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Hypothesis

Ethical Aspects of Human Induced Pluripotent Stem Cells and Alzheimer's Disease: Potentials and Challenges of a Seemingly Harmless Method

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Abstract. Dementia currently affects more than 55 million people worldwide, and scientists predict that this number will continue to rise. The most common form is Alzheimer's disease (AD), which is triggered, among other things, by dysfunctional cells in the human brain. Stem cell research attempts to counteract neurodegenerative processes, for example by replacing or treating diseased cells. In addition to human embryonic stem cells, since the successes of Takahashi and Yamanaka in 2006, there has been an increased focus on human induced pluripotent stem cells (hiPS cells). These cells avoid ethically challenging questions about the moral status of human embryos, but there are numerous problems, such as high production costs, side effects from the reprogramming process, or a potentially new moral status. These ethical issues will be examined primarily in relation to AD. The first part will be a discussion of hiPS cells and their importance for stem cell research, after which the focus turns to AD. Based on scientific studies, the relationship between hiPS cells and AD will be outlined as well as ethical implications presented. While potential limitations of hiPS cells have been discussed by numerous authors, an ethical perspective on the link between hiPS cells and AD seems to be neglected in the scientific community. The following risk analysis aims to identify a possible research agenda. In conclusion, the focus on individuals with AD may help to adopt an ethical stance that recognizes existing limitations and constructively engages with the possibilities of research.

Keywords: Alzheimer's disease, ethics, human induced pluripotent stem cells, moral issues, stem cells

INTRODUCTION

Human embryonic stem cells (hESCs) represent the absolute gold standard within research; however, ethical concerns have been raised about using these cells because the research involves destroying human embryos. In contrast, human induced pluripotent stem cells (hiPS cells) are derived from human cells in the human body (e.g., muscle, skin, blood, brain), thus avoiding the use of cells that could potentially become humans. There has been great hope in the use of hiPS cells—at least since the successes of Takahashi and Yamanaka in 2006 with murine

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iPS cells [1]. Currently, various transcription factors are required for the production of hiPS cells [2]. In addition to the unlimited possibility of differentiation, hiPS cells have an infinite self-renewal potential, which allows the formation of any cell in the human body [3]. Apart from promising fields of application, there are specific ethical concerns with these cells which will be discussed in this paper [4]. In order to assess these opportunities and challenges within biomedical research, the focus will be put on Alzheimer's disease (AD). This disease continuously leads to a reduction of self-determined living, for which biochemical mechanisms in the brain are partly responsible. These include, for example, the unwanted accumulation of amyloid-β (Aβ) and neurofibrillary tangles [5]. In this context, stem cell research is not only trying to better understand these processes, but also, for example, to replace malfunctioning or defective neurons and glial cells. hiPS cells offer an excellent opportunity for replacing hESCs because they can develop into these cells under suitable laboratory conditions [6]. Nevertheless, it seems necessary to take a closer look at these seemingly harmless cells and evaluate their potential for the *treatment* of AD. For this purpose, scientific articles and studies will be examined and an ethical evaluation process will identify which issues are crucial in the connection between hiPS cells and AD. While potential limitations of hiPS cells have been discussed by numerous authors, an ethical perspective on the link between hiPS cells and AD seems to be neglected by the scientific community [7]. From an ethical point of view, it is important to focus on the person with AD and to raise awareness of which scientific procedures cannot be justified and which are warranted.

THE POTENTIAL OF HIPS CELLS

Pluripotent stem cells are capable of differentiating into any cells of the human body. What, until a few years ago, could only be achieved exclusively by human embryonic stem cells can now also be implemented for adult stem cells after the successes of Takahashi and Yamanaka (mouse model [8]) in 2006. In the following years, the results of iPS cells were also confirmed in humans, which was achieved by different research groups, for instance Takahashi et al. (The results highlight the similarity of hiPS cells and hESCs. In addition, the formation of teratomas *in vivo* consisting of all three germ-layer-cells was experimentally demonstrated [9]) and Park et al. (Within the experiments, different transcription factors for the establishment of the pluripotent state were investigated. The relevant components seem to be Oct4 and Sox2. Although the protein c-Myc increases the efficiency of reprogramming, it could be avoided, mainly because it probably promotes the formation of tumors [10])). Currently, new cells can be produced from cells in the human body (muscle, skin, blood, brain), by suitable transcription factors in the laboratory, which are called hiPS cells [2]. Such cells are pluripotent, are in an undifferentiated state *in vitro* and are similar to hESCs in terms of self-renewal and differentiation potential [8].

The production of hiPS cells is based on the extraction of adult cells (e.g., skin, adipose tissue), which are then placed in a nutrient medium and their gene activity is influenced with the help of proteins. In the initial production of iPS cells, four transcription factors (Oct4, Sox2, Klf4, and c-Myc) were described as essential in achieving the pluripotent state and the transport systems were retroviruses [8, 11]. Through the influence of the transcription factors, certain genes are transcribed and activated respectively, resulting in a specific gene expression profile [12]. The combination of these factors is decisive for the subsequent status of the cell. For this purpose, the originally multipotent state of adult stem cells can be transformed into a pluripotent one. This is also accompanied by the possibility that already differentiated cells, which can only develop into certain cell types, take on an original unbiased state [13]. For example, neural stem cells can develop into neurons or glial cells, but not into cells of the blood system. However, it is precisely this restriction that is removed in the process of hiPS cell generation, which highlights its significance for stem cell research.

The transport systems used are viral vectors, electroporation, lipofection or, more recently, proteins, DNA and RNA transport systems [3, 14, 15]. In the reprogramming process, the cellular status is reverted to an undifferentiated state similar to that of hESCs. Unwanted side effects are possible, such as incomplete re-differentiation (molecular cell memory), contamination or mutation of the cells, or a short lifespan of the cells [1, 16]. The process by which the conversion from multipotent to pluripotent takes place, is currently by no means fully explainable. This means that the scientifically necessary sub-steps are not fully understood and certain influencing factors cannot be controlled [17]. This also implies that the associated risks cannot be fully predicted. The research-related goals with hiPS cells are, for exam-

ple, the testing of drugs *in vitro*, disease modeling or future cell replacement therapies [4].

In the initial generation of iPS cells in the mouse model, from adult/embryonic murine stem cells, Takahashi and Yamanaka identified the transcription factors Oct4, Sox2, c-Myc, and Klf4 as crucial [8]. Depending on the type of cell to be programmed, a different dosage and combination of transcription factors is needed, which contribute to a specific gene expression [3]. After that, specific markers for the pluripotent state are recognizable, such as Nanog or Oct4 [18]. With hiPS cells, the question of the moral status of human embryos becomes less important, because no embryo has to be killed to obtain these cells [1]. Another advantage of hiPS cells is the fact that they are the patient's own autologous cells, which allow a person-specific therapy. (Autologous in this context describes stem cells that come from the patient himself or herself. There is a genetic match between the cells generated in this way and the cells in the organism [19, 20]). In particular, treatment with immunosuppressors against defense reactions—which would be the case through the introduction of hESCs—of the patient's own body, can be avoided by these cells [14]. Drugs are also tested *in vitro* on personalized hiPS cells to determine their efficacy before actual application in humans. There are many preclinical studies with hiPS cells and their application in humans, but a widespread clinical practice and also a socially accepted method will require further studies and several years of research [21].

In this paper, the relevance of hiPS is mainly focused on their applicability and their general importance for the treatment of AD. In this disease, cellular and molecular—in addition to neurophysiological, there are genetic and epigenetic influences—processes in the human brain lead to a loss of neurons and thus also of cognitive performance [22]. Therefore, it seems obvious to use these cells for an understanding of neuronal processes, the explicit production of neurons or the adaptation of damaged cells. However, the integration of new cells into an existing environment (molecular niche) is difficult, which is particularly evident in the central nervous system due to its complexity [2, 14]. The extent to which such procedures can also be implemented in practice and which ethical aspects are associated with them must be considered [23].

For example, questions of information and consent of donors or the participating persons of an experiment have to be analyzed [20]. Information should not be limited to hopeful prospects but must equally consider the dangers for individuals and society. Also, with regard to considerations of justice theory, it must be determined who can afford these therapies, because they are often associated with a high financial effort. Relatives and perhaps only marginally affected people, whose interests may very well be affected, must be considered in all projects [24]. This includes family caregivers of people with AD, for example, when their lives are drastically changed by the care situation. Very often they are responsible for doctor's appointments, have to clarify legal matters, procure medications or organize everyday life. Of particular importance in this paper is their potential involvement in determining the will of persons with AD when their wishes are difficult to ascertain at an advanced stage of the disease. They then also have the responsibility to make the best possible (research) decision for the person affected, which is why their concerns must be taken into account [25]. In addition, from an ethical point of view, the aspects of autonomy and heteronomy are essential, because the possibility of a therapeutic intervention can also turn into a self-perceived necessity [26]. Due to the progression of the disease, it is quite conceivable that certain interventions with hiPS cells, which are currently still fraught with medical uncertainties, will be considered. Especially because there is currently no treatment for AD available, the person concerned might be tempted to exhaust all technical options, even if long-term consequences or risks cannot be completely determined. As a result, an additional option could also be understood as a necessity. Similarly, medical professionals or family members might recommend the available therapy, contributing to a heteronomy that at least limits autonomous decision-making, which would not be the case without the therapeutic intervention opportunity [27]. In order to reveal these ethical challenges, studies and experimental designs will be discussed in the following sections. This aims to show which procedures should be avoided within the research landscape, because no justification can be given from an ethical point of view.

ALZHEIMER'S DISEASE AND HIPS CELLS

This section addresses selected studies, research setups, and scientific experiments, regarding the link between AD and a hiPS cell approach. Due to the vari-

ety of possible forms of dementia (e.g., dementia with Lewy bodies, frontotemporal dementia, vascular disease [28, 29]), this paper narrows down to AD. Two forms can be distinguished: familial AD and sporadic AD. While the first variant can be traced back to inherited genetic causes, this is not possible with sporadic AD. However, it accounts for most cases (99%). If the aspects presented in the following do not apply to both variants, this is pointed out [18]. AD is the most common form of dementia and can be linked to hiPS in a special way. Numerous pathological findings suggest the relevance of Aβ and tau protein [7, 30]. On the one hand, extracellular plaques occur, disrupting the connection between different neurons, and on the other hand, there is an unwanted accumulation of neurofibrillary tangles within the cellular (neuronal cytoplasm [31]) structure. During this process, the number of neurons is reduced, synaptic connections are lost, and the activity of microglia increases within the human brain [5, 16].

Although the causes of AD are still unclear, according to Arber et al. there are indications that a mutation in the AβPP (amyloid-β protein precursor [32]) and the alteration of the Aβ occurrence are decisive [5]. From the previous presentations, a connection between AD and hiPS cells is apparent because neuronal processes, which start at the level of a single cell, can be better understood and possibly influenced with these versatile cells [2]. Moreover, it is precisely with these specific cells that one can generate a cell (or cell clusters [5]) corresponding to the respective human being without having to rely on sometimes costly and often impossible extractions of brain tissue [4, 33]. Also, the *in vivo* modeling of the described pathological processes, for example in rodents, can hardly be realized without epistemological limitations [34].

According to the current state of science, it is possible to remove single cells (e.g., fibroblasts, neural stem cells) from a person with AD in order to determine genetic dispositions, cellular changes or mutations [2, 35]. Any existing genetic makeup of the cell will remain after the hiPS-conversion process, for example, to neurons [23, 30, 33]. Thereafter, adaptation with CRISPR-Cas 9 and subsequent integration into the living organism would be just as possible as testing drugs *in vitro* [20]. Consequently, either specific mechanisms can be analyzed *in vitro* under laboratory conditions or integration of the produced cells into the human body takes place. In both cases, genetic adaptation with the CRISPR method is possible. For this purpose, reference is made to exemplary studies [32, 36, 37] already performed, which dealt with hiPS-derived neurons. It became apparent that the treatment with γ-secretase inhibitors can reduce the amount of Aβ and the use of β-secretase inhibitors simultaneously leads to a reduction of the tau concentration in the brain [5, 7, 30].

The genetic background can be investigated by producing hiPS cells in the laboratory to identify individual genes for AD, for example [2]. This is also linked to genetically determined risk factors, and this knowledge can be used to take appropriate measures. In this regard, study [5, 18, 31, 32] results indicate a link between AβPP and the *APOE3/E4* (apolipoprotein [18]) allele. If these alleles (proteins) are activated within the human genome, this leads to an accumulation of AβPP, which in turn results in the unwanted production of Aβ [22, 30]. Since such procedures can be modeled *in vitro* with the help of hiPS cells, it is possible to avoid the already mentioned postmortem biopsies. From an ethical point of view, it eliminates questionable issues regarding consent or intervention-related risk [4].

The complex processes in the brain of a person suffering from AD can be reconstructed on individual cells, corresponding to the respective person. There is a predisposition for AD in the newly generated cells, which means diseases can be modeled individually. Despite the high similarity to disease-damaged cells, they are in an undifferentiated state [16]. According to Preman et al., this leads to a significant advantage over previous methods, which often required animal experiments [23, 34]. In addition, science is concerned with endogenous influences on these neuronal processes and synaptic connections, for which the undifferentiated cells serve as starting material. After the extraction of adult stem cells, transcription factors are used for the hiPS-transformation into neurons, glial cells, or neuronal progenitor cells [16, 30]. Scientific investigation *in vitro* allows to draw conclusions about brain processes in order to mimic these endogenous processes in a laboratory setting [34].

During AD, different cell types are impaired in their function or destroyed due to the disease. According to Arber et al., the processes leading to the accumulation of tau protein are predominantly found in the hippocampus and neocortex. In contrast, the plaques or Aβ accumulation occurs within the isocortex [5]. When considering different cell types, which are affected during the progression of AD, the importance of hiPS cells becomes clear. They are not restricted to a specific cell type in the same way as adult (multipotent) stem cells but offer a wide range of

possible applications [31]. Current research projects aim at the generation, adaptation, and further development of glutamatergic cortical neurons, which can be differentiated from hiPS cells [5, 38].

Stem cells can be transplanted as a single-layer cell patch or as a three-dimensional structure. The second variant refers to the organoids described briefly below, which are named this way due to their similarity to the corresponding human organ. While two-dimensional cell cultures promote the formation of pure and functional neurons—which were derived from hiPS cells—the number of cells is limited and there are restrictions in terms of transfer from *in vitro* to *in vivo* processes [4]. In contrast, three-dimensional cultures provide a more balanced interaction and development of the cells, which in turn favors a better applicability of the results. However, the correct use of 3D materials, such as polymers, hydrogels, or Matrigel matrices, presents challenges [5]. The matrix selected in each case is decisive for the genesis of the cells contained in it [7]. In comparison, three-dimensional neuronal organoids visualize the *in vivo* processes in a better way than comparable 3D tissue structures. In particular, the interaction of different cell types is crucial and the accumulations of Aβ and pTau (phosphorylated tau [18]) can be better understood [15, 18]. Limitations include a fetal structure, heterogeneous cell population, and limited growth of cells [1]. According to Arber et al., the current use of organoids for modeling neurodegenerative diseases is low but will most likely increase in the near future. They have "[. . .] the potential to be an insightful new model in AD research, allowing researchers to experiment with more heterogenous, naturally organized 3D cell models." [5, p.4]. In this context, brain organoids would be an ideal way to model disease progression for AD analysis [2, 32].

ETHICAL ISSUES WITH HIPS CELLS

So far, the possible applications of hiPS cells and their relevance for the treatment of AD have been presented. However, what is very often neglected are ethical reflections that arise from the use of these, at first sight, harmless cells. For this reason, various aspects will be highlighted in the following section in order to point out current limitations of research—there is no therapy that can cure AD so far [31]—and also to raise awareness of the ethical relevance [39]. For this purpose, the moral status, questions of justice, animal experiments, and possible limitations are discussed. And this is not only because these issues are addressed by numerous authors, but because they represent the most fundamental challenges of hiPS cells. In addition, this analysis lays the foundation for the subsequent assessments regarding individuals with AD.

Moral status

The moral status of human embryos could also gain new importance for hiPS cells if reprogramming up to totipotency (Totipotency is the ability of a cell to form all cell types of a given organism, to develop into a complete organism, and to differentiate into extraembryonic tissue [40]) and the status of a zygote is possible [6]. The result would be, for example, a totipotent cell, which would not be established within the natural fusion of egg and sperm, but of an artificial reprogramming of adult cells in the human body. Nevertheless, the same question would exist as for the use of hESCs, namely whether the resulting embryo can be used for research. Moreover, it is not clear whether it makes a significant difference that the embryo was created by an artificial production process. Here it is especially important to draw attention to this status problem and to stimulate further considerations. According to Sawai et al., a potential is decisive for the moral status, and the distinction between natural or artificial cannot render moral consideration obsolete. They distinguish between active and passive potential, whereby the latter form would be assigned to the hypothetical embryos *in vitro* [41]. This account seems plausible because, for example, we do not differentiate between children born naturally and those born with the help of *in vitro* fertilization (IVF) in terms of moral status, responsibilities towards them or their moral rights. Moreover, the potential production of the embryo from hiPS cells prevents the use of oocytes, which are currently still essential for IVF. Apart from this proposal, the SKIP arguments should not remain unmentioned, which advocate protection from the fertilized egg through different approaches. (Reference is made here to an extensive work on SKIP arguments, as well as recent approaches to the moral status of embryos [42–44]). Körtner also points to ethical issues as a result of new opportunities, particularly related to recent successes with iPS cells [45]. In this recent study, artificial embryos were created from iPS cells. The authors emphasize the importance of the results for tracing human devel-

opment or understanding diseases, but these were experiments with mice [46]. From an ethical point of view, not only the equivalent consequence seems to be of importance here, but above all the intention of researching people or institutions [47]. If this approach is aimed at the complete elimination of hESC-dependency, this can also be regarded as a meaningful way out of the *embryo dilemma*. However, if the aim is only to make a profit, to exploit the scope of what is technically possible or to gain prestige, an ethical justification does not seem realistic [48]. Thus, exclusively subjective objectives would be in the center of the motivation for action, without taking into account the interests of the potentially human being. From an ethical point of view, an egoistic motivation cannot be sufficient to justify an act [49]. What is needed here is the consideration of the embryo or at least of the beings who can benefit from the selection of stem cells and ultimately from research. Their interests must be considered equally and not exclusively the individual pursuit of personal goals.

Justice

The hiPS cell approach involves an enormous financial investment before the potential benefits of these cells become apparent. In this regard, Arber et al. point out that special organoids "[. . .] are expensive to purchase and require large amounts of costly media." [5, p.4]. Due to the high cost of producing patient-specific hiPS cells, and derived products, the question of how to prioritize financially potent individuals, institutions, and organizations in terms of equity will need to be addressed [20, 23]. A quite comprehensible proposal would be given by the Fair-Opportunity Rule of Beauchamp and Childress, which is based on the ideas of justice of John Rawls. According to this rule, medical measures should primarily benefit those persons who have come into a bad situation due to misfortune or undeserved disadvantages [47]. For this purpose, prioritizing persons with AD in principle over mentally unimpaired persons would be justified from an ethical point of view, because their illness cannot be explained by self-infliction. In addition, they are in a worse situation due to the burden of the disease, possibly marked by suffering, which should be prevented. Although a healthy diet or sports activities can be considered preventive actions against dementia, the genetic influences already described also play an important role [50]. Nevertheless, the question remains unanswered to what extent a selection of individuals with AD, for example for a specific study, can be conducted according to just standards, mainly because of different and sometimes divergent notions of justice. It is not possible to provide a comprehensive account here, but it is important to note that individuals should not be selected for a study on the basis of financial resources or donors in the background. Such preferential treatment can in no way fulfill the requirement of nearly equal health care, in which all people, regardless of age, gender or, in this case, financial situation, have access to medical opportunities [51]. From an ethical point of view, it is important to refrain from actions and decisions that contradict these fundamental principles [52]. Besides that, it has to be considered that individual aspects such as the age of test subjects, the therapeutic prospects of success, sex and gender or even the stage of dementia are decisive for a scientific study and lead to unequal treatment that can be possibly justified.

Animal experiments

As a result of the use of starting material that is available in the adult organism, animal experiments no longer seem to play a role in the hiPS cell context. However, according to the current status, research still depends on these experiments with animals, because in most cases they are the precondition for an application in humans [1]. Such results and findings are still indispensable and provide a point of reference for hiPS cells. This is also evident within scientific contributions, when rodent models [5, 32] or animal models [23, 38] are briefly mentioned, without giving much attention to ethical aspects. A similar handling is revealed from the description of de Leeuw and Tackenberg, when it is often necessary to "[. . .] verify [. . .] data from animal studies [. . .]" [18, p.3] in order to gain knowledge and to predict possible applications in humans. The focus is mostly on scientific findings, the potential benefit for humans or commercial intentions. From an ethical point of view, we have the duty to avoid suffering and prevent harm to these living entities. Even if no moral right of animals can possibly be justified, it seems more than just allowed, but rather imperative, to prevent harmful actions [24]. When animal testing cannot be avoided, it is demanded from researchers that they take responsibility and direct their own activities not only to scientific, but also to ethical standards [52]. It should also be mentioned here that there are already numerous proposals for the ethi-

cally acceptable inclusion of animals in experimental setups. These include the three Rs, which involve reduction, replacement, and refinement, with respect to the use of non-human beings within research [53]. A current approach to animal handling in research settings is presented by Murray et al. [54] and the work of Ferrara et al. [55] demonstrates the importance of engaging different groups of people and disciplines in a discourse on animal welfare. Brink and Lewis expand the original approach to 12 Rs, which include, for example, responsibility and regulation [56]. Those works describe in general the importance that we have to sensitize ourselves to the potential suffering of animals and are ethically encouraged to refrain from any action that causes pain, especially but not only when there are suitable alternatives to look for.

Limitations

Depending on which transport systems are used for the transformation into a pluripotent state, side effects may occur. This includes the possible formation of tumors when retroviruses or lentiviruses are selected. Adenoviral vectors, on the other hand, would not promise complete safety with regard to permanent genetic adaptation, since certain genes are only activated in the genome for a short period of time [2, 16]. According to de Leeuw and Tackenberg, other techniques are currently also applicable, such as mRNA delivery systems, direct variants, or episomal vectors [3, 12, 14, 18]. Nevertheless, novel options reveal similar problems and may lead to long-term harm and suffering of recipients of these adapted cells. Above all, this involves providing information in a form that is easy to understand. What is needed is more intensive research, avoidance of risky procedures, and transparency for all people who may be affected [20]. Here, a balancing of benefits and costs becomes decisive, which, following Beauchamp and Childress, can be ethically justified if the good effects outweigh the negative effects [47]. For example, the possible formation of tumors by the retroviruses used would be justifiable if a desired state of health can only be established by permanent DNA modification, the persons concerned give their consent and alternative procedures are not possible. When reprogramming adult stem cells into hiPS cells, it often cannot be ruled out that certain characteristics of the already differentiated cells remain (molecular cell memory) or that these cells influence the constitution of the desired cell [1]. Especially when extracting cells which already show signatures of AD in terms of gene expression, those problems have to be considered [38]. This is illustrated by the results of Arber et al. insofar as hiPS-derived neurons were loaded with higher accumulations of Aβ and pTau [5]. However, differences exist with regard to the sporadic and familial forms of AD. According to de Leeuw and Tackenberg, a proof of pathological abnormalities is difficult to achieve, especially due to genetic diversity in the sporadic form [18]. However, exactly this variant (sporadic AD) is the most widespread within the society.

From an ethical perspective, researchers are encouraged to take this uncertainty into account and to use cells only when a sufficient level of safety can be offered [16]. Even if hiPS cells are considered as a hopeful alternative compared to hESCs, their equivalence is often pointed out [1, 3, 14]. Since, especially with reference to AD, no therapeutic treatments have been established by hESCs so far, it seems quite justified to claim similar things about the similar hiPS cells. Even as independently considered cells there are still tasks to be solved. The cells and tissues obtained are not sufficiently developed and seem comparable to fetal tissues. A general hiPS cell approach is not yet feasible through person-specific cell harvesting [18]. With that in mind, an *isogenic approach* is proposed by de Leeuw and Tackenberg to address this issue. (In this approach, hiPS cells are no longer compared with cells from healthy individuals, which leads to fundamental differences. Crucial is the use of, e.g., CRISPR-Cas 9 to cure sick hiPS cells or to make the cells of a healthy control subject sick. Thus, the comparison of both healthy or sick cells takes place [18]). This approach may also allow to push a general approach for the treatment of AD, because patient-specific conditions are reduced and pathological mechanisms of the disease become more recognizable in the model. Another challenge is the *in vitro* limited imitation of cell aging, resulting in higher disease susceptibility, which cells *in vivo* possess over time [5, 31]. These uncertainties highlight not only the research that still needs to be done but at the same time the limited information available to affected individuals [2]. When persons with AD provide their cells for research or therapeutical aims, these limitations must be addressed, even though this approach has tremendous potential. Otherwise, the requirement of informed consent could not be fulfilled, and there would probably be an intentional deception or even concealment of the truth, which is ethically unjustifiable [51, 57].

PERSONS WITH AD AND SPECIAL CONSIDERATIONS

With the presentation of promising options through hiPS cell research and their applicability for the *treatment* of AD, an understanding of its functionality and importance was represented. Alongside those positive implications, however, it is essential to address the potential challenges and complications in terms of an ethical evaluation [51]. The hurdles addressed are intended to raise awareness of the problems that still exist. Similar ethical concerns have already been discussed by a number of authors [58–60] who, however, focus on the specific mechanisms and applications of hiPS cells. An existing limitation appears to be the link between these cells, AD, and an ethical analysis, which is why this section has a special focus on it. What has not yet been mentioned in terms of imaginable difficulties is the role of people diagnosed with AD. However, they are the focus of attention, or at least they should be, when it comes to the usefulness and meaningfulness of hiPS cell research [30, 61]. Thus, as the cognitive performance of persons with AD is increasingly impaired as the disease progresses, and thus also their ability to understand and handle information, special consideration is required here [57]. The following section discusses the application of hiPS cells *in vivo*, a possible cell integration, an imaginable cell extraction, and an *in vitro* model system. Thus, the current application possibilities are outlined, and ethical challenges are revealed.

Using hiPS cells, it is possible to enable direct applicability *in vivo* by integrating them into an existing environment. This applies to the human brain, for example, when hiPS-derived neurons or glial cells can interact with pre-existing cells to help improve health status [32]. Duncan and Valenzuela rightly point out, however, that those cell interactions currently raise questions. In addition to the medical uncertainties about how exactly the existing cells react to new arrivals, what long-term consequences can be expected or whether only certain neuronal sections are treated, ethical questions arise [16]. In this context, it seems important to point out that the introduction of hiPS cells into the human brain of a person with AD should be understood as a permanent intervention. Especially the problem of irreversibility is a cause for concern from an ethical point of view, because many consequences of this cell-integration cannot be foreseen at the moment [62]. Huneman and Kaelin also address the concept of irreversibility in the context of death, emphasizing the importance of cells. The human body is to be regarded as a whole system, which, however, cannot be understood without the interaction of individual parts [63]. These considerations show that numerous processes are irreversible, unstoppable and belong to human nature. However, if this irreversibility is triggered by human intervention, which in this case would be the result of the integration of hiPS cells into the brain, it is a different risk than, for example, the use of drugs. Such substances can be metabolized by the human body and must be taken at regular intervals, making this danger reversible. The results of Pardridge and Agrawal et al. illustrate that drug treatments for AD are currently not very successful [64, 65]. With the *theoretical* integration of hiPS cells as a permanent solution, not only recurrent treatments seem obsolete, but rather the disease can be treated on a cellular level. Nevertheless, possible dangers, such as incorrect integration into the brain, disruption of other neuronal cells or undesired functionality cannot be ruled out. The danger is mainly increased by the fact that these cells then remain as a prerequisite, so to speak [48]. While with drugs one can observe whether they work or not and possibly simply stop the administration, this is not the same for the introduction of cells into the human brain. Moreover, the removal of these cells seems almost impossible since they are in a mixture of other neural cells. For these reasons, this particular danger will have to motivate ethical consideration before this step is even taken, because the consequences are permanent. Any new balancing of pros and cons of a specific AD treatment at later times, then refers to the possibly already negatively established basis of hiPS cells being in a person's brain. Thus, even a small number of new cells could lead to serious consequences. The side effects that may occur cannot be predicted, which makes it impossible to meet the requirements of safety and non-harm [66]. For this purpose, it is not only necessary to communicate such ambiguities accordingly, but rather to prevent any research projects that expose persons with AD to potential harm [47]. This is especially true when there are too many uncertainties and because such interventions can no longer be reversed.

If hiPS cells are to be *introduced* into the human brain, clinical studies will be required beforehand. These studies must prove the general safety and functionality of the cells. Within clinical trials, the general safety is usually tested in a small group of subjects. Afterwards, the dose and side effects are determined in a larger number of participating indi-

viduals. Only then do large-scale studies with a more extensive number of subjects follow to confirm efficacy. While in an experimental group, individuals receive an active ingredient or the treatment, this is not always the case in a control group [67]. Apart from clinical requirements, what seems particularly important is the potential risk for people with AD. For example, if the integration of hiPS cells into the brain is planned and a clinical study with experimental and control groups is necessary, several risks are imaginable [31]. These include, for example, the long-term consequences described above, side effects due to the procedure, a missing effect of the new cells, a general risk due to the procedure, psychological or physiological burdens, or immune reactions of the body [68, 69]. Such scenarios often involve surgeries that inherently involve a certain level of risk. However, if individuals with AD are included in such trials and subsequently become part of the control group, it is conceivable that a surgical procedure could be performed without even delivering hiPS cells. In such cases, a cost-benefit analysis is essential [52]. On the one hand, there is health, which is presented by Marckmann, for example, as a basic prerequisite for the realization of life chances [70]. However, the purely theoretical realization seems problematic, due to the currently still unclear functionality of hiPS cells in the human brain. On the other hand, there are numerous dangers such as the general surgical risk, the possible formation of tumors, a quite realistic ineffectiveness of the therapeutic intervention or long-term side effects [2]. While the considerations on the side of the individual seem to shift quite clearly to the negative side, it could at least be argued that future patients with AD could also benefit from these results. Nevertheless, mainly due to current limitations, with regard to long-term effects of the cell integration, of hiPS cells, the risks outweigh the benefits. De Leeuw and Tackenberg therefore also encourage further experiments and warn against exaggerated hopes [18]. For this purpose, it would be at least theoretically conceivable to announce the operation to the test persons (in the control group), but not to actually perform it. Whether this can be done according to scientific standards and ethical considerations for clinical studies and raise fewer concerns is another question. In any case, there can be no general solution here, as rather the person- and situation-related circumstances must be analyzed. The acceptance of such risks does not seem reasonable according to the current state of knowledge and cannot be justified in the course of an ethical analysis, only to promote a potential well-being or to possibly preserve the good of health [48].

In order to understand disease-relevant mechanisms or to test drugs, cells are often *extracted* from the human body to generate the described hiPS cells [5]. In AD, however, this extraction becomes much more complicated because the source is the human brain. In this way, cellular and molecular structures for this disease, could be modeled *in vitro* from directly affected brain cells [5]. Nevertheless, it is also necessary to look at the people concerned. For cognitively healthy individuals, this requires informed consent, which, however, comes with additional obstacles for individuals with AD. Hall, Prochazka, and Fink declare three essential components of informed consent for the clinical domain: comprehension, use of information, and autonomy [71]. (In recent years, there have been many proposals as to how exactly informed consent can be understood. According to most views, the aim is to support the person concerned in forming his or her own will and to respect this. Nevertheless, numerous problems of implementation arise in practice, for example when information is too general or a paternalistic approach undermines autonomous decision-making. The following works are suggested for further study [72, 73]). Comparable requirements are presented at least implicitly by Bazzano, Durant, and Brantley: comprehension, Key Information, and Freedom [74]. Those views are comparable to numerous proposals by other authors. Nevertheless, it seems difficult to apply such demands to persons with AD when the sequence of informing, understanding, and deciding is involved. A healthcare professional may be able to communicate information in the best possible way, but this by no means ensures the informedness of a person with AD. The requirement of sufficient comprehensibility or general understanding seems to be similarly difficult, since those people are increasingly limited in their cognitive capacity in the course of the disease. Decision-making can and very often is understood as being free from external and internal influences [75]. Even if people in the social environment are not tempted to paternalism, it cannot be denied that inner influences, caused by the disease, make it difficult to form a free will on the basis of information and one's own considerations [76]. On the one hand, persons with AD do not seem to be able to meet the requirements of informed consent, especially in later phases of the disease, and on the other hand, meeting the criteria would lead to an avoidable excessive demand [77]. This avoid-

ance seems to be necessary from an ethical point of view, because it concerns a risky intervention which, actually, is not directly relevant for the person concerned. Through the analysis and an understanding of the cells obtained, it is predominantly people who will suffer from AD in the future who can benefit. In addition, it is conceivable that relatives could exert pressure or believe that they know what they consider to be the presumed will [76]. Such procedures can in no way be understood in the sense of dignified and just treatment, in which all human beings are to be taken into account, regardless of cognitive limitations in this case [47]. A Possible solution would be the use of advance directives [47] or also surrogates, which establish the will of a person suffering from AD at an early stage or shortly after diagnosis, even if these necessary criteria for an informed consent can no longer be fulfilled when the disease progresses.

For the production of patient-specific tissue or even three-dimensional brain organoids, cells are required which can be of human or animal origin. Due to the fact that the discussed animal models will not disappear from the biomedical context in the foreseeable future, animal products are also the starting point in this consideration [23]. Here, the described three-dimensional cell assemblies or two-dimensional structures could be used to integrate them into the brain of a person with AD [32]. In addition to medically relevant questions of immune compatibility, possible tumor formation or scientifically confirmed differences between humans and animals, ethical questions open up [34, 78]. These concern, for example, the general image of the human being, should animal cells be introduced into a brain. If the new cells are iPS cells, this can be considered chimeric tissue, since it involves the mixing of several species. Chimeras result from the combination of different zygotes. A mixing of several species occurs, whereby the corresponding cells originate from at least two genomes that can be distinguished from each other. These cells are then simultaneously present in a new organism [79]. This would be the case, for example, if iPS-derived brain cells from a mouse were introduced into the brain of a human with AD. Apart from the possible suffering of animals, which has already been mentioned, the question may be asked whether the image of humankind could change as a result of the introduction of such cells. Kreß also points out that we often associate the brain with aspects of personality, freedom, or morality. Through personal reflections, thoughts, and desires, we give expression to our personality [52]. This fundamental freedom can be traced back to the fact that subjective considerations are not influenced in any (unwanted) way [29]. Whether this would be the case by integrating iPS cells from a foreign organism, which subsequently interact with already existing brain cells, seems not easy to answer, but should at least be discussed. If we see ourselves superior to animals through the described capacities such as reason, or conscience, the integration of animal cells could at least challenge this notion. Polgar, Müller, and Morciniec rightly point out that moral decisions are not exclusively based on causal processes in the brain [80]. The same can be said of rational considerations, which are not to be understood only biologically determined. Nevertheless, brain research currently seems unable to clarify many questions that are relevant from an ethical point of view. Should chimeric tissue be established, the description of a human being can be criticized because non-human components are also present in the brain. But does this also change the image of humankind? Would we be more inclined to tolerate such an approach in individuals with AD? If cognitive abilities are progressively diminished by the disease, is it easier to justify an intervention that could affect cognitive performance in particular? Kitwood rightly emphasizes that people with dementia have equal value, needs, and rights [29]. The mindless inclusion of individuals with AD to such experimental settings, (maybe) due to cognitive limitations, should be understood as a discrimination. Such actions are not only morally wrong, but can in no way be justified from an ethical perspective [47]. There is a comparable discussion in the case of xenotransplantation, insofar as these animal organs could lead to a different conception of what it means to be human. Johnson points to the importance of xenotransplantation if this procedure could be used as a reaction to the organ-shortage [78]. Kögel and Marckmann, on the other hand, suggest that the use of animal organs in the human body might not change our conception of humanity, but it might influence the human-animal relationship [69]. Entwistle, Sade, and Drake also focus on the ethical issues surrounding these biomedical interventions, such as animal welfare, assessment of risks, access, financial requirements, or regulatory oversight institutions [81]. In both xenotransplantation and chimeric tissue formation, it is important to keep in mind not only dangers to humans but also to animals. The previous accounts seem to make it clear that humans are not considered persons only because of individual cells and their functioning, but because of specific

capacities, reason, or even moral agency. Following Burger, it is not only our brain that decides, thinks and feels, but the entire human being [82].

CONCLUSION

The above considerations should illustrate the complexity of the difficulties that arise from an ill-considered use of hiPS cells in general. The listed aspects of possible side effects due to reprogramming techniques, limitations due to the transport systems used, enormous costs for patient specific hiPS-cells or limited medical knowledge, need to be communicated in an environment of trust [2]. However, as mentioned before, this provision of information comes with additional barriers for people with AD. A scientific explanation of how exactly the CRISPR method enables genetic modifications, or why adenoviruses are used instead of retroviruses is likely to push many non-professionals to the limits of their ability to comprehend these vast amounts of information [3, 13]. However, if the persons addressed here are confronted with increasing deficits with regard to their own cognitive, mental and verbal abilities because of the disease, it becomes much more difficult to deal with the given information [31, 57]. A sensitive approach is needed for patients who are considered to have special needs due to their exceptional situation [39]. If they are considered for an experiment, if their stem cells are analyzed *in vitro*, or if they must agree to the collection of adult stem cells in general, a conventional explanation is not sufficient [52]. An ethically oriented approach must focus on the human being in this specific life situation. If there is no adequate understanding of the risks and dangers during the explanation or the person cannot follow the general intentions, any procedures which harm or are likely to harm this person are prohibited [47]. It is also important that people with this disease, according to the current state of biomedical science, cannot directly benefit from the application possibilities of hiPS cells [33]. The results of *in vitro* investigations, the understanding of disease-related mechanisms or potential cell replacement therapies, are primarily aimed at future patients. It is clear from these considerations that the potentials of hiPS cells should not obscure the work that remains to be done, for all people involved in the research process. The focus on persons with AD highlights additional challenges that should be addressed from an ethical perspective [29]. Apart from these limitations and difficulties, refusing research projects involving people with AD is not a proper or ethically required solution [26]. These individuals themselves can also benefit, at least in part, from research findings or recognize something important in them for other affected persons. In this context, Kreß refers to advance directives, which are used to determine a person´s wishes or preferences even before cognitive impairment occurs [24]. This may be a possible strategy, but ultimately, it requires an ethical analysis of the respective research project, followed by an evaluation of the pros and cons. What has to be integrated as a guideline into all actions and decisions is an open, sensitive attitude containing ethical points of reference, which is oriented towards the individual needs of the persons addressed [61].

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CONFLICT OF INTEREST

The author has no conflict of interest to report.

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