreatitis among patients with *TPMT* variants; however we could identify a trend toward an effect for *GST-M1* deletion: patients with this genetic feature tended to have a reduced incidence of pancreatitis during azathioprine treatment [24].

Inosine triphosphate-pyrophosphatase (ITPA) is another enzyme involved in thiopurine inactivation, putatively by preventing accumulation of potentially toxic thioinosine-triphosphate metabolites, by conversion to thioinosine-monophosphate. Previous studies have shown an increased incidence of pancreatitis among IBD patients treated with thiopurines and with an *ITPA* genetic variant associated with reduced enzymatic activity [46].
A recent study performed a genome-wide analysis to identify genetic determinants of TIP [29]. This study enrolled patients with IBD that had presented pancreatitis within 3 months of starting thiopurines from 168 hospitals worldwide. The genome-wide association analysis considered 172 cases and 2,035 controls with IBD. By this approach, the authors established a strong association of rs2647087 within the class II HLA region and development of TIP (odds ratio 2.59, 95% confidence interval 2.07–3.26, P = 2 × 10^{-16}). This finding was validated in an independent cohort of 78 cases and 472 controls with IBD matched for drug exposure. Fine mapping of the HLA region further characterized the association with the HLA-DQA1*02:01–HLA-DRB1*07:01 haplotype. This study showed that after administration of a thiopurine, patients heterozygous for rs2647087 have a 9% risk of developing pancreatitis, whereas the risk for homozygotes was 17%. In this study with an agnostic approach, TPMT and ITPA candidate variants were not associated with an increased incidence of pancreatitis. For GST-M1 deletion, no conclusion could be made, since this kind of genetic alteration was not considered by the study.

**Induced pluripotent stem cells (iPSC) as a ground-breaking tool for personalized medicine**

Somatic cells can be reprogrammed into pluripotent stem cells [47], capable of differentiating to all cell types present in the human body [48,49,50]. These cells can provide an *in vitro* model to explore cellular and molecular mechanisms involved in disease pathogenesis, including adverse drug reactions, which could bring innovative medications or be applied to predict peculiar drug responses in specific patients [42]. The technology has a particularly strong appeal to investigate clinical issues which occur in cell types that cannot be easily collected from patients, such as cardiomyocytes or neurons.
In particular iPSC technology has been recently applied to the study of chemotherapy-induced peripheral neuropathy (CIPN), a severe adverse effect characteristic of several anti-cancer agents [53]. No effective biomarker for CIPN is currently available. Therefore, human neurons derived from iPSC have been used to develop a human neuronal model to investigate the effect of various chemotherapeutics. In neurons derived from human iPSC (iCell Neurons), morphological alterations were assessed following treatment with drugs associated with CIPN, paclitaxel, vincristine, cisplatin, using high-content imaging of neurite outgrowth; in addition, cell viability was tested using an appropriate colorimetric assay (CellTiterGlo). Upon in vitro exposure of neurons derived from iPSC to these chemotherapeutic agents for 72 hours, a reproducible reduction in cell median neurite process length was observed (12-14%, 6-18% and 2-4% decrease respectively for paclitaxel, vincristine or cisplatin). Hydroxyurea, a drug not associated with neuropathy, did not induce any decrease in neurite length in this in vitro model. Vincristine treatment displayed the stronger effect on neurite outgrowth at low doses, paclitaxel showed an intermediate effect while cisplatin had a detectable effects only at the highest (i.e., micromolar) doses. This model system may constitute a tool to investigate the mechanisms of CIPN and to validate candidate genes involved in neuropathy [54,55]. Indeed, Diouf et al. recently validated in human neurons derived from iPSC findings emerging from a genome-wide association study to identify germline variants related to the occurrence and severity of CIPN associated with vincristine therapy in pediatric patients with acute lymphoblastic leukemia. This analysis identified a variant in the promoter of CEP72, a gene encoding for a centrosomal protein involved in microtubule formation, as significantly associated with vincristine-induced peripheral neuropathy, and neurons derived from iPSC were successfully used to evaluate the effects
of CEP72 hindered expression on vincristine sensitivity. Indeed, knocking-down CEP72 mRNA in human neurons augmented their in vitro response to vincristine cytotoxic effects [56].

Even hepatocytes differentiated from human iPSC have been shown recently to be useful to model interindividual variability in drug biotransformation. Activity of cytochrome P450 (CYP) enzymes and drug effects in human hepatocytes derived from iPSC were significantly associated with those of primary human hepatocytes, suggesting that hepatocytes derived from iPSC retain donor-specific CYP biotransformation activity and drug sensitivity. This study also indicated that the interindividual differences, which are due to variants in specific CYP genes, could also be recapitulated by primary human hepatocytes derived from iPSC [57]. Similar approaches could be applied in order to create a human pancreatic model to study drug induced pancreatitis and in particular TIP.

Exocrine pancreatic cells from patients’ iPSC as most appropriate cell types to model TIP

Exocrine pancreatic cells are involved in the pathogenesis of pancreatitis and represent a cell type that is difficult to collect from patients or human probands. Therefore, pancreatic exocrine cells generated from patients’ iPSC are potentially a model of great interest for testing susceptibility or elucidating underlying mechanisms of drug induced pancreatitis. The molecular mechanisms that regulate pancreatic acinar cell development remain unknown [58]. Until now, production of pancreatic cells from human embryonic stem (hES) or iPSC has focused more on the differentiation of endocrine rather than exocrine cells [59,60]. Indeed, several iPSC lines have been recently established from patients with various types of diabetes, and these could be differentiated into insulin-secreting β cells
hinting at a potential cure for diabetes in the future [61,62]. However recent studies have consolidated the evidence that exocrine pancreatic cells can also be generated starting from human embryonic stem cells using a three step approach (Figure 1): Step I, differentiation of hES cell colonies to definitive endoderm (DE) by treatment with activin A; Step II, stimulation with all trans retinoic acid to induce differentiation to pancreatic progenitor cells, after re-plating of the cells of Step I onto 24-well plates at high density; Step III, differentiation of pancreatic exocrine cells by exposure to fibroblast growth factor 7 (FGF7), glucagon-like peptide 1 (GLP-1) and nicotinamide (NA) in combination [63]. In both Step I and II, up-regulation of endodermal markers such as Sox17, Foxa2 and of gut tube endoderm marker HNF1β could be observed. The expression level of Pdx1, a transcription factor necessary for pancreatic development, arises during Step II. From day 8 in Step III, cells immunohistochemically positive for pancreatic exocrine cell products, amylase and carboxypeptidase A, are induced by FGF7, in proximity of pancreatic progenitor Pdx1-positive cells. Thus, this three step culture protocol effectively determines the differentiation of human stem cells to pancreatic exocrine cells [63]; these cells could be tested as an in vitro model of drug induced pancreatitis and in particular TIP.

Understanding the molecular mechanism of TIP using patient specific iPSC derived exocrine pancreatic cells
TIP may be related to thiopurine induced direct damage to the exocrine pancreatic cells or to accumulation of a toxic metabolite (biotransformation hypothesis). Pancreatitis due to direct toxicity may manifest later than that due to an immunological mechanism; for thiopurines, pancreatitis occurs relatively early after the start of treatment, generally within 30 days [27,29], therefore the mechanism is likely immunological and may involve
the innate or the adaptative immunity. However, a direct toxicity of thiopurines or their
metabolites on patients’ pancreatic cells cannot be completely excluded, particularly in
patients with IBD [64].

Biotransformation hypothesis

To test a specific sensitivity to thiopurines of exocrine pancreatic cells from patients that
developed TIP, pancreatic cells derived from patients iPSC may be grown in vitro and
exposed to thiopurines: cells sensitivity could then be measured with adequate outputs
(e.g., cell proliferation by thymidine incorporation assay, cell survival by assays that
measure mitochondrial activity such as 3-(4,5-dimethylthiazol-2-yl)-2,5-
diphenyltetrazolium bromide (MTT) assay or ATP concentration). Enzymes directly or
indirectly involved in oxidative stress production (e.g., glutathione-S-transferase, GST and
xanthine oxidase) contribute to thiopurine biotranformation and these agents may
therefore induce oxidative stress at the cellular level. Tentatively, this could represent one
additional mechanism of thiopurine cytotoxicity. This seems to be proven especially for
azathioprine during its conversion to mercaptopurine. This reaction can occur
spontaneously [65]; however in vitro studies have shown an increased rate of conversion in
the presence of the GST enzymes [66], particularly the GST-A1/2 and GST-M1 isoforms
[67]. One of the main differences between azathioprine and mercaptopurine, from the
pharmacological point of view, is that azathioprine conversion to mercaptopurine may
deplete intracellular reduced glutathione, leading to a significant increase of reactive
oxygen species (ROS) [68]. This phenomenon could explain the fact that azathioprine may
induce pancreatitis at a higher rate than mercaptopurine in patients with Crohn’s disease
and that after TIP on azathioprine, some subjects may be safely treated with
mercaptopurine [69,70]. Therefore azathioprine in some patients, particularly in those with Crohn’s disease, may induce pancreatitis with a drug specific mechanism, different from mercaptopurine, leading to ROS production. Therefore, in vitro studies on thiopurine induce pancreatitis in Crohn’s disease should consider to mimic even these processes. The higher rate of TIP described in some studies for Crohn’s disease patients may also be related to a defect at the level of autophagy, present in many patients with Crohn’s disease [71], that could not allow a proper protection from oxidative stress induced by azathioprine [72].

Innate immunity hypothesis

Crohn’s disease is related to abnormalities in innate immunity, which involves monocyte activation [22]. TIP incidence is higher when thiopurines are used in Crohn’s disease than in other conditions (e.g., autoimmune hepatitis) [27], even if not all reports are consistent on this in the literature [73]. As mentioned previously in this review, the higher frequency of TIP when thiopurines are used to treat Crohn’s disease may be suggestive that molecular mechanisms involved in Crohn’s disease pathogenesis, such as innate immunity, may contribute also to development of TIP. To test this hypothesis, patients’ monocyte activation in the presence of pancreatic cells treated or not with thiopurines or their metabolites could be evaluated, and compared to stimuli for innate immunity such as lipopolysaccharide (LPS). A similar approach has been applied to investigate whether oxidatively modified, autologous red blood cells (RBCs) modulate monocyte cytokine responses in humans [74]. Oxidatively modified RBCs (OX-RBC) or vehicle-treated RBCs (VT-RBC) were exposed to monocytes, also in combination with innate immunity activating agents, such as LPS. OX-RBC alone augmented cellular complexity, evaluated
by flow cytometry, of CD14-monocytes but did not induce cytokine production. LPS alone
induced cytokine production with no effect on cell complexity. The combined treatment
(OX-RBC-LPS), induced both an increase in monocytes complexity and in their production
of TNF-alpha. Therefore, the interaction between oxidatively damaged autologous
erythrocytes and monocytes is important for innate responses in human cells. Similar
pathogenetic processes may be at the base of TIP development during azathioprine
treatment, especially in patients with Crohn’s disease.

Adaptive immunity hypothesis

Drug hypersensitivity reaction are known to occur through mechanisms involving
adaptive immunity, in particular through antibodies directed against red blood cells or
platelets, for drug induced anemia or thrombocytopenia [75]. Activation of patients’
lymphocytes by co-culture with pancreatic cells, even obtained by differentiation from
patients’ iPSC, may shed light on the relevance of adaptive immunity for TIP
pathogenesis. Lymphocytes’ activation may be measured by in vitro assays, such as
thymidine incorporation [76]. These experiments would be particularly informative if
performed on purified lymphocytes subpopulations, including T lymphocytes (CD3+)
alone, T lymphocytes (CD3+) with B lymphocytes (CD19+) or T lymphocytes (CD3+) with
monocytes (CD14+), to evaluate the relevance of antigen presenting cells’ presence for TIP
development and the contribution of specific adaptive immunity functional cells [77,78].

Caveats of exocrine pancreatic cells derived from patients’ iPSC as a model for TIP

One aspect to consider when using iPSC-derived cells as functional models for
pharmacological studies is the very low efficiency of the reprogramming processes and
that *in vitro* redifferentiated cells may be heterogeneous, expressing for example in part fetal markers, even at low level [79]. Recent insights on the molecular mechanism of reprogramming, obtained by genome-wide characterization of transcriptomic, epigenomic and proteomic data describing the cellular routes leading fibroblast to induced pluripotency. These results will likely lead to improved efficiency in reprogramming, providing human models derived from induced pluripotent cells more quickly and with reduced costs [80,81].

These *in vitro* models may lack intercellular communication, known to be crucial for organogenesis. The generation of complex vascularized organs, such as endoderm derivatives, indeed depends on coordinated signals deriving from endodermal, epithelial, mesenchymal and endothelial progenitors. Therefore, specific challenges to recapitulate organ development *in vitro*, such as liver and pancreas, are the induction of *in vitro* organ formation by co-culturing endothelial and mesenchymal progenitors and simulation of blood perfusion for stimulating intercellular communication. This approach has been recently applied to the development of liver-like tissue from iPSC in culture. Specific human hepatic cells condensed and self-organized into 3D-iPSC-derived liver buds (rudimentary/miniature liver), when cocultured with endothelial and mesenchymal progenitors, display also gene expression patterns similar to those found in relevant embryonic and endothelial tissues (e.g., inner branched endothelium) [44].

From the pharmacological point of view, it is important to note that thiopurines are prodrugs requiring conversion to thioguanine nucleotides to exert their cellular effects [65]: after oral administration azathioprine is completely converted to mercaptopurine during first pass metabolism in the liver and even mercaptopurine has a very short half-life and is transformed to thionucleotides [66]. The main effects of these medications are
indeed due to thionucleotides. Therefore, in Crohn's disease patients, after oral administration of thiopurines, pancreatic cells are reached through the blood stream by a mix of thionucleotides, including thioguanosine, thioinosine, methylthioinosine, which should be responsible for TIP in susceptible patients. On these bases, drug sensitivity, drug metabolism and immunological assays should be performed with azathioprine and mercaptopurine, and even with their active metabolites. Moreover, a medium conditioned by a stabilized cell line of human hepatocytes (e.g., IHH), exposed to azathioprine or mercaptopurine, could be used, as representative of a mix of thiopurines' active metabolites produced by the human liver after oral administration.

Patients’ iPSC for preventing TIP

Human cells derived from iPSC have been shown to be useful as a model for drug sensitivity of tissues that are not easily accessible, such as cardiac muscle, brain and liver. In vitro cellular models based on patients’ iPSC have great potential in developing agents and predicting toxicity in the field of cardiovascular medicine and neuroscience, as discussed recently in excellent reviews [82-84]. Using iPSCs to predict toxicity has been streamlined in some pharmaceutical companies [85].

TIP is a significant, potentially life-threatening, clinical issue for Crohn’s disease patients treated with azathioprine or other thiopurines. Current strategies to prevent TIP consist mainly in clinically monitoring by measuring in patients’ peripheral blood the concentration of amylase and lipase, especially in the first weeks of treatment: in case of increase in the concentration of circulating pancreatic enzymes, azathioprine treatment is promptly interrupted. In our hospital, amylase/lipase concentrations are evaluated weekly during the first month of thiopurine therapy, then monthly for the second and
third month and then every three months, since it is known that TIP occurs early after therapy start. While practitioners accept this strategy, it poses significant risks for the patient since pancreatitis is detected only after the beginning of thiopurine-induced pancreatic damage and, moreover, therapy with azathioprine has to be promptly interrupted during the early phases, suspending IBD treatment and increasing the risk of missing the “window of opportunity” for optimal therapy, leading to disease progression and intestinal damage. Pancreatic cells derived from patients’ iPSC, as a model of human pancreas, may provide strategies to identify, before treatment, patients predisposed to TIP in Crohn’s disease (Figure 2). Patients at high risk could then be treated with a different medication, preventing this severe adverse drug reaction and inefficacious patients’ treatment, with the associated risk of disease progression. This approach could also result in saving significant resources related to treatment failure and medical care of TIP.

Conclusion

In this review, we have reflected upon the future research applications of exocrine pancreatic cells generated from patient specific iPSC. Such pancreatic cells can provide excellent insights into the molecular mechanism of TIP. In particular three hypotheses on the mechanism of TIP could be considered: on a role of drug biotransformation, on innate immunity and on adaptative immunity. Hence, our proposed model system could also be extended as a paradigm to study pancreatitis induced by other medications and in other conditions.
Conflict of interest

The authors declare no conflict of interest.
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Figure 1: Summary of procedure to differentiate human iPSC to pancreatic exocrine cells (modified from [63]). Exocrine pancreatic cells can be generated starting from human stem cells using a three step approach: stem cell colonies are differentiated to definitive endoderm (DE) by treatment with 100 ng/ml activin A and 25 ng/ml Wnt3A in RPMI medium supplemented with 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin. After 24 h, the medium is switched to 100 ng/ml activin A in RPMI medium supplemented with ITS (i.e., 5 μg/ml insulin, 50 μg/ml transferrin, 30nM selenium chloride), 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin for 48 h. Step II of the culture procedure results in the differentiation of pancreatic progenitor cells from the DE cells. These are re-plated onto 24-well plates and treated with 1 μM all-trans retinoic acid in RPMI1640 medium supplemented with 2% fetal bovine serum (FBS), 50 U/ml penicillin and 50 μg/ml streptomycin for 3 days. Step III of the culture procedure achieved the final differentiation of cells containing pancreatic exocrine enzymes. The pancreatic progenitor cells are cultured in DMEM/F12 supplemented with 15 ng/ml fibroblast growth factor 7 (FGF7), 10 mM nicotinamide (NA), 100 ng/ml glucagon-like peptide 1 (GLP-1) (7–36 amide), N2 supplement, B27 supplement, 50 U/ml penicillin and 50 μg/ml streptomycin. Cell markers: pancreatic progenitor cell marker (Pdx1), pancreatic exocrine cell marker (amylose)
Figure 2: schematic representation of exocrine pancreatic cells derived from patients' iPSC as a model for TIP (modified from [51]): exocrine pancreatic can be differentiated from iPSC generated from lymphocytes of patients with Crohn's disease susceptible or not to TIP. In order to assess differences in thiopurine sensitivity and biotransformation, these can be treated with thiopurines and their metabolites. In order to identify immunological activation, patients' lymphocytes can be exposed to exocrine pancreatic cells obtained from patients' iPSC, with and without thiopurines.