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REVIEW

Dysregulation of Immune Tolerance to Autologous iPSCs and Their Differentiated Derivatives

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Abstract—Induced pluripotent stem cells (iPSCs), capable of differentiating into any cell type, are a promising tool for solving the problem of donor organ shortage. In addition, reprogramming technology makes it possible to obtain a personalized, i.e., patient-specific, cell product transplantation of which should not cause problems related to histocompatibility of the transplanted tissues and organs. At the same time, inconsistent information about the main advantage of autologous iPSC-derivatives – lack of immunogenicity – still casts doubt on the possibility of using such cells beyond immunosuppressive therapy protocols. This review is devoted to immunogenic properties of the syngeneic and autologous iPSCs and their derivatives, as well as to the reasons for dysregulation of their immune tolerance.

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INTRODUCTION

Human pluripotent stem cells (PSCs), which include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), can unlimitedly proliferate, and differentiate into almost any type of somatic cells [1, 2]. These unique properties make them an attractive and promising tool for modeling various diseases and drug development [3, 4]. Hopes are pinned on differentiated derivatives of PSCs as a source of material for cell therapy, which should solve the problem of shortage of donor organs and tissues [5].

It is still too early to discuss widespread introduction of the PSC technology into clinical practice. Only 26 years have passed since the discovery of human ESCs in 1998 [1], and active work is currently ongoing to improve protocols for differentiating PSCs into specialized cells and obtaining three-dimensional structured tissues *in vitro* [4]. Another stumbling block to integrating PSCs into the clinic is high cost of the technology. According to the recent estimate, generating a clinical grade iPSCs line under good manufacturing practice (GMP) costs approximately U$800,000 [4]. Another limiting factor is long time required to obtain a new iPSC line and its subsequent differentiation into the desired cell type [6]. Additionally, there are currently no developed standardization parameters that would be applied to both iPSCs [7] and their differ-

Abbreviations: B2M, beta-2-microglobulin; CD, clusters of differentiation; ESCs, embryonic stem cells; HLA, human leukocyte antigen; iPSCs, induced pluripotent stem cells; KIR, killer-cell immunoglobulin-like receptor; NK, natural killer cells; PSCs, pluripotent stem cells; RPE, retinal pigment epithelium; SMCs, smooth muscle cells.

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entiated derivatives [8]. Thus, at least in the coming years, patient-specific iPSC-based therapy is unlikely to become widespread. Interestingly, almost half of the clinical trials of PSC-derivatives used cell products derived from only five ESC lines; however, the number of studies of cells derived from iPSCs has increased significantly over the past few years [9]. According to the Federal Law No. 180, using ESCs to develop, produce, and apply biomedical cell products is prohibited in Russia. Therefore, only iPSC-derivatives can be used in clinical practice.

Despite the obvious economic advantages associated with production and "scaling-up" of allogeneic PSC-derivatives, the issue of immune rejection remains unresolved due to the high polymorphism of the genes of the major histocompatibility complex (HLA). To prevent immune rejection during allogeneic tissue and organ transplantation, patients must undergo lifelong immunosuppressive treatment and its associated side effects [10]. Initially, it was believed that personalized therapy based on autologous iPSC-derivatives could circumvent the problem of histocompatibility [11]. However, some researchers report that immune response against the syngeneic and autologous iPSCs is still possible, which casts doubt on the main advantage of autologous iPSCs – lack of immunogenicity [12-15]. The reasons for this phenomenon have yet to be thoroughly studied. In this review, we tried to shed light on the mechanisms of impaired immune tolerance to autologous iPSCs. It is worth emphasizing that understanding effects of the significant immune effector cells – T and NK-cells – on various types of cells and, in the long-term perspective, tissues derived from iPSCs will help to find approaches to their suppression and will be of great importance for successful development of translational medicine.

IMMUNOGENICITY OF SYNGENEIC AND AUTOLOGOUS iPSCs AND THEIR DERIVATIVES TOWARD T-CELLS

The possibility that cells differentiated from autologous iPSCs can provoke an immune response was widely considered only after the publication of Zhao et al. [12]. In this study, the authors showed that subcutaneous administration of iPSCs to syngeneic, i.e., linear, mice led to formation of teratomas, where T-cell infiltration zones were found. Moreover, the process was accompanied by subsequent necrosis and regression of the resulting teratomas. At the same time, teratomas formed after administration of the syngeneic ESCs with the same genetic background caused an immune response much less often. The authors noted that the teratomas from iPSCs were rarely rejected when episomal reprogramming was used. However, in this case, the formed teratomas were rejected with the frequency of 10-20%, and most of them were also infiltrated by T-cells. Nevertheless, these results were met with some skepticism by the scientific community, mainly because undifferentiated iPSCs are not considered as a source of cells for clinical use [11].

More recent studies have been somewhat contradictory, although most still indicated lack of immunogenicity of the syngeneic iPSCs derivatives. The opposite findings were reported only in a few studies. Thus, additional evidence of immunogenicity of the cells derived from syngeneic iPSCs was presented in 2013 by Araki et al. [16]. The authors reported similar frequency of teratoma rejection formed by both syngeneic iPSCs and ESCs. It was suggested that the immune response to teratomas is potentially related to the expression of genes regulating pluripotency. In particular, the authors relied on the previously obtained data that the transcription factor OCT4 may have immunogenic properties [17]. In addition, Araki et al., for the first time, found signs of immune response to the terminally differentiated derivatives of syngeneic iPSCs. Transplantation of cardiomyocytes differentiated from the iPSCs led to the significant T-cell infiltration of the graft in the syngeneic mice [16].

Another study reported complete survival of teratomas formed by the syngeneic ESCs, although some teratomas still showed areas of T-cell infiltration [18]. Furthermore, the authors compared immunogenicity of endothelial cells, hepatocytes, and neuron precursors differentiated from the syngeneic ESCs and iPSCs. Differentiated iPSCs derivatives did not induce signs of specific T-cell response either in the *in vitro* model or after transplantation into the syngeneic mice. Thus, Guha et al. [18] showed that the degree of immunogenicity of iPSCs can decrease in the process of differentiation. The authors of another work [19] came to the same conclusion. Analysis of the functional state of immune cells found in the transplantation area showed that teratomas were infiltrated predominantly by the cytotoxic T-cells, while endothelial cells differentiated from iPSCs – by the regulatory T-cells and macrophages [19]. Another study reported complete survival of teratomas formed by the syngeneic ESCs, although some teratomas still showed areas of T-cell infiltration [18]. Moreover, the authors compared immunogenicity of endothelial cells, hepatocytes, and neuron precursors differentiated from the syngeneic ESCs and iPSCs. Differentiated iPSCs derivatives did not induce signs of specific T-cell response either in the *in vitro* model or after transplantation into syngeneic mice. Thus, Guha et al. [18] showed that the degree of immunogenicity of iPSCs can decrease in the differentiation process. The authors of another work [19] came to the same conclusion. Analysis of the functional state of immune cells found in the transplantation area showed that

teratomas were infiltrated predominantly by the cytotoxic T-cells, but endothelial cells differentiated from iPSCs – by the regulatory T-cells and macrophages [19].

Another study with the non-human primate model was published in 2013 [20]. The authors compared immune response to autologous and allogeneic transplantation of the iPSC-differentiated midbrain dopaminergic neurons into *Macaca fascicularis* brains [20]. It was found that the significant amounts of microglia and T-cells infiltrated the allografts, while autologous neurons elicited minimal immune cell response. Similar work was done with the neural precursors of iPSCs, where no significant infiltration was observed in the autologous cell transplantation areas [21, 22]. Interestingly, Morizane et al. detected a limited T-cell response to the autologous dopaminergic neurons in some experimental groups, if they were differentiated from the iPSCs derived by retroviral transfection [20]. However, when the iPSCs were obtained by the episomal reprogramming system, their derivatives did not lead to the immune response of autologous T-cells. These data indicate that viral integration of pluripotency factors during the iPSCs production may affect immunogenicity of the cell products.

Some limitations of the work of Morizane et al. [20] are worth noting. First, the authors were able to analyze immune response only after euthanasia of the animals, approximately 3-4 months after transplantation. Attempts were made to track dynamics of the immune response using positron emission tomography and measuring cytokine content in blood and cerebrospinal fluid. However, the results were highly variable and correlated poorly with the postmortem histologic data. Additional time points could more accurately determine likelihood of the immune response to transplantation of autologous iPSC-derivatives. Moreover, although the authors demonstrated that the degree of immune response was higher when the allogeneic dopaminergic neurons were transplanted, rejection was not observed even without immunosuppressive therapy. This phenomenon could be explained by the brain being an immune-privileged organ. Moreover, these data are consistent with the clinical observations of long-term survival of the allogeneic dopaminergic neurons derived from the fetal material in the patients with Parkinson's disease who received only short-term or no immunosuppression [23, 24].

All previous studies have described immunogenicity of the animal iPSCs – mice and primates. However, studying immunogenicity of the autologous human iPSC-derivatives is essential for clinical application. In 2015, Zhao et al. studied this issue in a humanized mouse model with the reconstructed human immune system [13]. They found T-cell infiltration and tissue necrosis areas in the most teratomas formed from iPSCs. However, the degree of immune response to the autologous iPSCs was weaker than to the allogeneic ESCs. Therefore, the authors hypothesized that only certain derivatives of iPSCs could induce rejection. In addition, deep sequencing of the T-cell receptor (TCR) repertoire of the infiltrating lymphocytes revealed their oligoclonal character, pointing to the antigen-specific response of T-lymphocytes to the autologous iPSCs. For allogeneic ESCs, the polyclonal TCR repertoire was revealed.

Furthermore, histological sections of the teratomas infiltrated with T-cells were analyzed to identify potentially immunogenic tissues. The authors found that the desmin-positive smooth muscle cells (SMCs) were significantly more frequently surrounded by the infiltrating T-cells. In contrast, the retinal pigment epithelial cells (RPE) were almost never infiltrated by T-cells. Next, the authors compared immunogenicity of the two cell types, SMCs and RPE. It turned out that the autologous SMCs were more immunogenic due to dysregulated expression of the tumor-associated genes, particularly *HORMAD1* and *ZG16*. Ectopic expression of *ZG16* in the RPE cells resulted in the significant T-cell response in autologous recipients.

Thus, the data on immunogenicity of syngeneic and autologous iPSCs derivatives against T-cells are contradictory. Nevertheless, most of them are encouraging, such as, for example, relatively recent works performed with the pigs [25], monkeys [26], humanized mice [27] models as well as *in vitro* studies [15, 28], where immune tolerance to the cellular products derived from iPSCs was demonstrated.

IMMUNOGENICITY OF SYNGENEIC AND AUTOLOGOUS iPSCs AND THEIR DERIVATIVES TOWARD NK-CELLS

While primary function of T-lymphocytes is to recognize foreign molecules, including neoepitopes, NK-cells have a different principle of immunological recognition. The classical "missing self" hypothesis assumes that NK-cells recognize and destroy all cells lacking HLA class I molecules [29]. Based on the current viewpoint, activation of NK-cells is a more complex concept and is determined by interaction of the signals from two types of receptors on their surface: activating and inhibitory [30]. Predominance of the inhibitory signals upon interaction with the target cell does not disturb the anergy of NK-cells, while predominance of the activating signals triggers their cytotoxic program. In turn, predominance of the activating signals can be caused by increase in the amount (level) of the ligands for activating NK-cell receptors on the target cells and decrease in the inhibitory ligands, mainly HLA class I molecules. Such imbalance in physiological conditions is determined by various pathological

processes, including oncogenesis, viral and bacterial infections, and stress [31].

Activity of NK-cells against undifferentiated PSCs, including syngeneic and autologous ones, was previously highlighted in several *in vitro* studies [32, 33]. Generally, low expression of HLA class I molecules is a characteristic feature of PSCs [34], so high activity of NK-cells is primarily due to the absence of inhibitory signals. However, some studies indirectly noted contribution of the activating ligands. For example, Frenzel et al. found that the preliminary blocking of activating receptor NKG2D significantly reduces cytotoxicity of the NK-cells co-cultured with the syngeneic mouse ESCs [35]. Another study showed that the NK-cells with knockout of the *Klrk1*$^{-/-}$ gene encoding the NKG2D receptor lysed a significantly lower percentage of the ESCs than the wild-type NK-cells [36]. Interestingly, experiments with blocking antibodies have shown a role for another activating receptor, DNAM-1, for human PSCs [33]. High sensitivity of PSCs to NK-cells is due to two factors simultaneously: low expression of HLA-I molecules and increased expression of activating ligands.

As for the *in vivo* immune response, NK-cells are known to limit teratoma formation after subcutaneous injection of both syngeneic [37] and autologous iPSCs [38]. These findings suggest that the residual PSCs, which could potentially remain in the graft among the differentiated cells, would fail to form teratomas and would be rejected by the NK-cells. Melendez et al. reported that NK-cells can act as an internal barrier during reprogramming *in vitro* and *in vivo* [39]. It was demonstrated that NK-cells can recognize and destroy the partially reprogrammed cells shown to express the ligands for activating NKG2D and DNAM-1 receptors. Further, using the transgenic mouse line [40] expressing four reprogramming factors from the Yamanaka cocktail (OSKM) under doxycycline treatment, the authors showed that partial reprogramming *in vivo* occurs more efficiently when NK-cells are depleted and, on the contrary, is significantly reduced when they are adoptively transferred [39].

Response of the syngeneic and autologous NK-cells to differentiated iPSC-derivatives needs to be better understood. For example, increased sensitivity of the hepatocyte-like cells differentiated from the murine iPSCs (iPS-HLC) to syngeneic NK-cells *in vitro* has been reported [41]. At the same time, syngeneic somatic cells – hepatocytes – practically did not induce the NK-cell response. Interestingly, this work also determined immune response of NK-cells to the hepatocyte-like cells derived from ESCs (ES-HCs). The authors found that the lysed ES-HCs percentage was almost twice as high as that of the lysed iPS-HCs. ES-HCs, but not iPS-HCs, appeared to have ligands for activating the NKG2D receptor. In addition, knockout of the NKG2D receptors in the NK-cells significantly reduced percentage of the lysed ES-HSCs but not iPS-HSCs. Thus, this work confirmed previous findings that elimination of the murine PSCs and their differentiated derivatives is mainly due to interaction of the NKG2D receptor with its ligands [35, 36, 42].

In another work, the NK-cell response to transplantation of cardiomyocytes differentiated from the syngeneic iPSCs (miPSC-CMs) was studied in a mouse model [14]. It was shown that survival rate of the subcutaneously transplanted miPSC-CMs was significantly higher in the NK-cell-depleted mice. In the control mice, in addition to infiltration of the graft by NK-cells, there were signs of the NK-cell degranulation and rejection of the miPSC-CMs. Analysis of the NK-cell ligand expression showed that the miPSC-CMs weakly expressed the MHC class I molecules and were stained with antibodies to the ligands for NKG2D and DNAM-1 receptors. Blocking of the NKG2D and DNAM-1 receptors or increasing the MHC-I expression by the IFNγ (interferon gamma) pretreatment mitigated cytotoxic properties of the NK-cells *in vitro*. Also, it decreased the NK-cell infiltration into the transplanted areas and necrosis of miPSC-CMs *in vivo*.

At the same time, immunogenicity of the human iPSC-derivatives to NK-cells is poorly investigated. It was studied mainly upon engineering of the immune-evasive or “universal” PSCs. This approach is believed to be an alternative to the traditional immunosuppressive therapy, since derivatives of such cells will be suitable for any recipient [43, 44]. The most commonly used strategy to create hypoimmunogenic cells is to completely “turn off” expression of HLA molecules, both classes I and II. To suppress the HLA class I expression, the beta-2-microglobulin (*B2M*) gene, which encodes the light subunit required for stable heterodimer formation, is usually knocked out [45-47]. To suppress the HLA class II expression, the *CIITA* gene, transcription factor required for the HLA-II expression, is usually knocked out [48-50]. Cells devoid of HLA molecules should become completely invisible to the recipient’s T-cells, both $CD8^+$ and $CD4^+$ [51]. On the other hand, elimination of the HLA class I molecules makes the PSC-derivatives sensitive to cytotoxic properties of NK-cells [46, 52, 53]. Therefore, obtaining PSC lines with the reduced immunogenicity usually involves two steps: first, HLA expression should be suppressed, and then additional factors should be added to avoid NK-cell response [53-57].

A priori studies of low-immunogenic iPSC-derivatives were performed with an allogeneic model. Since NK-cells can not recognize foreign molecules, immune tolerance to the iPSC-derivatives can be evaluated using NK-cells of allogeneic origin but with one stipulation. It is known that the NK-cells alloreactivity can theoretically be caused by mismatch of the

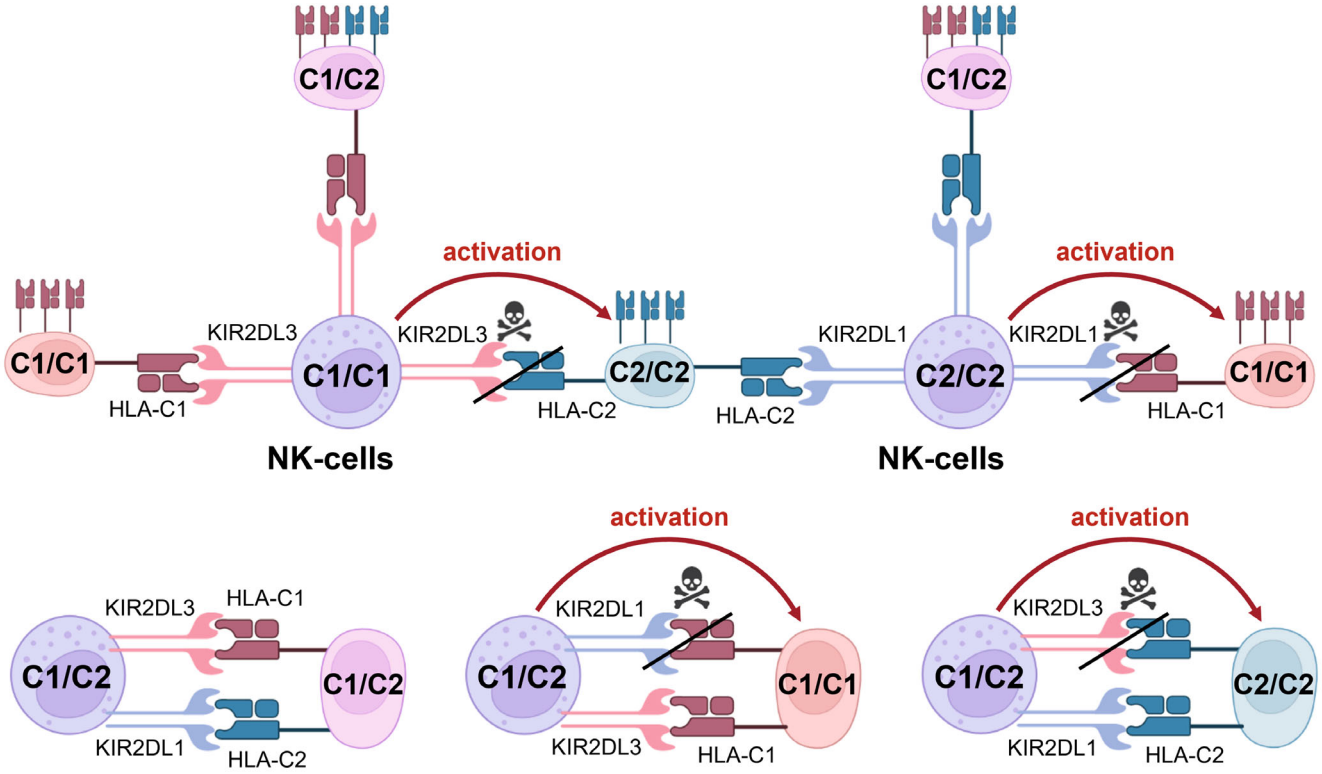

Fig. 1. Mechanism of NK-cell alloreactivity exemplified by the ligand mismatch for KIR2DL receptors. HLA-C1/C1 NK-cells are activated by interaction with HLA-C2/C2 target cells (no inhibitory signal via KIR2DL3-receptor). HLA-C2/C2 NK-cells are activated by interaction with HLA-C1/C1 target cells (no inhibitory signaling through KIR2DL1 receptor). HLA-C1/C2 NK-cells are activated by interaction with HLA-C1/C1 target cells (no inhibitory signaling through KIR2DL1-receptor) and by interaction with HLA-C2/C2 target cells (no inhibitory signaling through KIR2DL3-receptor).

KIR family (killer-cell immunoglobulin-like receptor) ligands via the mechanism of "missing-self" recognition. All HLA-C alleles are divided into two groups – HLA-C1 and HLA-C2 – depending on the sequence of amino acids at positions 77 and 80 of the alpha chain, which determines their ability to bind to the NK-cell receptors KIR2DL3 and KIR2DL1, respectively [58]. According to this principle, all donors and recipients can be categorized into the following groups: HLA-C1/C1, HLA-C1/C2, and HLA-C2/C2. NK-cells, in the process of licensing or "learning" during maturation, acquire tolerance to the specific set of HLA-C alleles on their own cells. The conditions of NK-cell response to HLA-C allele mismatch by interaction with the KIR2DL receptors are presented in Fig. 1. Thus, if derivatives differentiated from the HLA-homozygous iPSC lines are used as target cells, heterozygous HLA-C1/C2 NK-cells will respond to the absence of any of the KIR-ligands. It was confirmed in the work by Ichise et al. [59]. The authors showed [59] that the HLA-C1/C2 NK-cells isolated from the blood of healthy donors lysed T-cells and endothelial cells differentiated from the HLA-C1/C1 iPSCs. In turn, ectopic expression of HLA-C2 in the differentiated HLA-C1/C1 derivatives diminished the NK-cell response. In addition to alloreactivity to the mismatched ligands of the KIR2DL receptors, the NK-cell responses to the absence of ligands for KIR3DL1 (epitope Bw4) and KIR3DL2 (HLA-A3, A11 alleles) receptors have also been reported [60]. Thus, the allogeneic model can be used to assess immunogenicity of the iPSCs derivatives toward NK-cells; however, only under condition that the donors participating in the study are typed.

Interestingly, in some studies with hypoimmunogenic PSC-derivatives, no significant difference was found in the response of NK-cells to PSC knockout derivatives and wild-type PSC-derivatives. However, it should be noted that in all these studies, the target cells and the donors involved were not typed. Differences in cytotoxicity and number of $CD107a^{+}$, i.e., degranulated NK-cells cocultured with the wild-type SMCs or SMCs not expressing HLA class I, were statistically insignificant [55]. In turn, the wild-type RPE cells induced extremely high NK-cell cytotoxicity, comparable to the knockout PSC-derivatives [50]. Moreover, percentage of the degranulated NK-cells also did not differ between the wild-type and knockout RPE cells, although it varied greatly depending on the donor. High cytotoxicity of NK-cells was also observed against the cardiomyocytes differentiated from ESCs [57].

Endothelial cells induced the same level of NK-cell degranulation regardless of the HLA class I expression, although NK-cell cytotoxicity was higher against the ESC-derivatives with the *B2M* gene knockout [61]. It should be noted that in the studies mentioned above, the authors did not focus on the NK-cell response to the wild-type PSC-derivatives. Instead, they described absence of hypersensitivity of the HLA-negative cells to the NK-cells.

In our recent work, we also found that the fibroblast-like iPSC-derivatives with the *B2M* gene knockout (ΔiPS-fibro) show the same degree of sensitivity to allogeneic and autologous NK-cells as the wild-type fibroblast-like cells – iPS-fibro [15]. Unlike other authors, we used parental fibroblasts as a negative control for the NK-cell reaction. This comparison enabled us to detect absence of complete immunologic tolerance to the differentiated iPSC-derivatives from the autologous NK-cells. Transcriptome analysis revealed a significant imbalance of the NK-cell ligands in the iPS-fibro. Compared to the parental fibroblasts, iPS-fibro simultaneously showed significant decrease in the expression of HLA-I molecules and increase in the expression of ligands for activating DNAM-1 and NKG2D receptors. Further in this work, it was shown that the NK-cell ligands in the differentiated iPSC-derivatives can be balanced by pretreatment of the cell cultures with IFNγ [15].

Another study noted sensitivity of the renal iPSC-derivatives to the autologous NK-cells [28]. The authors found that percentage of the activated NK-cells cocultured with the proximal epithelial cell precursors was lower than with the more “mature” iPSC-derivatives. However, these differences were not discussed in detail in the article. According to the RNA-sequencing data, the level of HLA class I transcripts increased in the proximal epithelial cells during prolonged culturing. These data are in agreement with the results obtained with iPS-fibro [15] as well as with some activating NK-cell ligands, particularly *MICA* and *NECTIN2*. It explains predominance of the activating signals and initiation of cytotoxic program in the NK-cells. It is also interesting to note that, in contrast to the earlier work [33], Rossbach et al. [28] did not observe high activity of NK-cells against the undifferentiated human iPSCs.

It should be noted that the role of NK-cells in the solid organ transplantation remains quite controversial [60, 62]. There is evidence that some subsets of NK-cells may play a role in regulation of allograft tolerance, and that NK-cells are, nevertheless, involved in the T-cell-mediated and antibody-mediated allograft rejection [63]. Without immunosuppressive therapy, which affects cytotoxic activity and adjusts degranulation properties, the activated NK-cells produce IFNγ that could contribute to the development of chronic inflammation and enhance the predominantly T-cell-mediated immune response [64]. Thus, the iPSC-based cell therapies should also consider immunogenicity of the iPSC-derivatives toward NK-cells.

POSSIBLE REASONS OF THE IMMUNE RESPONSE TO AUTOLOGOUS IPSC DERIVATIVES

It is still unclear what the crucial factor for sometimes-observed immunogenicity of the autologous iPSC-derivatives is. Generally, the T-cell response can be explained by formation of neoantigens and aberrant gene expression (Fig. 2a), and the NK-cell response can be explained by imbalance between the activating and inhibitory ligands in the target cells (Fig. 2b).

It was initially assumed that using different reprogramming vectors could be the reason for iPSC immunogenicity. Even in the first work, it was shown that the teratomas formed by retroviral iPSCs were more often rejected in the syngeneic hosts [12]. In turn, using episomes as a reprogramming vector significantly reduced percentage of the rejected teratomas. Similar results were obtained during transplantation of dopaminergic neurons into the primate brain [20]. It is well-known that the retroviral and lentiviral constructs are predominantly integrated into the transcriptionally active sites that could cause mutations, genome instability, and chromosomal aberrations. In addition, there is evidence that the subsequent activation of transgenes correlates with the aberrant production of the immunogenic protein OCT4 [17].

It is assumed that immunogenicity of the “integration-free” iPSCs should be lower than of the iPSCs obtained by retro- and lentiviral transfection. However, to the best of our knowledge, there are no detailed studies on this topic. The data comparing genomic instability in the iPSC lines obtained by various reprogramming methods are contradictory. Thus, some researchers report a similar number of point mutations [65], as well as copy number variations (CNVs) [66]. On the contrary, others showed 2-fold lower number of mutations in the integration-free” iPSCs [67, 68], which means lower probability of neoepitope formation. The frequency of genetic variations was also low in the human iPSCs obtained with episomal constructs [69]. In the recent years, other methods of reprogramming without integration have been suggested, particularly involving endogenous pluripotent genes using the CRISPR/Cas9 system [70]. However, as far as we know, no additional information about their genomic instability has been provided. In any case, non-integrative reprogramming methods are currently the safest and most effective for further clinical use [4].

Commonly, differences in immunogenicity are explained by mutations and, consequently, by formation of neoepitopes [71]. First, these may be mutations that

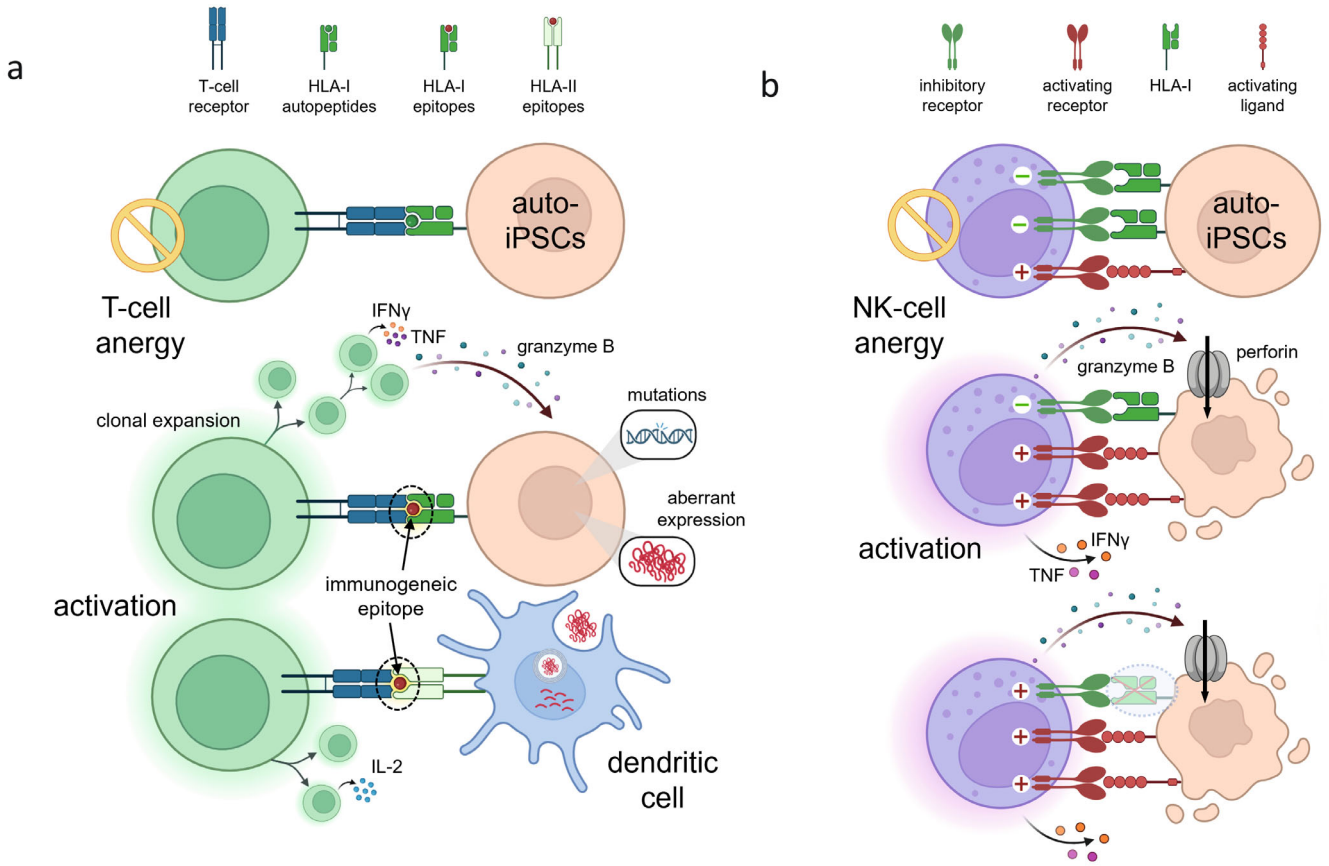

Fig. 2. Immunogenicity of autologous iPSCs and their derivatives. a) Immune response of T-cells to autologous iPSCs and their differentiated derivatives could be due to recognition of immunogenic neoepitopes formed as a result of mutations or aberrant expression of immunogenic genes. b) Immune response of NK-cells to autologous iPSCs and their differentiated derivatives could be due to imbalance of the NK-cell ligands in the target cells. Predominance of activating signals triggers cytotoxic program in NK-cells.

existed in the parental somatic cells. Thus, fibroblasts are one of the most frequently used sources of cells for reprogramming [72]. It has been reported that mutations acquired due to UV-induced mutagenesis are present in ~50% of human iPSCs reprogrammed from the skin fibroblasts [73]. Such mutations are characterized by the C-to-T or CC-to-TT substitutions and are often observed in melanomas [74]. Other somatic cells can act as an alternative to fibroblasts. For example, it has been reported that the iPSCs obtained from hematopoietic stem cells contain significantly fewer point mutations, insertions, and deletions than the iPSCs obtained from the skin fibroblasts [75]. Peripheral blood cells are also very often used as a source of cells for reprogramming. Rouhani et al. revealed that the blood-derived iPSCs contained fewer mutations than the fibroblast-derived iPSCs [76]. At the same time, there is evidence that mutations in the blood cells also accumulate with age [77]. Thus, the work using 16 iPSC lines obtained from the blood cells of donors aged 21-100 years demonstrated that the frequency of mutations in the iPSC increases linearly with the donor age [78]. In addition, frequency of the mitochondrial DNA mutations in human iPSCs also increases with the donor age, and this can lead to metabolic defects in iPSCs [79]. Although *de novo* mutations in the mitochondrial DNA are usually rare for iPSCs [80, 81], they can lead to formation of immunogenic neoepitopes and provoke immune response even during the autologous transplantation, as was shown earlier [82].

Thus, somatic cells of the young donors may have a comparative advantage for deriving iPSCs. This is also confirmed by the results of recent work, where iPSCs were obtained from the umbilical cord blood erythroblasts and did not contain mutations in the protein-coding regions [83]. In addition, age of the donors could affect cultural properties of iPSCs. Thus, it was shown that the iPSCs obtained from the older mice proliferated not as good as the iPSCs obtained from the young mice [84].

Mutations in iPSCs could also occur during reprogramming. Such mutations are usually similar to the mutations caused by oxidative stress (C-to-A substitutions) and are predominantly found in the lamina-associated domains – condensed regions of heterochromatin positioned at the nuclear periphery [85].

Earlier studies showed that up to 75% of point mutations in iPSCs occur during reprogramming [86, 87]. Presence of various mutations in the isogenic iPSC clones and lower frequency of point mutations in the isogenic ESC presumably indicates that such mutations were not obtained from the parental somatic cells [67]. Additional information on the mutations that occur during reprogramming was provided by Rouhani et al. [88]. The authors identified unique mutations in the isogenic iPSC lines derived from the monoclonal-origin endothelial precursors.

It is believed that mutations occur at the earliest stages of reprogramming before the first cell division after the introduction of reprogramming factors or immediately after the first or second division [67, 83]. The authors of the latest work also found that temporary deficit of the control components of the G1/S cell cycle at the initial stage of the reprogramming process leads to accumulation of mutations [83]. At the same time, the data on mutations that occur during reprogramming are pretty ambiguous. Recent studies have shown that up to 90% of the various SNPs and indels in iPSCs originate from somatic cells [73, 89]. Moreover, Kosanke et al. showed that only 2% of the mutations detected in iPSCs were not detected in the parental endothelial cells used for reprogramming [90].

The third reason for mutations in iPSCs is long-term cultivation. Such mutations are formed stochastically and are much less common than the pre-existing somatic mutations or reprogramming-induced mutations. Mutations caused by prolonged cultivation are believed to provide proliferative benefits [91, 92]. For example, one iPSCs line carried four additional point mutations in the late passages compared with the cells of early passages [86]. Nevertheless, according to the recent data, frequency and spectrum of the mutations induced by long-term cultivation of iPSCs do not differ from the mutations occurring at the pre-gastrulation stage of embryogenesis [93]. Another study showed that the rate of accumulated mutations in the long-term cultured iPSCs is lower than in the intestinal and liver stem cells [94]. The authors found that more than a third of mutations were caused by the C-to-A substitutions associated with oxidative stress. Cell cultivation under hypoxic conditions (3% oxygen) for three months significantly reduced the number of single nucleotide substitutions. Similar results were obtained for the ESCs: the authors observed a more than two-fold decrease in the frequency of mutations under hypoxia [95]. The obtained data can be used to optimize conditions of IPSC cultivation.

For clinical use, it is crucial to understand how mutations can affect the iPSC phenotype, including whether they can trigger carcinogenesis. There are studies indicating that point mutations are predominantly found in the cancer-associated genes [86]. Repeated mutations in the *TP53* tumor suppressor gene were detected both by the whole exome sequencing of ESC and by analyzing the publicly available RNA sequencing data from 120 PSC lines [92]. In addition to mutations in the *TP53* gene, repeated mutations were found in other tumor-associated genes, such as *CDK12*, *EGFR*, and *PATZ1* [96, 97]. A recent study revealed mutations in the *BCOR* gene, often found in hematological diseases, in more than 25% of the analyzed iPSC lines [76]. In contrast, no association with the tumor-associated genes has been found in other studies [65, 69, 73, 94]. Point mutations were mostly found in the areas of inactive chromatin, so they were unlikely to cause undesirable effects. However, unique mutations in various isogenic iPSC clones were usually found in active promoters and could alter gene expression [73]. This, in turn, could lead to formation of immunogenic determinants or affect effectiveness of differentiation into the desired cell type [98], which is also essential for regenerative medicine.

In addition to mutations induced by reprogramming or cultivation, epigenetic changes that regulate expression of various proteins should also be considered. First of all, it applies to disruption in DNA methylation in the PSC lines. Some studies noted that aberrant methylation pattern in iPSCs may be similar to the tumor cells [99-101]. Moreover, it was shown that methylation deregulation can persist in differentiated cells [102]. However, it is worth noting that DNA methylation in the PSCs can be dynamic, respond to culture conditions, and vary depending on the cell line [95].

It is also believed that some cells are not fully undergoing the reprogramming process, and iPSCs could to a large extent retain transcriptional and epigenetic memory of their origin [103]. Nevertheless, results of the studies on this topic are quite contradictory, but according to the modern concepts, molecular and functional differences in different iPSC lines are lost during prolonged cultivation [104]. At the same time, it was shown in the recent study that reprogramming through the stage of naive iPSCs (TNT-reprogramming) completely erases epigenetic memory and corrects epigenetic aberrations that have arisen [105]. Such TNT-iPSCs turned out to be more similar to ESCs from the molecular and functional point of view than the iPSCs obtained by the standard method.

Epigenetic peculiarities could explain abnormal expression of immunogenic proteins. Thus, at least two studies have demonstrated that the “somatic memory” phenomenon could influence immunogenicity of the iPSCs [106, 107]. Mouse iPSCs obtained from the Sertoli cells, anatomically related to the immune-privileged regions, formed teratomas more efficiently than the iPSCs obtained from the embryonic fibroblasts [106]. Moreover, differentiated derivatives of the syngeneic ESCs demonstrated a reduced *in vitro* activation

of allogeneic T-cells compared to the iPSCs obtained from the embryonic fibroblasts. However, it is worth noting that in the later passages, the authors did not observe differences in immunogenicity of the iPSCs obtained from different somatic cells. These results confirm that "somatic memory" in the iPSCs may be present only in early passages [106]. Another study showed that the umbilical cord mesenchymal cells are a less immunogenic source of cells for reprogramming than the skin fibroblasts [107].

Impaired expression of the genes associated with the NK-cell response is another reason for immunogenicity of iPSCs and their derivatives [15]. Both increase in the signals from activating receptors and decrease in the signals from inhibitory receptors can trigger cytotoxic program of NK-cells. In other words, proper balance between the inhibitory and activating ligands could make the target cell invisible to NK-cells [108]. In contrast, an impaired balance of the NK-cell ligands in the iPSC-derivatives could cause excessive activation of NK-cells. Thus, intensity of the HLA class I molecule expression and activating ligands and adhesion molecules would influence the degree of immune response. Previously, we showed that all these factors were responsible for the increased NK-cell response to iPSC-derivatives [15]. First, we observed a relatively low gene expression of the HLA-I molecules, major inhibitory ligands in the fibroblast-like iPSC-derivatives (iPS-fibro). Second, the genes coding for the main activating NK-cell ligands were upregulated in the iPS-fibro. Expression of the stress-induced molecule MICA (NKG2D ligand) gene was more than 1.5 times higher in the iPS-fibro than in their parental fibroblasts. The DNAM-1 ligands, *NECTIN2* (CD112) and *PVR* (CD155), and the NKp30 ligand, *NCR3LG1* (B7-H6), underwent a more noticeable increase in the gene expression with more than 3-fold-change in the iPS-fibro. Third, the genes of some adhesion molecules were also overexpressed in iPS-fibro. Interaction of the adhesion molecules with their receptors on NK-cells facilitates formation of tight junctions between the NK-cell and the target cell and leads to assembly of immunological synapses essential for the target cell killing [109]. The *ICAM-1* (LFA-1 ligand) and *VCAM-1* (VLA-4 or α4β1 integrin ligand) genes were upregulated in the iPSC-derivatives. Hence, imbalance between the NK-cell ligands in iPSC-derivatives was determined simultaneously by low intensity of the inhibitory signals and elevated intensity of the activating signals [15].

Vulnerability to the action of NK-cells can be explained by insufficient maturity of the differentiated iPSC-derivatives and low level of the HLA-I class molecules compared to the parental somatic cells. Thus, increase in the HLA-I expression was shown during prolonged cultivation or passaging, at least for the RPE cells [50], proximal renal epithelium cells [28], and iPS-fibro [15]. Another risk of immature phenotype is expression of embryonic or fetal proteins, which are also typical for some cancers (for example, alpha-fetoprotein) [110]. Despite the active development of differentiation protocols, several cell types can be differentiated *in vitro* only to an immature phenotype, in particular, cardiomyocytes [111], hepatocytes [112], or beta-cells [113].

Increased expression of the activating NK-cell ligands is worth noting separately. Analysis of the publicly available RNA-seq datasets [114-116] showed that expression of the *NECTIN2*, *PVR*, *CADM1*, and *CD70* genes was upregulated in the independently derived fibroblast-like cells compared to the isogenic fibroblasts used for reprogramming [15]. Imperfect microenvironment during *in vitro* differentiation may affect proper balance between the ligands for the NK-cell receptors in this type of iPSC-derivatives. In addition, high levels of the *MICA* and *NECTIN2* gene expression were observed in the proximal epithelial cells of the kidney [28]. Since each cell type expresses its own set of proteins, it will be necessary to determine expression pattern of the ligands of the NK-cell receptors for clinical use. It is worth noting that the cells that belong to immune-privileged tissues could have immunomodulatory functions to suppress immune response. It was shown that some types of the differentiated PSC-derivatives, in particular RPE cells [117, 118], retinal ganglion cells [119], neuron precursors [120-122], neural crest cells [123, 124], and chondrocytes [125] demonstrate reduced immunogenicity even to allogeneic lymphocytes.

Different cultivation conditions could affect immunogenicity of iPSCs and their derivatives. As mentioned earlier, prolonged cultivation could lead to accumulation of mutations in the cells at later passages [86, 94]. The cryo-pause method, i.e., storing iPSCs as ready-to-use aliquots from one passage, can reduce frequency of genomic aberrations caused by passaging and prolonged cultivation of iPSCs [126]. Considering that oxidative process during reprogramming and prolonged cultivation could lead to C-to-A substitutions [85, 88, 94], antioxidants could reduce mutagenic load in the iPSCs. In particular, antioxidants were reported to reduce CNVs in the iPSCs [127]. A recent study also indicated that introduction of antioxidant transgenes, such as superoxide dismutase 1 (*SOD1*) and 2 (*SOD2*), glutathione peroxidase 1 (*GPX1*), and N-acetylcysteine (*NAC*), reduced the number of transversions in iPSCs [83].

Selection of the reagents used for cultivation and differentiation could affect immunogenic properties of iPSCs and their derivatives. Using xenogeneic materials for PSC cultivation could complicate further clinical use of the PSC-derivatives. For example, ESCs and embryoid bodies were shown to absorb

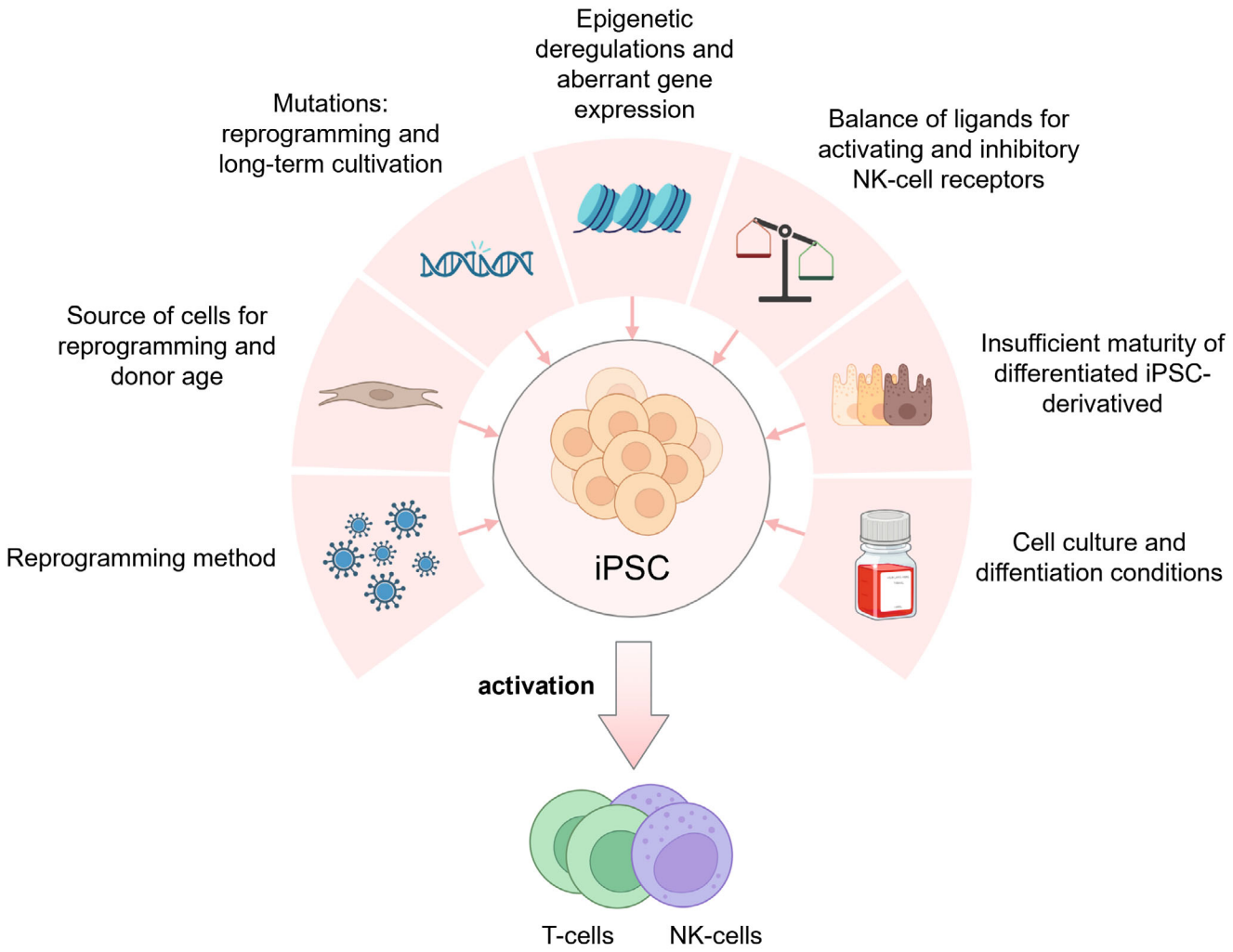

Fig. 3. Potential reasons of impaired immune tolerance to the autologous iPSCs and their differentiated derivatives.

N- glycolylneuraminic acid (Neu5Gc) from the feeder layers of embryonic fibroblasts and from nutrient media containing animal serum [128]. This poses a significant risk since antibodies to Neu5Gc circulate in human blood [129]. Currently, PSCs are usually cultivated under feeder-free conditions. In addition, reprogramming and differentiation protocols have been developed without using animal components (xeno-free), in which the amount of Neu5Gc is reduced or it is completely absent. However, these methods are more expensive [130, 131]. On the other hand, commercial xeno-free media may contain elevated levels of ascorbate, which could affect methylation of the *CD30* promoter, a marker of malignant neoplasms [132]. However, it is worth noting that CD30 more likely is the marker of undifferentiated cells than the marker of transformed cells [133]. In any case, the risk is not limited to animal products. For new formulations of nutrient media, it is necessary to determine their biological effects on the cultured cells, including balance of the NK-cell ligands.

Hence, several factors could potentially affect immunogenicity of the final cellular product obtained from PSCs (Fig. 3). Systematization of the possible reasons listed above should contribute to the development of quality criteria to ensure safety of the PSC-based therapy in clinical practice. Since each type of the differentiated cells expresses different genes and proteins, we assume that screening of each cell type for immunogenicity would be necessary for subsequent clinical use [134].

CONCLUSION

Accumulating data on the lack of complete immune tolerance to derivatives of autologous iPSCs [13-15] raises concerns about their transplantation without immunosuppression. Nevertheless, the most eloquent response to these concerns are the results of ongoing clinical trials. According to the https://clinicaltrials.gov, about 20 cell products based on autologous iPSCs are

undergoing clinical trials. Primary results of the three of them have been published [135-137]. In the first two cases, RPE cells were transplanted to treat age-related macular degeneration; in the third, dopaminergic progenitor cells were transplanted to treat Parkinson's disease. Immunosuppressive therapy was not used in any transplantation, but no side effects were reported. It should be noted that in all cases transplantation of homogeneous cultures of the iPSC-derivatives was carried out into the immune-privileged organs – eye and brain. It remains to be studied whether the undesirable immune reactions would occur during transplantation of autologous iPSC-derivatives, including complex cellular products, into the organs and tissues deprived of immune-privileged status. More recently, there has been a report on the absence of side effects in the case of transfusion of the platelets differentiated from autologous iPSCs [138].

Impaired immune tolerance and high cost and time necessary for deriving a new iPSC line currently do not allow us to consider personalized therapy as a promising tool for broad medical practice. In this regard, allogeneic derivatives of PSCs are currently the preferred source for regenerative medicine. In the recent years, it was suggested that derivation of the "universal" PSCs could solve the problem of histocompatibility and prevent immune rejection since their derivatives would be suitable for any recipient [43, 44, 51]. As mentioned earlier, various "immune masking" strategies are used to obtain such iPSCs, from elimination of the HLA molecules to inhibit T-lymphocytes [45-50] to introduction of the immunomodulatory factors to suppress NK-cells [53-57]. It was shown that the differentiated derivatives of the modified iPSCs with reduced immunogenicity demonstrate long-term survival in the fully immunocompetent animals: 50 days in the mouse model [56] and 40 weeks in rhesus monkeys [139]. In both studies, "blinding" of the allogeneic immune system was achieved by blocking expression of the HLA class I and II by knocking out the *B2M* and *CIITA* genes and introducing the *CD47* transgene as an immune checkpoint to NK-cells [140]. In the recent study, simultaneous introduction of 8 immunomodulatory factors (*Pdl1*, *Cd200*, *Cd47*, *H2-M3*, *Fasl*, *Serpinb9*, *Ccl21*, and *Mfge8*) to the mouse ESC ensured long-term survival of teratomas in the allogeneic model [141]. In some groups of animals, the observation period was nine months. In February 2022, ViaCyte and CRISPR Therapeutics announced launch of the Phase I trials of VCTX210, ESC-based therapy for type 1 diabetes without the need for immunosuppression. The used CyT49 line has a *B2M* gene knockout and expresses the *CD274* transgene encoding immunological checkpoint PD-L1 for additional protection against the T-cell attack [142].

Despite attractiveness of the "universal" approach, the problems associated with safety of such therapy are worth noting [43, 143, 144]. First, they relate to immune evasion, which means increase in the risks of tumor transformation of such cells. By itself, absence of the MHC/HLA molecules should not contribute to oncogenesis [145]. Therefore, the probability of malignant degeneration is unlikely to exceed that in the adult tissues. However, it is evident that in the HLA-negative cells, it would be more challenging to eliminate it using conventional immune mechanisms. It was proposed to solve this problem by introducing "suicide cassettes" that can be activated in the case of malignant transformation or viral infection of the graft [43, 51]. The herpes simplex virus 1 thymidine kinase (HSV-TK) gene can be introduced under the promoter of both pluripotent genes [146] and the genes that play a key role in regulation of the cell cycle, such as the *CDK1* gene [147]. The latter approach has recently been applied to the immunomodified human ESCs [141]. Another approach is a suicidal system based on the inducible caspase-9 (iCas9) [148]. Taking into account this issue, the current Viacyte clinical trial is safer since a semipermeable membrane encapsulates beta-cells and cannot exit the capsule in the case of degeneration [149]. In any case, it is evident that for safety reasons it is necessary to thoroughly investigate "universal" lines for potential oncogenic mutations and activating proto-oncogenes [51]. Still, despite the unresolved issues, development of the genetically modified pluripotent stem cells can contribute to the large-scale production of the "off-the-shelf" cell products and solve the problem of tissue and organ shortage.

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REFERENCES

1. Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., et al. (1998) Embryonic stem cell lines derived from human blastocysts, *Science*, **282**, 1145-1147, doi: 10.1126/science.282.5391.1145.

2. Takahashi, K., and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, *Cell*, **126**, 663-676, doi: 10.1016/j.cell.2006.07.024.
3. Lebedeva, O. S., and Lagarkova, M. A. (2018) Pluripotent stem cells for modelling and cell therapy of Parkinson's disease, *Biochemistry (Moscow)*, **83**, 1318-1330, doi: 10.1134/S0006297918090067.
4. Doss, M. X., and Sachinidis, A. (2019) Current challenges of iPSC-based disease modeling and therapeutic implications, *Cells*, **8**, 403, doi: 10.3390/cells8050403.
5. Bogomazova, A. N., Vassina, E. M., Kiselev, S. I., Lagarkova, M. A., Lebedeva, O. S., et al. (2015) Genetic cell reprogramming: a new technology for basic research and applied usage, *Genetika*, **51**, 466-478, doi: 10.7868/S0016675815040025.
6. Huang, C. Y., Liu, C. L., Ting, C. Y., Chiu, Y. T., Cheng, Y. C., et al. (2019) Human iPSC banking: barriers and opportunities, *J. Biomed. Sci.*, **26**, 87, doi: 10.1186/s12929-019-0578-x.
7. Rehakova, D., Souralova, T., and Koutna, I. (2020) Clinical-grade human pluripotent stem cells for cell therapy: characterization strategy, *Int. J. Mol. Sci.*, **21**, 2435, doi: 10.3390/ijms21072435.
8. Sullivan, S., Stacey, G. N., Akazawa, C., Aoyama, N., Baptista, R., et al. (2018) Quality control guidelines for clinical-grade human induced pluripotent stem cell lines, *Regen. Med.*, **13**, 859-866, doi: 10.2217/rme-2018-0095.
9. Kobold, S., Guhr, A., Mah, N., Bultjer, N., Seltmann, S., et al. (2020) A manually curated database on clinical studies involving cell products derived from human pluripotent stem cells, *Stem Cell Rep.*, **15**, 546-555, doi: 10.1016/j.stemcr.2020.06.014.
10. Bolton, E. M., and Bradley, J. A. (2015) Avoiding immunological rejection in regenerative medicine, *Regen. Med.*, **10**, 287-304, doi: 10.2217/rme.15.11.
11. Barrilleaux, B., and Knoepfler, P. S. (2011) Inducing iPSCs to escape the dish, *Cell Stem Cell*, **9**, 103-111, doi: 10.1016/j.stem.2011.07.006.
12. Zhao, T., Zhang, Z. N., Rong, Z., and Xu, Y. (2011) Immunogenicity of induced pluripotent stem cells, *Nature*, **474**, 212-215, doi: 10.1038/nature10135.
13. Zhao, T., Zhang, Z. N., Westenskow, P. D., Todorova, D., Hu, Z., et al. (2015) Humanized mice reveal differential immunogenicity of cells derived from autologous induced pluripotent stem cells, *Cell Stem Cell*, **17**, 353-359, doi: 10.1016/j.stem.2015.07.021.
14. Nakamura, Y., Miyagawa, S., Yoshida, S., Sasawatari, S., Toyofuku, T., et al. (2019) Natural killer cells impede the engraftment of cardiomyocytes derived from induced pluripotent stem cells in syngeneic mouse model, *Sci. Rep.*, **9**, 10840, doi: 10.1038/s41598-019-47134-3.
15. Bogomiakova, M. E., Sekretova, E. K., Anufrieva, K. S., Khabarova, P. O., Kazakova, A. N., et al. (2023) iPSC-derived cells lack immune tolerance to autologous NK-cells due to imbalance in ligands for activating and inhibitory NK-cell receptors, *Stem Cell Res. Ther.*, **14**, 77, doi: 10.1186/s13287-023-03308-5.
16. Araki, R., Uda, M., Hoki, Y., Sunayama, M., Nakamura, M., et al. (2013) Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells, *Nature*, **494**, 100-104, doi: 10.1038/nature11807.
17. Dhodapkar, K. M., Feldman, D., Matthews, P., Radfar, S., Pickering, R., et al. (2010) Natural immunity to pluripotency antigen OCT4 in humans, *Proc. Natl. Acad. Sci. USA*, **107**, 8718-8723, doi: 10.1073/pnas.0915086107.
18. Guha, P., Morgan, J. W., Mostoslavsky, G., Rodrigues, N. P., and Boyd, A. S. (2013) Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells, *Cell Stem Cell*, **12**, 407-412, doi: 10.1016/j.stem.2013.01.006.
19. de Almeida, P. E., Meyer, E. H., Kooreman, N. G., Diecke, S., Dey, D., et al. (2014) Transplanted terminally differentiated induced pluripotent stem cells are accepted by immune mechanisms similar to self-tolerance, *Nat. Commun.*, **5**, 3903, doi: 10.1038/ncomms4903.
20. Morizane, A., Doi, D., Kikuchi, T., Okita, K., Hotta, A., et al. (2013) Direct comparison of autologous and allogeneic transplantation of iPSC-derived neural cells in the brain of a non-human primate, *Stem Cell Rep.*, **1**, 283-292, doi: 10.1016/j.stemcr.2013.08.007.
21. Emborg, M. E., Liu, Y., Xi, J., Zhang, X., Yin, Y., et al. (2013) Induced pluripotent stem cell-derived neural cells survive and mature in the nonhuman primate brain, *Cell Rep.*, **3**, 646-650, doi: 10.1016/j.celrep.2013.02.016.
22. Hallett, P. J., Deleidi, M., Astradsson, A., Smith, G. A., Cooper, O., et al. (2015) Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease, *Cell Stem Cell*, **16**, 269-274, doi: 10.1016/j.stem.2015.01.018.
23. Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B., and Olanow, C. W. (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease, *Nat. Med.*, **14**, 504-506, doi: 10.1038/nm1747.
24. Mendez, I., Viñuela, A., Astradsson, A., Mukhida, K., Hallett, P., et al. (2008) Dopamine neurons implanted into people with Parkinson's disease survive without pathology for 14 years, *Nat. Med.*, **14**, 507-509, doi: 10.1038/nm1752.
25. Strnadel, J., Carromeu, C., Bardy, C., Navarro, M., Platoshyn, O., et al. (2018) Survival of syngeneic and allogeneic iPSC-derived neural precursors after spinal grafting in minipigs, *Sci. Transl. Med.*, **10**, eaam6651, doi: 10.1126/scitranslmed.aam6651.
26. Lu, M., Peng, L., Ming, X., Wang, X., Cui, A., et al. (2019) Enhanced wound healing promotion by immune response-free monkey autologous iPSCs and exosomes

vs. their allogeneic counterparts, *EBioMedicine*, **42**, 443-457, doi: 10.1016/j.ebiom.2019.03.011.

27. Benabdallah, B., Désaulniers-Langevin, C., Goyer, M. L., Colas, C., Maltais, C., et al. (2021) Myogenic progenitor cells derived from human induced pluripotent stem cell are immune-tolerated in humanized mice, *Stem Cells Transl. Med.*, **10**, 267-277, doi: 10.1002/sctm.19-0452.
28. Rossbach, B., Hariharan, K., Mah, N., Oh, S. J., Volk, H. D., et al. (2022) Human iPSC-derived renal cells change their immunogenic properties during maturation: implications for regenerative therapies, *Cells*, **11**, 1328, doi: 10.3390/cells11081328.
29. Ljunggren, H. G., and Kärre, K. (1990) In search of the 'missing self': MHC molecules and NK cell recognition, *Immunol. Today*, **11**, 237-244, doi: 10.1016/0167-569990097-s.
30. Lanier, L. L. (2005) NK cell recognition, *Annu. Rev. Immunol.*, **23**, 225-274, doi: 10.1146/annurev.immunol.23.021704.115526.
31. Smyth, M. J., Cretney, E., Kelly, J. M., Westwood, J. A., Street, S. E., et al. (2005) Activation of NK cell cytotoxicity, *Mol. Immunol.*, **42**, 501-510, doi: 10.1016/j.molimm.2004.07.034.
32. Dressel, R., Nolte, J., Elsner, L., Novota, P., Guan, K., et al. (2010) Pluripotent stem cells are highly susceptible targets for syngeneic, allogeneic, and xenogeneic natural killer cells, *FASEB J.*, **24**, 2164-2177, doi: 10.1096/fj.09-134957.
33. Kruse, V., Hamann, C., Monecke, S., Cyganek, L., Elsner, L., et al. (2015) Human induced pluripotent stem cells are targets for allogeneic and autologous natural Killer (NK) cells and killing is partly mediated by the activating NK receptor DNAM-1, *PLoS One*, **10**, e0125544, doi: 10.1371/journal.pone.0125544.
34. Drukker, M., Katz, G., Urbach, A., Schuldiner, M., Markel, G., et al. (2002) Characterization of the expression of MHC proteins in human embryonic stem cells, *Proc. Natl. Acad. Sci. USA*, **99**, 9864-9869, doi: 10.1073/pnas.142298299.
35. Frenzel, L. P., Abdullah, Z., Kriegeskorte, A. K., Dieterich, R., Lange, N., et al. (2009) Role of natural-killer group 2 member D ligands and intercellular adhesion molecule 1 in natural killer cell-mediated lysis of murine embryonic stem cells and embryonic stem cell-derived cardiomyocytes, *Stem Cells*, **27**, 307-316, doi: 10.1634/stemcells.2008-0528.
36. Gröschel, C., Hübscher, D., Nolte, J., Monecke, S., Sasse, A., et al. (2017) Efficient killing of murine pluripotent stem cells by natural killer (NK) cells requires activation by cytokines and partly depends on the activating NK receptor NKG2D, *Front. Immunol.*, **8**, 870, doi: 10.3389/fimmu.2017.00870.
37. Dressel, R., Schindehütte, J., Kuhlmann, T., Elsner, L., Novota, P., et al. (2008) The tumorigenicity of mouse embryonic stem cells and in vitro differentiated neuronal cells is controlled by the recipients' immune response, *PLoS One*, **3**, e2622, doi: 10.1371/journal.pone.0002622.
38. Benabdallah, B., Désaulniers-Langevin, C., Colas, C., Li, Y., Rousseau, G., et al. (2019) Natural killer cells prevent the formation of teratomas derived from human induced pluripotent stem cells, *Front. Immunol.*, **10**, 2580, doi: 10.3389/fimmu.2019.02580.
39. Melendez, E., Chondronasiou, D., Mosteiro, L., Martínez de Villarreal, J., Fernández-Alfara, M., et al. (2022) Natural killer cells act as an extrinsic barrier for in vivo reprogramming, *Development*, **149**, dev200361, doi: 10.1242/dev.200361.
40. Abad, M., Mosteiro, L., Pantoja, C., Cañamero, M., Rayon, T., et al. (2013) Reprogramming in vivo produces teratomas and iPS cells with totipotency features, *Nature*, **502**, 340-345, doi: 10.1038/nature12586.
41. Cisneros, T., Dillard, D. W., Qu, X., Arredondo-Guerrero, J., Castro, M., et al. (2019) Differential role of natural killer group 2D in recognition and cytotoxicity of hepatocyte-like cells derived from embryonic stem cells and induced pluripotent stem cells, *Am. J. Transplant.*, **19**, 1652-1662, doi: 10.1111/ajt.15217.
42. Phillips, L. K., Gould, E. A., Babu, H., Krams, S. M., Palmer, T. D., and Martinez, O. M. (2013) Natural killer cell-activating receptor NKG2D mediates innate immune targeting of allogeneic neural progenitor cell grafts, *Stem Cells*, **31**, 1829-1839, doi: 10.1002/stem.1422.
43. Zheng, D., Wang, X., and Xu, R. H. (2016) Concise review: one stone for multiple birds: generating universally compatible human embryonic stem cells, *Stem Cells*, **34**, 2269-2275, doi: 10.1002/stem.2407.
44. Bogomiakova, M. E., Eremeev, A. V., and Lagarkova, M. A. (2019) At home among strangers: is it possible to create hypoimmunogenic pluripotent stem cell lines? *Mol. Biol.*, **53**, 725-740, doi: 10.1134/S0026898419050045.
45. Riolobos, L., Hirata, R. K., Turtle, C. J., Wang, P. R., Gornalusse, G. G., et al. (2013) HLA engineering of human pluripotent stem cells, *Mol. Ther.*, **21**, 1232-1241, doi: 10.1038/mt.2013.59.
46. Wang, D., Quan, Y., Yan, Q., Morales, J. E., and Wetsel, R. A. (2015) Targeted disruption of the β2-microglobulin gene minimizes the immunogenicity of human embryonic stem cells, *Stem Cells Transl. Med.*, **4**, 1234-1245, doi: 10.5966/sctm.2015-0049.
47. Bogomiakova, M. E., Sekretova, E. K., Eremeev, A. V., Shuvalova, L. D., Bobrovsky, P. A., et al. (2021) Derivation of induced pluripotent stem cells line (RCPCMi007-A-1) with inactivation of the beta-2-microglobulin gene by CRISPR/Cas9 genome editing, *Stem Cell Res.*, **55**, 102451, doi: 10.1016/j.scr.2021.102451.
48. Chen, H., Li, Y., Lin, X., Cui, D., Cui, C., et al. (2015) Functional disruption of human leukocyte antigen II in human embryonic stem cell, *Biol. Res.*, **48**, 59, doi: 10.1186/s40659-015-0051-6.

49. Mattapally, S., Pawlik, K. M., Fast, V. G., Zumaquero, E., Lund, F. E., et al. (2018) Human leukocyte antigen class I and II knockout human induced pluripotent stem cell-derived cells: universal donor for cell therapy, *J. Am Heart Assoc.*, **7**, e010239, doi: 10.1161/JAHA.118.010239.
50. Petrus-Reurer, S., Winblad, N., Kumar, P., Gorchs, L., Chrobok, M., et al. (2020) Generation of retinal pigment epithelial cells derived from human embryonic stem cells lacking human leukocyte antigen class I and II, *Stem Cell Rep.*, **14**, 648-662, doi: 10.1016/j.stemcr.2020.02.006.
51. Lanza, R., Russell, D. W., and Nagy, A. (2019) Engineering universal cells that evade immune detection, *Nat. Rev. Immunol.*, **19**, 723-733, doi: 10.1038/s41577-019-0200-1.
52. Kim, A., Lee, K. G., Kwon, Y., Lee, K. I., Yang, H. M., et al. (2021) Off-the-shelf, immune-compatible human embryonic stem cells generated via CRISPR-mediated genome editing, *Stem Cell Rev Rep*, **17**, 1053-1067, doi: 10.1007/s12015-020-10113-7.
53. Wang, B., Iriguchi, S., Waseda, M., Ueda, N., Ueda, T., et al. (2021) Generation of hypoimmunogenic T cells from genetically engineered allogeneic human induced pluripotent stem cells, *Nat. Biomed. Eng.*, **5**, 429-440, doi: 10.1038/s41551-021-00730-z.
54. Gornalusse, G. G., Hirata, R. K., Funk, S. E., Riolobos, L., Lopes, V. S., et al. (2017) HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells, *Nat. Biotechnol.*, **35**, 765-772, doi: 10.1038/nbt.3860.
55. Han, X., Wang, M., Duan, S., Franco, P. J., Kenty, J. H., et al. (2019) Generation of hypoimmunogenic human pluripotent stem cells, *Proc. Natl. Acad. Sci. USA*, **116**, 10441-10446, doi: 10.1073/pnas.1902566116.
56. Deuse, T., Hu, X., Gravina, A., Wang, D., Tediashvili, G., et al. (2019) Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients, *Nat. Biotechnol.*, **37**, 252-258, doi: 10.1038/s41587-019-0016-3.
57. Shi, L., Li, W., Liu, Y., Chen, Z., Hui, Y., et al. (2020) Generation of hypoimmunogenic human pluripotent stem cells via expression of membrane-bound and secreted β2m-HLA-G fusion proteins, *Stem Cells*, **38**, 1423-1437, doi: 10.1002/stem.3269.
58. Moffett, A., and Colucci, F. (2015) Co-evolution of NK receptors and HLA ligands in humans is driven by reproduction, *Immunol. Rev.*, **267**, 283-297, doi: 10.1111/imr.12323.
59. Ichise, H., Nagano, S., Maeda, T., Miyazaki, M., Miyazaki, Y., et al. (2017) NK Cell Alloreactivity against KIR-ligand-mismatched HLA-haploidentical tissue derived from HLA haplotype-homozygous iPSCs, *Stem Cell Rep.*, **9**, 853-867, doi: 10.1016/j.stemcr.2017.07.020.
60. Zamir, M. R., Shahi, A., Salehi, S., and Amirzargar, A. (2022) Natural killer cells and killer cell immunoglobulin-like receptors in solid organ transplantation: Protectors or opponents? *Transplant. Rev. (Orlando)*, **36**, 100723, doi: 10.1016/j.trre.2022.100723.
61. Song, C., Wang, L., Li, Q., Liao, B., Qiao, W., et al. (2022) Generation of individualized immunocompatible endothelial cells from HLA-I-matched human pluripotent stem cells, *Stem Cell Res. Ther.*, **13**, 48, doi: 10.1186/s13287-022-02720-7.
62. Villard, J. (2011) The role of natural killer cells in human solid organ and tissue transplantation, *J. Innate Immun.*, **3**, 395-402, doi: 10.1159/000324400.
63. Pontrelli, P., Rascio, F., Castellano, G., Grandaliano, G., Gesualdo, L., and Stallone, G. (2020) The role of natural killer cells in the immune response in kidney transplantation, *Front. Immunol.*, **11**, 1454, doi: 10.3389/fimmu.2020.01454.
64. Adenugba, A. (2017) NK cells in transplantation, *Transplantation*, **101**, 2262-2264, doi: 10.1097/TP.0000000000001914.
65. Bhutani, K., Nazor, K. L., Williams, R., Tran, H., Dai, H., et al. (2016) Whole-genome mutational burden analysis of three pluripotency induction methods, *Nat. Commun.*, **7**, 10536, doi: 10.1038/ncomms10536.
66. Popp, B., Krumbiegel, M., Grosch, J., Sommer, A., Uebe, S., et al. (2018) Need for high-resolution genetic analysis in iPSC: results and lessons from the ForIPS consortium, *Sci. Rep.*, **8**, 17201, doi: 10.1038/s41598-018-35506-0.
67. Sugiura, M., Kasama, Y., Araki, R., Hoki, Y., Sunayama, M., et al. (2014) Induced pluripotent stem cell generation-associated point mutations arise during the initial stages of the conversion of these cells, *Stem Cell Rep.*, **2**, 52-63, doi: 10.1016/j.stemcr.2013.11.006.
68. Kang, X., Yu, Q., Huang, Y., Song, B., Chen, Y., et al. (2015) Effects of integrating and non-integrating reprogramming methods on copy number variation and genomic stability of human induced pluripotent stem cells, *PLoS One*, **10**, e0131128, doi: 10.1371/journal.pone.0131128.
69. Cheng, L., Hansen, N. F., Zhao, L., Du, Y., Zou, C., et al. (2012) Low incidence of DNA sequence variation in human induced pluripotent stem cells generated by nonintegrating plasmid expression, *Cell Stem Cell*, **10**, 337-344, doi: 10.1016/j.stem.2012.01.005.
70. Weltner, J., Balboa, D., Katayama, S., Bespalov, M., Krjutškov, K., et al. (2018) Human pluripotent reprogramming with CRISPR activators, *Nat. Commun.*, **9**, 2643, doi: 10.1038/s41467-018-05067-x.
71. Liu, X., Li, W., Fu, X., and Xu, Y. (2017) The immunogenicity and immune tolerance of pluripotent stem cell derivatives, *Front. Immunol.*, **8**, 645, doi: 10.3389/fimmu.2017.00645.
72. Hanna, J. H., Saha, K., and Jaenisch, R. (2010) Pluripotency and cellular reprogramming: facts, hypotheses, unresolved issues, *Cell*, **143**, 508-525, doi: 10.1016/j.cell.2010.10.008.

73. D'Antonio, M., Benaglio, P., Jakubosky, D., Greenwald, W. W., and Matsui, H. (2018) Insights into the mutational burden of human induced pluripotent stem cells from an integrative multi-omics approach, *Cell Rep.*, **24**, 883-894, doi: 10.1016/j.celrep.2018.06.091.
74. Martincorena, I., Roshan, A., Gerstung, M., Ellis, P., Van Loo, P., et al. (2015) Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin, *Science*, **348**, 880-886, doi: 10.1126/science.aaa6806.
75. Wang, K., Guzman, A. K., Yan, Z., Zhang, S., Hu, M. Y., et al. (2019) Ultra-high-frequency reprogramming of individual long-term hematopoietic stem cells yields low somatic variant induced pluripotent stem cells, *Cell Rep.*, **26**, 2580-2592.e7, doi: 10.1016/j.celrep.2019.02.021.
76. Rouhani, F. J., Zou, X., Danecek, P., Badja, C., Amarante, T. D., et al. (2022) Substantial somatic genomic variation and selection for BCOR mutations in human induced pluripotent stem cells, *Nat. Genet.*, **54**, 1406-1416, doi: 10.1038/s41588-022-01147-3.
77. Genovese, G., Kähler, A. K., Handsaker, R. E., Lindberg, J., Rose, S. A., et al. (2014) Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence, *N. Engl. J. Med.*, **371**, 2477-2487, doi: 10.1056/NEJMoa1409405.
78. Lo Sardo, V., Ferguson, W., Erikson, G. A., Topol, E. J., Baldwin, K. K., and Torkamani, A. (2017) Influence of donor age on induced pluripotent stem cells, *Nat. Biotechnol.*, **35**, 69-74, doi: 10.1038/nbt.3749.
79. Kang, E., Wang, X., Tippner-Hedges, R., Ma, H., Folmes, C. D., et al. (2016) Age-related accumulation of somatic mitochondrial DNA mutations in adult-derived human iPSCs, *Cell Stem Cell*, **18**, 625-636, doi: 10.1016/j.stem.2016.02.005.
80. Wei, W., Gaffney, D. J., and Chinnery, P. F. (2021) Cell reprogramming shapes the mitochondrial DNA landscape, *Nat. Commun.*, **12**, 5241, doi: 10.1038/s41467-021-25482-x.
81. Kosanke, M., Davenport, C., Szepes, M., Wiehlmann, L., Kohrn, T., et al. (2021) iPSC culture expansion selects against putatively actionable mutations in the mitochondrial genome, *Stem Cell Rep.*, **16**, 2488-2502, doi: 10.1016/j.stemcr.2021.08.016.
82. Deuse, T., Hu, X., Agbor-Enoh, S., Koch, M., Spitzer, M. H., et al. (2019) De novo mutations in mitochondrial DNA of iPSCs produce immunogenic neoepitopes in mice and humans, *Nat Biotechnol.*, **37**, 1137-1144, doi: 10.1038/s41587-019-0227-7.
83. Araki, R., Hoki, Y., Suga, T., Obara, C., Sunayama, M., et al. (2020) Genetic aberrations in iPSCs are introduced by a transient G1/S cell cycle checkpoint deficiency, *Nat. Commun.*, **11**, 197, doi: 10.1038/s41467-019-13830-x.
84. Wang, B., Miyagoe-Suzuki, Y., Yada, E., Ito, N., Nishiyama, T., et al. (2011) Reprogramming efficiency and quality of induced pluripotent stem cells (iPSCs) generated from muscle-derived fibroblasts of mdx mice at different ages, *PLoS Curr.*, **3**, RRN1274, doi: 10.1371/currents.RRN1274.
85. Yoshihara, M., Araki, R., Kasama, Y., Sunayama, M., Abe, M., et al. (2017) Hotspots of *de novo* point mutations in induced pluripotent stem cells, *Cell Rep.*, **21**, 308-315, doi: 10.1016/j.celrep.2017.09.060.
86. Gore, A., Li, Z., Fung, H. L., Young, J. E., Agarwal, S., et al. (2011) Somatic coding mutations in human induced pluripotent stem cells, *Nature*, **471**, 63-67, doi: 10.1038/nature09805.
87. Ji, J., Ng, S. H., Sharma, V., Neculai, D., Hussein, S., et al. (2012) Elevated coding mutation rate during the reprogramming of human somatic cells into induced pluripotent stem cells, *Stem Cells*, **30**, 435-440, doi: 10.1002/stem.1011.
88. Rouhani, F. J., Nik-Zainal, S., Wuster, A., Li, Y., Conte, N., et al. (2016) Mutational history of a human cell lineage from somatic to induced pluripotent stem cells, *PLoS Genet.*, **12**, e1005932, doi: 10.1371/journal.pgen.1005932.
89. Kwon, E. M., Connelly, J. P., Hansen, N. F., Donovan, F. X., Winkler, T., et al. (2017) iPSCs and fibroblast subclones from the same fibroblast population contain comparable levels of sequence variations, *Proc. Natl. Acad. Sci. USA*, **114**, 1964-1969, doi: 10.1073/pnas.1616035114.
90. Kosanke, M., Osetek, K., Haase, A., Wiehlmann, L., Davenport, C., et al. (2021) Reprogramming enriches for somatic cell clones with small-scale mutations in cancer-associated genes, *Mol. Ther.*, **29**, 2535-2553, doi: 10.1016/j.ymthe.2021.04.007.
91. International Stem Cell Initiative, Amps, K., Andrews, P. W., Anyfantis, G., Armstrong, L., et al. (2011) Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage, *Nat. Biotechnol.*, **29**, 1132-1144, doi: 10.1038/nbt.2051.
92. Merkle, F. T., Ghosh, S., Kamitaki, N., Mitchell, J., et al. (2017) Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations, *Nature*, **545**, 229-233, doi: 10.1038/nature22312.
93. Hasaart, K. A. L., Manders, F., Ubels, J., Verheul, M., van Roosmalen, M. J., et al. (2022) Human induced pluripotent stem cells display a similar mutation burden as embryonic pluripotent cells *in vivo*, *iScience*, **25**, 103736, doi: 10.1016/j.isci.2022.103736.
94. Kuijk, E., Jager, M., van der Roest, B., Locati, M. D., Van Hoeck, A., et al. (2020) The mutational impact of culturing human pluripotent and adult stem cells, *Nat. Commun.*, **11**, 2493, doi: 10.1038/s41467-020-16323-4.
95. Thompson, O., von Meyenn, F., Hewitt, Z., Alexander, J., Wood, A., et al. (2020) Low rates of mutation in clinical grade human pluripotent stem cells under

different culture conditions, *Nat. Commun.*, **11**, 1528, doi: 10.1038/s41467-020-15271-3.

96. Avior, Y., Lezmi, E., Eggan, K., and Benvenisty, N. (2021) Cancer-related mutations identified in primed human pluripotent stem cells, *Cell Stem Cell*, **28**, 10-11, doi: 10.1016/j.stem.2020.11.013.
97. Merkle, F. T., Ghosh, S., Genovese, G., Handsaker, R. E., Kashin, S., et al. (2022) Whole-genome analysis of human embryonic stem cells enables rational line selection based on genetic variation, *Cell Stem Cell*, **29**, 472-486.e7, doi: 10.1016/j.stem.2022.01.011.
98. Puigdevall, P., Jerber, J., Danecek, P., Castellano, S., and Kilpinen, H. (2023) Somatic mutations alter the differentiation outcomes of iPSC-derived neurons, *Cell Genom.*, **3**, 100280, doi: 10.1016/j.xgen.2023.100280.
99. Ohm, J. E., Mali, P., Van Neste, L., Berman, D. M., Liang, L., et al. (2010) Cancer-related epigenome changes associated with reprogramming to induced pluripotent stem cells, *Cancer Res.*, **70**, 7662-7673, doi: 10.1158/0008-5472.CAN-10-1361.
100. Planello, A. C., Ji, J., Sharma, V., Singhania, R., Mbabaali, F., et al. (2014) Aberrant DNA methylation reprogramming during induced pluripotent stem cell generation is dependent on the choice of reprogramming factors, *Cell Regen.*, **3**, 4, doi: 10.1186/2045-9769-3-4.
101. Weissbein, U., Plotnik, O., Vershkov, D., and Benvenisty, N. (2017) Culture-induced recurrent epigenetic aberrations in human pluripotent stem cells, *PLoS Genet.*, **13**, e1006979, doi: 10.1371/journal.pgen.1006979.
102. Ruiz, S., Diep, D., Gore, A., Panopoulos, A. D., Montserrat, N., et al. (2012) Identification of a specific reprogramming-associated epigenetic signature in human induced pluripotent stem cells, *Proc. Natl. Acad. Sci. USA*, **109**, 16196-16201, doi: 10.1073/pnas.1202352109.
103. Chin, M. H., Mason, M. J., Xie, W., Volinia, S., Singer, M., et al. (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures, *Cell Stem Cell*, **5**, 111-123, doi: 10.1016/j.stem.2009.06.008.
104. Poetsch, M. S., Strano, A., and Guan, K. (2022) Human induced pluripotent stem cells: from cell origin, genomic stability, and epigenetic memory to translational medicine, *Stem Cells*, **40**, 546-555, doi: 10.1093/stmcls/sxac020.
105. Buckberry, S., Liu, X., Poppe, D., Tan, J. P., Sun, G., et al. (2023) Transient naive reprogramming corrects hiPS cells functionally and epigenetically, *Nature*, **620**, 863-872, doi: 10.1038/s41586-023-06424-7.
106. Wang, X., Qin, J., Zhao, R. C., and Zenke, M. (2014) Reduced immunogenicity of induced pluripotent stem cells derived from Sertoli cells, *PLoS One*, **9**, e106110, doi: 10.1371/journal.pone.0106110.
107. Liu, P., Chen, S., Li, X., Qin, L., Huang, K., et al. (2013) Low immunogenicity of neural progenitor cells differentiated from induced pluripotent stem cells derived from less immunogenic somatic cells, *PLoS One*, **8**, e69617, doi: 10.1371/journal.pone.0069617.
108. Pegram, H. J., Andrews, D. M., Smyth, M. J., Darcy, P. K., and Kershaw, M. H. (2011) Activating and inhibitory receptors of natural killer cells, *Immunol. Cell Biol.*, **89**, 216-224, doi: 10.1038/icb.2010.78.
109. Netter, P., Anft, M., and Watzl, C. (2017) Termination of the activating NK Cell immunological synapse is an active and regulated process, *J. Immunol.*, **199**, 2528-2535, doi: 10.4049/jimmunol.1700394.
110. Simpson, A. J., Caballero, O. L., Jungbluth, A., Chen, Y. T., and Old, L. J. (2005) Cancer/testis antigens, gametogenesis and cancer, *Nat. Rev. Cancer*, **5**, 615-625, doi: 10.1038/nrc1669.
111. Hong, Y., Zhao, Y., Li, H., Yang, Y., Chen, M., et al. (2023) Engineering the maturation of stem cell-derived cardiomyocytes, *Front. Bioeng. Biotechnol.*, **11**, 1155052, doi: 10.3389/fbioe.2023.1155052.
112. Tricot, T., Verfaillie, C. M., and Kumar, M. (2022) Current status and challenges of human induced pluripotent stem cell-derived liver models in drug discovery, *Cells*, **11**, 442, doi: 10.3390/cells11030442.
113. Diane, A., Mohammed, L. I., and Al-Siddiqi, H. H. (2023) Islets in the body are never flat: transitioning from two-dimensional (2D) monolayer culture to three-dimensional (3D) spheroid for better efficiency in the generation of functional hPSC-derived pancreatic β cells *in vitro*, *Cell Commun. Signal.*, **21**, 151, doi: 10.1186/s12964-023-01171-8.
114. Ma, H., Folmes, C. D., Wu, J., Morey, R., Mora-Castilla, S., et al. (2015) Metabolic rescue in pluripotent cells from patients with mtDNA disease, *Nature*, **524**, 234-238, doi: 10.1038/nature14546.
115. Cacchiarelli, D., Trapnell, C., Ziller, M. J., Soumillon, M., Cesana, M., et al. (2015) Integrative analyses of human reprogramming reveal dynamic nature of induced pluripotency, *Cell*, **162**, 412-424, doi: 10.1016/j.cell.2015.06.016.
116. Choi, J., Lee, S., Mallard, W., Clement, K., Tagliazucchi, G. M., et al. (2015) A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs, *Nat. Biotechnol.*, **33**, 1173-1181, doi: 10.1038/nbt.3388.
117. Idelson, M., Alper, R., Obolensky, A., Yachimovich-Cohen, N., Rachmilewitz, J., et al. (2018) Immunological properties of human embryonic stem cell-derived retinal pigment epithelial cells, *Stem Cell Rep.*, **11**, 681-695, doi: 10.1016/j.stemcr.2018.07.009.
118. Yamasaki, S., Sugita, S., Horiuchi, M., Masuda, T., Fujii, S., et al. (2021) Low immunogenicity and immunosuppressive properties of human ESC- and iPSC-derived retinas, *Stem Cell Rep.*, **16**, 851-867, doi: 10.1016/j.stemcr.2021.02.021.
119. Edo, A., Sugita, S., Futatsugi, Y., Sho, J., Onishi, A., et al. (2020) Capacity of retinal ganglion cells derived from human induced pluripotent stem cells to

suppress T-cells, *Int. J. Mol. Sci.*, **21**, 7831, doi: 10.3390/ijms21217831.

120. Liu, J., Götherström, C., Forsberg, M., Samuelsson, E. B., Wu, J., et al. (2013) Human neural stem/progenitor cells derived from embryonic stem cells and fetal nervous system present differences in immunogenicity and immunomodulatory potentials *in vitro*, *Stem Cell Res.*, **10**, 325-337, doi: 10.1016/j.scr.2013.01.001.
121. Itakura, G., Ozaki, M., Nagoshi, N., Kawabata, S., Nishiyama, Y., et al. (2017) Low immunogenicity of mouse induced pluripotent stem cell-derived neural stem/progenitor cells, *Sci. Rep.*, **7**, 12996, doi: 10.1038/s41598-017-13522-w.
122. Ozaki, M., Iwanami, A., Nagoshi, N., Kohyama, J., Itakura, G., et al. (2017) Evaluation of the immunogenicity of human iPS cell-derived neural stem/progenitor cells *in vitro*, *Stem Cell Res.*, **19**, 128-138, doi: 10.1016/j.scr.2017.01.007.
123. Fujii, S., Yoshida, S., Inagaki, E., Hatou, S., Tsubota, K., et al. (2019) Immunological properties of neural crest cells derived from human induced pluripotent stem cells, *Stem Cells Dev.*, **28**, 28-43, doi: 10.1089/scd.2018.0058.
124. Mehler, V. J., Burns, C. J., Stauss, H., Francis, R. J., and Moore, M. L. (2020) Human iPSC-derived neural crest stem cells exhibit low immunogenicity, *Mol. Ther. Methods Clin. Dev.*, **16**, 161-171, doi: 10.1016/j.omtm.2019.12.015.
125. Kimura, T., Yamashita, A., Ozono, K., and Tsumaki, N. (2016) Limited immunogenicity of human induced pluripotent stem cell-derived cartilages, *Tissue Eng. Part A*, **22**, 1367-1375, doi: 10.1089/ten.TEA.2016.0189.
126. Wong, K. G., Ryan, S. D., Ramnarine, K., Rosen, S. A., Mann, S. E., et al. (2017) CryoPause: a new method to immediately initiate experiments after cryopreservation of pluripotent stem cells, *Stem Cell Rep.*, **9**, 355-365, doi: 10.1016/j.stemcr.2017.05.010.
127. Ji, J., Sharma, V., Qi, S., Guarch, M. E., Zhao, P., et al. (2014) Antioxidant supplementation reduces genomic aberrations in human induced pluripotent stem cells, *Stem Cell Rep.*, **2**, 44-51, doi: 10.1016/j.stemcr.2013.11.004.
128. Martin, M. J., Muotri, A., Gage, F., and Varki, A. (2005) Human embryonic stem cells express an immunogenic nonhuman sialic acid, *Nat. Med.*, **11**, 228-232, doi: 10.1038/nm1181.
129. Tangvoranuntakul, P., Gagneux, P., Diaz, S., Bardor, M., Varki, N., et al. (2003) Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid, *Proc. Natl. Acad. Sci. USA*, **100**, 12045-12050, doi: 10.1073/pnas.2131556100.
130. Rodríguez-Pizà, I., Richaud-Patin, Y., Vassena, R., González, F., Barrero, M. J., et al. (2010) Reprogramming of human fibroblasts to induced pluripotent stem cells under xeno-free conditions, *Stem Cells*, **28**, 36-44, doi: 10.1002/stem.248.
131. Swistowski, A., Peng, J., Liu, Q., Mali, P., Rao, M. S., et al. (2010) Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions, *Stem Cells*, **28**, 1893-1904, doi: 10.1002/stem.499.
132. Chung, T. L., Turner, J. P., Thaker, N. Y., Kolle, G., Cooper-White, J. J., et al. (2010) Ascorbate promotes epigenetic activation of CD30 in human embryonic stem cells, *Stem Cells*, **28**, 1782-1793, doi: 10.1002/stem.500.
133. Lagarkova, M. A., Volchkov, P. Y., Philonenko, E. S., Pfannkuche, K., Prokhorovich, M. A., et al. (2008) CD 30 is a marker of undifferentiated human embryonic stem cells rather than a biomarker of transformed hESCs, *Cell Cycle*, **7**, 3610-3612, doi: 10.4161/cc.7.22.6981.
134. Kaneko, S., and Yamanaka, S. (2013) To be immunogenic, or not to be: that's the iPSC question, *Cell Stem Cell*, **12**, 385-386, doi: 10.1016/j.stem.2013.03.008.
135. Mandai, M., Watanabe, A., Kurimoto, Y., Hirami, Y., Morinaga, C., et al. (2017) Autologous induced stem-cell-derived retinal cells for macular degeneration, *N. Engl. J. Med.*, **376**, 1038-1046, doi: 10.1056/NEJMoa1608368.
136. Takagi, S., Mandai, M., Gocho, K., Hirami, Y., Yamamoto, M., et al. (2019) Evaluation of transplanted autologous induced pluripotent stem cell-derived retinal pigment epithelium in exudative age-related macular degeneration, *Ophthalmol. Retina*, **3**, 850-859, doi: 10.1016/j.oret.2019.04.021.
137. Schweitzer, J. S., Song, B., Herrington, T. M., Park, T. Y., Lee, N., et al. (2020) Personalized iPSC-derived dopamine progenitor cells for Parkinson's disease, *N. Engl. J. Med.*, **382**, 1926-1932, doi: 10.1056/NEJMoa1915872.
138. Sugimoto, N., Kanda, J., Nakamura, S., Kitano, T., Hishizawa, M., et al. (2022) iPLAT1: the first-in-human clinical trial of iPSC-derived platelets as a phase 1 autologous transfusion study, *Blood*, **140**, 2398-2402, doi: 10.1182/blood.2022017296.
139. Hu, X., White, K., Olroyd, A. G., DeJesus, R., Dominguez, A. A., et al. (2023) Hypoimmune induced pluripotent stem cells survive long term in fully immunocompetent, allogeneic rhesus macaques, *Nat. Biotechnol.*, doi: 10.1038/s41587-023-01784-x.
140. Deuse, T., Hu, X., Agbor-Enoh, S., Jang, M. K., Alawi, M., et al. (2021) The SIRPα-CD47 immune checkpoint in NK cells, *J. Exp. Med.*, **218**, e20200839, doi: 10.1084/jem.20200839.
141. Harding, J., Vintersten-Nagy, K., Yang, H., Tang, J. K., Shutova, M., et al. (2023) Immune-privileged tissues formed from immunologically cloaked mouse embryonic stem cells survive long term in allogeneic hosts, *Nat. Biomed. Eng.*, doi: 10.1038/s41551-023-01133-y.
142. Viacyte. CRISPR Therapeutics and ViaCyte, Inc. announce first patient dosed in phase 1 clinical trial of novel gene-edited cell replacement therapy for treatment of type 1 diabetes (T1D). February 2, 2022,

URL: https://crisprtx.com/about-us/press-releases-and-presentations/crispr-therapeutics-and-viacyte-inc-announce-first-patient-dosed-in-phase-1-clinical-trial-of-novel-gene-edited-cell-replacement-therapy-for-treatment-of-type-1-diabetes-t1d.

143. González, B. J., Creusot, R. J., Sykes, M., and Egli, D. (2020) How safe are universal pluripotent stem cells? *Cell Stem Cell*, **26**, 307-308, doi: 10.1016/j.stem.2020.02.006.
144. Harding, J., Vintersten-Nagy, K., and Nagy, A. (2020) Universal stem cells: making the unsafe safe, *Cell Stem Cell*, **27**, 198-199, doi: 10.1016/j.stem.2020.07.004.
145. Zimmer, J., Andrès, E., Donato, L., Hanau, D., Hentges, F., and de la Salle, H. (2005) Clinical and immunological aspects of HLA class I deficiency, *QJM*, **98**, 719-727, doi: 10.1093/qjmed/hci112.
146. Hara, A., Aoki, H., Taguchi, A., Niwa, M., Yamada, Y., Kunisada, T., and Mori, H. (2008) Neuron-like differentiation and selective ablation of undifferentiated embryonic stem cells containing suicide gene with Oct-4 promoter, *Stem Cells Dev.*, **17**, 619-627, doi: 10.1089/scd.2007.0235.
147. Liang, Q., Monetti, C., Shutova, M. V., Neely, E. J., Hacibekiroglu, S., et al. (2018) Linking a cell-division gene and a suicide gene to define and improve cell therapy safety, *Nature*, **563**, 701-704, doi: 10.1038/s41586-018-0733-7.
148. Wu, Y., Chang, T., Long, Y., Huang, H., Kandeel, F., and Yee, J. K. (2019) Using gene editing to establish a safeguard system for pluripotent stem-cell-based therapies, *iScience*, **22**, 409-422, doi: 10.1016/j.isci.2019.11.038.
149. Viacyte. CRISPR Therapeutics and ViaCyte present positive in vitro data towards a potential immune-evasive cell replacement therapy for diabetes at EASD 2019. September 17, 2019, URL: https://crisprtx.com/about-us/press-releases-and-presentations/crispr-therapeutics-and-via-cyte-present-positive-in-vitro-data-towards-a-potential-immune-evasive-cell-replacement-therapy-for-diabetes-at-easd-2019.