Review

Junmo Wu, Yuxi Shi, Shanshan Yang, Zengli Tang, Zifan Li, Zhuoyao Li, Jiawei Zuo, Weizhi Ji* and Yuyu Niu*

Current state of stem cell research in non-human primates: an overview

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Abstract: The remarkable similarity between non-human primates (NHPs) and humans establishes them as essential models for understanding human biology and diseases, as well as for developing novel therapeutic strategies, thereby providing more comprehensive reference data for clinical treatment. Pluripotent stem cells such as embryonic stem cells and induced pluripotent stem cells provide unprecedented opportunities for cell therapies against intractable diseases and injuries. As continue to harness the potential of these biotechnological therapies, NHPs are increasingly being employed in preclinical trials, serving as a pivotal tool to evaluate the safety and efficacy of these interventions. Here, we review the recent advancements in the fundamental research of stem cells and the progress made in studies involving NHPs.

Keywords: non-human primates; cell therapy; stem cell

Introduction

Non-human primates (NHPs) provide an indispensable platform for preclinical testing. There are currently over 300 species of NHPs, with chimpanzees, rhesus monkeys, cynomolgus monkey and common marmosets historically being the most frequently utilized in scientific research, each making significant contributions to the field. Among these, chimpanzees share the closest evolutionary relationship with humans. However, the use of chimpanzees and other great apes in experiments is currently restricted due to ethical considerations. Under ethically and legally permissible conditions, common marmoset, cynomolgus monkey and rhesus monkeys have become the most widely used NHPs in biomedical research. Scientists have leveraged these species to make impactful contributions in various domains, including neuroscience and developmental biology [1].

Their close genetic relation to humans, coupled with their complex behaviors, permits the evaluation of the efficacy and safety of novel therapy protocols in a context that closely approximates human physiology and disease progression. Specifically, NHPs have shown to be invaluable in the development of vaccines for diseases such as HIV [2]. The complex immune responses of NHPs, which closely mirror those of humans, have provided key insights into vaccine efficacy and safety that could not be obtained from other model organisms. Furthermore, their long lifespan relative to other model organisms allows for the study of long-term outcomes following the transplantation. Moreover, the manifestation of disease symptoms in NHPs is often more akin to that observed in human patients compared to rodent models and other species. This enables a more nuanced understanding of disease pathogenesis and progression, ultimately facilitating the development of more effective therapeutic strategies. In summary, the use of NHPs in biomedical research not only contributes to a deeper understanding of disease mechanisms but also plays a pivotal role in the development of new therapeutic interventions. As such, they continue to represent an essential resource in the ongoing quest to combat human disease.

Pluripotent stem cells (PSCs) are distinguished by their ability to undergo limitless proliferation and their capacity to differentiate into any cell type. The PSCs derived from NHPs exhibit identical markers and properties to those of human embryonic stem cells (ESCs), making them an invaluable transitional model. This characteristic uniquely equips researchers with the means to effectively address the

***Corresponding authors: Weizhi Ji and Yuyu Niu**, State Key Laboratory of Primate Biomedical Research; Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming 650500, Yunnan Province, China; and Yunnan Key Laboratory of Primate Biomedical Research, Kunming 650500, Yunnan Province, China, E-mail: wji@lpbr.cn (W. Ji), niuyy@lpbr.cn (Y. Niu).https://orcid.org/0000-0003-2550-4224 (W. Ji), https://orcid.org/0000-0002-8797-4152 (Y. Niu)
Junmo Wu, Yuxi Shi, Shanshan Yang, Zengli Tang, Zifan Li, Zhuoyao Li and Jiawei Zuo, Kunming University of Science and Technology, Kunming, Yunnan Province, China; and Yunnan Key Laboratory of Primate Biomedical Research, Kunming, Yunnan Province, China

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safety concerns associated with the clinical application of human ESCs. NHP models provide critical insights into potential risks, side effects, and effectiveness before progressing to human trials, thereby enhancing the safety profile of future clinical applications in humans. As such, PSCs derived from NHPs are an essential link in translating stem cell research into potential therapeutic strategies. The emergence of human induced pluripotent stem cells (iPSCs) has propelled the progress of stem cell-based therapies significantly, demonstrating promising results in clinical trials. Since the development of human iPSCs, stem cell-based therapy has advanced significantly and has shown promise in clinical trials [3, 4].

Expectations are soaring in the public sphere for cell transplantation in regenerative medicine, fueled by the remarkable advancements of scientists have achieved over the past few decades in this field. Two primary modes of action for cell transplantation have been thoroughly investigated: bystander effects and replacement therapy. In the case of bystander effects, transplanted cells often have a short survival span, but they release cytokines and exosomes that serve to mediate pharmaceutical-like effects, including the suppression of cell death, inflammation, and other harmful impacts on surviving tissues that result from disease or injury. This mechanism is chiefly employed in cell-based therapies that involve transplanting mesenchymal stem cells or mesenchymal stromal cells (MSCs) into the body. On the other hand, replacement therapy relies on transplanted cells that survive for extended periods-potentially for the recipient's remaining lifespan-and work to restore cellular functions lost due to disease or injury. This approach is the objective of most cell therapies currently being explored that use differentiated cells derived from PSCs [5]. More than 10 diseases, including Parkinson's disease (PD), age-related macular degeneration, and diabetes, have been the subjects of worldwide clinical trials using PSCs. Nonetheless, before this therapeutic strategy can be accepted as a standard care approach, its safety and efficacy need to be demonstrated conclusively in pre-clinical trials.

In this review, we strive to consolidate the most advanced applications of stem cell technologies in primate cell therapy research, which includes foundational research, disease modeling, and personalized medicine. Stem cell therapy, though a fairly recent advent, is poised for significant breakthroughs owing to the intensive research dedicated to this field.

Stem cell research in NHP

The unique attributes of NHP PSCs

NHPs are closer to humans in terms of genetic, physiological, and behavioral aspects compared to other animal models. This makes the data obtained from studies on NHP PSCs more predictive and relevant to human biology and disease. First, performing gene editing at the embryonic level in NHPs paves the way for establishing various gene-edited stem cell lines. These stem cell lines can serve as invaluable tools for studying gene function, investigating the mechanisms of genetic diseases, and developing potential therapeutic interventions [6]. By introducing precise genetic modifications to NHP embryos, and then deriving stem cells from these embryos, researchers can generate PSC lines carrying specific genetic alterations. These stem cell lines can be further differentiated into various cell types, providing a platform to study the effects of these genetic modifications in the context of specific cell lineages or tissues. Such approaches can enhance our understanding of gene function in the context of complex primate biology and provide crucial insights for the translation of genetic findings into human health and disease. It also offers the potential to create better disease models, which are crucial for preclinical testing of gene therapies [7]. Meanwhile, NHP PSCs can be used to study early development and organogenesis, processes that are difficult to study in humans for ethical and practical reasons. NHP PSCs can be differentiated into various cell types and used to generate three-dimensional organ-like structures, often called organoids. These organoids can recapitulate key aspects of organ development and function, providing a platform for studying developmental processes in a controlled *in vitro* environment [8]. Organoids, which are 3-dimensional structures grown from stem cells in the lab, are increasingly being used to model human development and disease. However, when these organoids are derived from human PSCs and start to resemble early-stage human embryos, ethical concerns can arise [9]. Moreover, PSCs derived from NHPs are playing an increasingly vital role in disease modeling and therapeutic studies. NHP PSC-derived cells could be transplanted into diseased or damaged tissues to restore function. Since these cells originate from the same individual, the results of autologous transplantation experiments can provide valuable insights into the safety and effectiveness of human autologous cell treatments.

The type of stem cells

In 1981, Martin et al. first isolated a pluripotent cell line from mouse embryos [10], a significant discovery that laid the foundation for the clinical application of human ESCs. In 1995, Thomson et al. successfully isolated ESCs from Rhesus monkey blastocysts and established the first ESCs line in primates [11]. In 1998, the Thomson laboratory successfully cultured stem cells from human embryos [12]. This finding drew unprecedented attention to PSCs in clinical research. Further propelling stem cell research was the work of Shinya Yamanaka. In 2006, he successfully used a viral vector to introduce four transcription factors (Oct4, Sox2, Klf4, and c-Myc) into differentiated somatic cells, inducing them to reprogram into iPSCs, similar to ESCs [3, 4]. This groundbreaking discovery led scientists worldwide to explore and discover other methods to create iPSCs, further driving the rapid advancement of stem cell research (Figure 1A).

Nuclear transfer technology, also known as somatic cell nuclear transfer (SCNT), involves the transplantation of a donor cell nucleus into an enucleated oocyte, which is then activated to divide and develop without the need for sexual processes like sperm contact. This process allows for the complete replication of the donor cell's genes. After a period of cultivation, the developing oocyte can be transplanted into a human or animal body. Nuclear transfer technology can be used for cell transplantation and xenotransplantation, and can treat various diseases caused by cell function defects (Figure 1B). In 2007, Byrne et al. successfully isolated two ESCs from cloned embryos created by transferring the nucleus of adult Rhesus monkey skin fibroblasts. These ESCs not only had a normal karyotype, but also demonstrated pluripotency with the ability to differentiate into all three germ layers both *in vitro* and *in vivo* [13]. This breakthrough paved the way for potential future human applications, particularly for patients with genetic diseases. In 2013, under the leadership of Shoukhrat

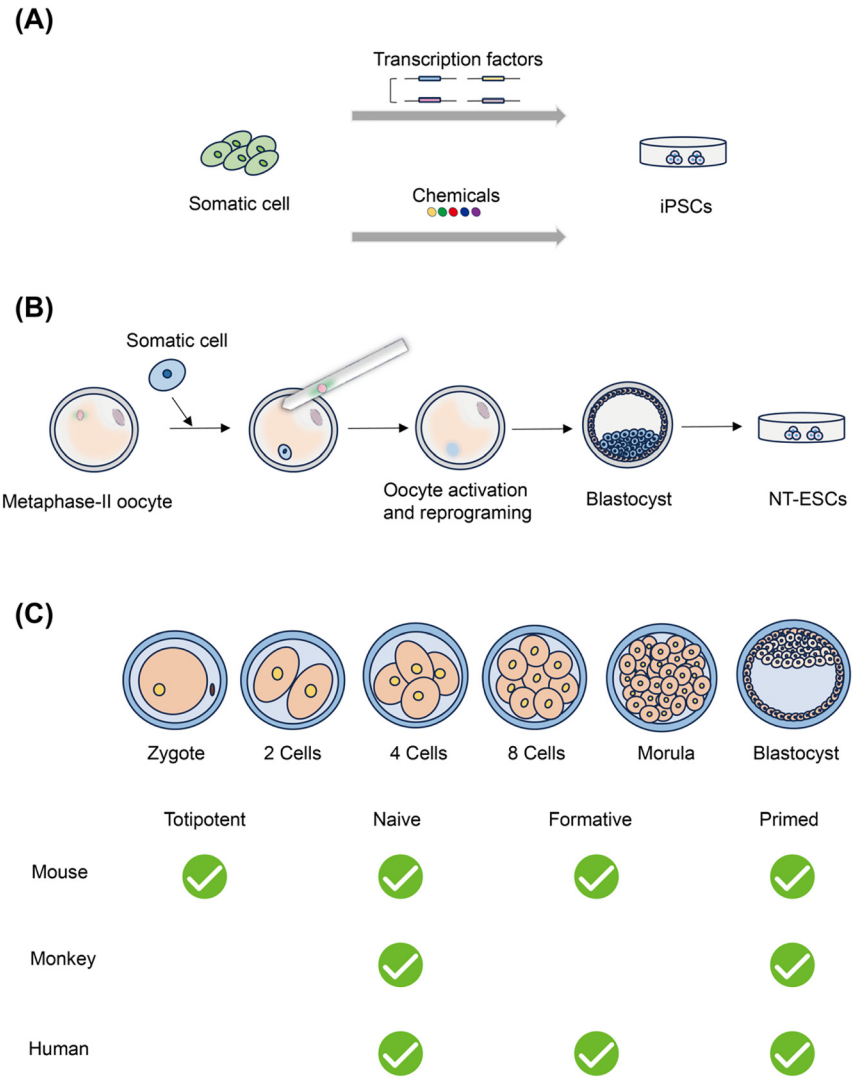

Figure 1: The source of ESCs *in vitro* and the pluripotency state corresponding to embryonic development *in vivo*. (A) Reprogramming methods to generate iPSCs. Transcription factors: Sendai virus mediated cell fate reprogramming; Chemicals: Chemical reprogramming. (B) Generation of stem cells by somatic cell nuclear transfer. (C) Illustration of early embryonic development and pluripotency of cells from different species. Compared with mice, the research of primate totipotent stem cells, 2C like stem cells, naïve PSCs, and formative PSCs is very scarce, showing the importance of NHP stem cell pluripotency research. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; PSCs, pluripotent stem cells; NT-ESCs, nuclear transfer embryonic stem cells.

Mitalipov, human embryos were successfully created using SCNT technology, and stem cells were obtained from these embryos [14]. This marked the first successful acquisition of stem cells from human embryos using SCNT technology by scientists. Since the birth of Dolly the sheep, the first mammal cloned by SCNT in 1986 [15], this technology has been used to clone a variety of large livestock including horses, cows, sheep, pigs, and camels, as well as a variety of experimental animals including mice, rats, rabbits, cats, and dogs. Although there have been reports of NHPs SCNT research since 2002, this field of research has not successfully produced cloned monkeys from SCNT for many years [13]. Recently, two research teams have reported successful cloning of individuals using monkey skin fibroblasts for nuclear transfer [7, 16]. This breakthrough is significant because NHPs are considered the best research models for human diseases. The genetic consistency of cloning can eliminate individual differences in traditional model monkeys, providing more precise data and observations for scientific research.

Since the first derivation of human ESCs in 1998, stem cell therapy has remained a focal point in the field of regenerative medicine. However, the procurement and usage of ESCs accompany several contentious issues, including genetic background concerns, potential contamination, and the greatest obstacle-the ethical implications of cultivating embryos from human fertilized eggs. These issues have significantly limited the research and application of stem cells. Nevertheless, the technology of iPSCs provides a potential solution to these challenges [17]. By reprogramming an individual's skin cells, blood cells, or other cells into iPSCs, they can then differentiate into any cell type needed for regeneration. This personalized approach to disease treatment not only bypasses the risk of immune rejection but also avoids the ethical controversy associated with the use of ESCs. In 1987, research on cell reprogramming reached a significant milestone with the discovery of a single factor that could reprogram cellular identity, known as MyoD [18]. This was the first transcription factor found to be used for cell reprogramming, the expression of which could convert fibroblasts into contracting muscle cells.

In 2007, Takahashi et al. screened 24 candidate genes specifically expressed in ESCs and identified four critical genes (Oct3/4, Klf4, c-Myc, and Sox2) in the induction of pluripotency in fibroblasts. By transfecting these four factors into fibroblasts through a retrovirus, they produced iPSCs exhibiting characteristics of ESCs, and transplantation into nude mice led to teratomas containing tissues of all three germ layers [3]. However, this virus-based method poses problems such as low induction efficiency and possible genetic mutations. In 2007, Yamanaka and colleagues obtained adult chimeras from iPSC clones. Still, some offspring developed tumors due to the reactivation of the c-Myc gene [19]. As the viral vector is integrated into the host genome, the re-expression of the transgenic pluripotency factors could potentially cause tumors in the transplanted cells. Thus, resolving the issue of reactivation of pluripotency factors is a significant concern in the research application of virus-transfected iPSCs.

Methods such as nuclear transfer and the introduction of exogenous genes can lead to reprogramming of somatic cells, while chemical small molecule reprogramming enables the transformation of cell fate with higher precision. This approach uses chemical small molecules to mimic signals from the internal and external environment of the organism, driving cells to change their identity and functional state step by step. Compared to traditional nuclear transfer and gene import methods, the advantage of chemical small molecule reprogramming lies in its greater control over cell fate change. It is also simpler to operate, safer, and more efficient. In 2013, Hou et al. first reported that mouse somatic cells could be reprogrammed into PSCs, termed chemically induced pluripotent stem cells (CiPSCs), using seven exogenous small molecules, without relying on oocytes or endogenous substances like transcription factors [20]. This discovery opened a new path for somatic cell reprogramming technology. In 2015, Deng's team further elucidated the process of CiPSC generation, pointing out that in the early stages of reprogramming, cells undergo an intermediate state similar to the extraembryonic endoderm (XEN-like) of early embryonic development [21]. It was found that promoting this intermediate state can significantly enhance the efficiency of chemical reprogramming. By 2022, Deng's group achieved another breakthrough, successfully inducing human adult cells into PSCs using chemical small molecules [22]. Then in March 2023, they discovered a new combination of chemical small molecules that could significantly speed up the reprogramming process, reducing the induction process from an original 50 to 30 days, or even as short as 16 days [23]. This new induction scheme is not only fast, efficient, and stable but also satisfies clinical needs better due to its defined components and lack of exogenous sources.

In summary, due to its non-integration into the genome, reversible action, and simplicity of operation, the technology of chemical small molecule reprogramming is safer, simpler, and easier to standardize. It has a broad prospect for clinical application.

The pluripotent state of mouse, monkey and human stem cells

In 2009, Nichols and Smith proposed a theoretical framework distinguishing between two states of pluripotency—termed the 'primed state' and the 'naïve or ground state'—based on a cell's developmental stage within the epiblast (EPI), functional characteristics, biological properties, and gene expression patterns [24]. According to this model, mouse PSCs are categorized as being in the naïve or ground state, while epiblast stem cells (EpiSCs) and traditional primate PSCs are classified as being in the primed state. These two states of pluripotency represent different developmental stages of the EPI: the naïve state corresponds to the pre-implantation EPI (Pre-EPI), while the primed state corresponds to the post-implantation EPI (Post-EPI).

Totipotent cells, which have the highest developmental potential, are typically referred to as the fertilized eggs, 2-cell and 4-cell blastomeres in mammals. To date, the capture and maintenance of totipotent stem cells *in vitro*, similar to endogenous totipotent blastomeres on a molecular and cellular level, has not been achieved. However, scientists have been striving to capture and maintain stem cells with higher potential *in vitro* (Figure 1C). In mice, the fertilized eggs and blastomeres from 2-cell embryos are the only genuine totipotent stem cells (TotiSCs), capable of generating all differentiated cells in embryonic and extraembryonic tissues and forming a complete organism. In 2017, Yang et al. established expanded pluripotent stem (EPS) cells using a combination of small molecules called LCDM [25]. In 2021, Shen et al. established and cultured mouse totipotent stem cells *in vitro* by suppressing the spliceosome. These cells, close in molecular and functional terms to *in vivo* 2-cell and 4-cell stage embryos, were thus named totipotent blastomere-like cells (TBLCs) [26]. In 2022, Yang et al. reported a novel mouse totipotent-like stem cell (TLSC) [27]. In 2022, Xu et al. established a new culture condition for TotiSCs: the CPEC combination can support the direct establishment of TotiSCs from mouse two-cell embryos and EPS cells *in vitro* [28]. In 2022, Hu et al. reported a new small molecule drug combination TAW that can induce mouse PSCs to become TotiSCs with the potential to transform into a complete organism. The stem cells induced by the TAW combination, named as chemically induced totipotent stem cells (ciTotiSCs), are similar to mouse totipotent two-cell embryonic cells at the transcriptomic, epigenomic, and metabolomic levels [29].

Compared to the mouse PSC culture system, the development of the human PSC and monkey PSC culture systems is relatively slow, and there is almost no culture system for PSCs that is close to a totipotent state. In 2022, the first time a non-genetic, fast, and controllable method to induce human PSCs into human totipotent stem cells similar to a fertilized egg developing for 3 days, 8-cell embryo-like cells (8CLC) [30]. In 2021, Hanna further optimized the culture conditions of naïve human ESCs, resulting in enhanced feeder-free naïve human ESCs [31].

In 2014, Austin Smith first proposed the existence of a new state between Naïve and Primed [32]. Cells in this state should resemble embryos during the early implantation phase and exhibit the following characteristics: they can chimerize into the embryonic inner cell mass (ICM) and be induced *in vitro* to become primordial germ cell-like cells (PGCLCs). This state was named the Formative state. However, since this intermediate state was proposed, the scientific community has yet to establish a stable cell line that possesses the main characteristics of the Formative state. In 2020, Yu et al. established stable stem cell lines with "intermediate" characteristics in multiple species [33]. By simultaneously activating the FGF/Erk, TGF-β/Smad, and WNT/β-Catenin signaling pathways, they established a stable cell line with "formative" features. Experimental evidence and multi-omics data confirmed that this cell line indeed represents an intermediate state between naïve and primed, possessing characteristics of both states, can be directly transformed from the naïve state, and can directly transition to the Primed state. Most importantly, this cell line can chimerize efficiently and can directly respond to the action of the BMP4 signal to transform into primordial germ cells. Based on these characteristics, these cells were named XPSCs. In 2021, Austin Smith reported the establishment of a stable stem cell line with formative state characteristics (formative stem cell, FS cells) from mouse embryonic epiblasts and ESCs using a newly developed low growth factor culture system. This system can also be used to induce and expand human FS-like cells [34].

In the realm of PSCs research involving NHPs, often model animals such as cynomolgus monkey, rhesus monkey, or common marmoset, the progress has been slower compared to human PSCs studies. In 2014, a research team reported that a 4i/LIF+bFGF culture condition is suitable for cultivating rhesus monkey iPSCs. Under this system, rhesus monkey iPSCs can be transitioned into a naïve pluripotent state. Furthermore, these naïve iPSCs were shown to be capable of integrating into mouse embryos [35]. A research team has reported that by modifying the rabbit PSC culture system (K5cLD) and using a feeder layer along with DOX-induced endogenous expression of KLF2 and NANOG, cynomolgus monkey ESCs can exhibit partial features of naïve pluripotency. These features include the formation of dome-shaped colonies and the expression of naïve

pluripotent state markers REX1, KLF4, and KLF17. However, when these cells were injected into mouse embryos, no chimerism was detected in the adult mice. Additionally, the dependence on DOX induction for endogenous KLF2 and NANOG expression constrains further applications [36]. Moreover, another research group has shown that employing a feeder layer and adding bFGF as well as IWR-1 (WNT pathway inhibitor) in a cultivation protocol they've dubbed AITS-IF20, allows for the sustained culture of cynomolgus monkey ESCs. Remarkably, these cynomolgus monkey ESCs exhibit mRNA profiles closely mirroring those of post-implantation EPI in the same species. These cynomolgus monkey ESCs have the capability to differentiate into PGCLCs specific to cynomolgus monkey. A salient feature of the AITS-IF20 culture regimen is its intentional avoidance of WNT/β-catenin signaling activation. This is crucial, as existing research indicates that extended activation of WNT signaling can induce unstable X-chromosome inactivation, a condition adverse for the successful differentiation of PGCLCs and their subsequent development into oocytes [37]. In principle, due to the close biological and genetic alignment between NHPs and humans, it is reasonable to consider that methods effective for culturing human PSCs might also be suitable for NHP PSCs. Confirming this idea, some protocols originally developed for human PSCs have indeed found utility in the culture and differentiation of NHP PSCs. For instance, the feeder layer method supplemented with KSR/bFGF media has seen broad use in NHP PSCs studies. Importantly, this approach has demonstrated its adaptability, accommodating the stem cell maintenance needs of diverse primate species such as cynomolgus monkey, rhesus monkey and common marmoset [38, 39]. Furthermore, research groups have demonstrated that the inclusion of ascorbic acid in the established human PSCs culturing protocol, known as NHSM, yields a modified method termed NHSMV. In this enhanced setting, ESCs derived from cynomolgus monkey exhibit dome-shaped colonies, which are generally regarded as a hallmark of naïve pluripotency. Notably, these NHSMV-cultured cells not only maintain their foundational pluripotent attributes, as evidenced by the continued expression of key markers OCT4, SOX2, and NANOG, but also display specific naïve pluripotency markers such as KLF4 and PRDM14. Significantly, cells cultured under these NHSMV conditions possess the capability to integrate seamlessly into same-species embryos [40]. Similarly, some research teams have reported modifications to the PXGL culturing system, specifically by omitting Go6983 (PKCi) and additionally incorporating Activin A and ascorbic acid, thereby creating a new protocol called PLAXA. Under these PLAXA conditions, ESCs derived from common marmoset form dome-shaped colonies and express the marker KLF17. Importantly, when subjected to RA+FGF and BMP4 induction, these PLAXA-cultured common marmoset ESCs have demonstrated the ability to form structures resembling both the embryonic disc and amniotic sac [41]. Additionally, some research groups have reported applying the 4CL culture method to the cultivation of cynomolgus monkey ESCs. These 4CL-cultured cells not only express naïve pluripotency markers KLF4 and KLF17 at the protein level but also demonstrate the ability to form blastoids [8].

In conclusion, a comprehensive examination of the signaling pathways influenced by varying components in pluripotent stem cell culturing protocols for both humans and NHPs could yield invaluable insights into the evolutionary divergences between these species. This scrutiny may also unveil underlying mechanisms that account for the observed discrepancies in the maintenance of pluripotency across human, non-human primate, and murine models.

The application of CRISPR/Cas9 in NHP models

Indeed, despite the unparalleled relevance of NHPs in biomedical research due to their genetic, physiological, developmental, and cognitive similarities to humans, there are challenges in creating transgenic models with these species (Figure 2A). Classical disease modeling in NHPs typically relied on the study of naturally occurring diseases or disease induction through chemical agents and surgical interventions. In recent times, however, the advent of precise gene modification techniques, notably the broadly employed CRISPR/Cas9 system, has revolutionized this field. The deployment of these precise genome editing tools has facilitated the creation of NHP models that closely mimic human diseases. The first-ever report of a gene-modified monkey through CRISPR/Cas9 emerged in 2014 [6]. Researchers achieved precise gene targeting in cynomolgus monkeys by co-injecting Cas9 mRNA and sgRNAs into one-cell-stage embryos. They demonstrated the capability of this system to concurrently disrupt two target genes (peroxisome proliferator-activated receptor gamma (PPARG) and recombination activating gene 1 (RAG1)) in a single step, with no detectable off-target effects. Furthermore, they affirmed the potential for germline transmission in the Cas9-manipulated monkeys by investigating gene targeting in gonads and germ cells [42]. However, the resultant transgenic monkey exhibited mosaic mutations, with different tissues containing the wild type allele. This raised questions regarding the impact of these mosaic mutations on functional studies. Subsequently, Chen et al. employed Cas9 to disrupt the dystrophin gene in rhesus monkeys.

The monkeys displayed severely diminished dystrophin and muscle degeneration (DMD), mirroring early-stage DMD [43]. This highlighted that CRISPR/Cas9 can effectively generate monkey models of human diseases, regardless of the inheritance patterns (Figure 2B).

Validating the safety and efficacy of cellular therapies based on NHPs

Stem cell therapy for Alzheimer's disease (AD)

AD is the most common neurodegenerative brain disorder, clinically characterized by memory confusion, cognitive and behavioral dysfunction, among other dementia-like symptoms. The underlying mechanisms of the disease are not fully understood, although some researchers speculate that the accumulation of amyloid beta protein and Tau protein may contribute to extensive neuron death in the brain. Thus, cell therapy has immense potential, where stem cell-derived neurons can replace damaged ones, greatly benefiting individuals who cannot gain from drugs. Studies have shown that neural stem cells (NSCs) can deliver gene-enhancing neurotrophic factors, potentially altering the course of AD's development. When mouse ESC-derived NPCs were transplanted into the basal nucleus of AD mice, their learning and memory abilities were both improved, and cholinergic neurons derived from ESCs could enhance cognitive capabilities [44]. MSCs have been used to treat Alzheimer's animal models and other neurodegenerative diseases. After treatment with bone marrow-derived MSCs, memory loss and cognitive dysfunction in models were significantly improved, likely through mechanisms involving neurogenesis, cell apoptosis, angiogenesis, inflammation, and immune regulation [45]. If AD progresses to a state of hyperphosphorylation of Tau protein and cognitive impairment, therapeutic strategies using MSCs should be considered [46]. Moreover, Extracellular vesicles (EVs) are a form of cell communication where parental cell materials can be transferred to recipient cells, leading to changes in molecular and signaling levels of the recipient cells [47]. Bone marrow-derived mesenchymal EVs interact with amyloid-beta through lipid membranes, enhancing the phagocytic action of microglia on plaques and directly reducing plaque buildup; these vesicles also contain a protease that directly degrades amyloid-beta, indirectly reducing cellular amyloid-beta accumulation [48]. Studies have shown that EVs derived from NSCs can enhance mitochondrial function, increase SIRT1 protein levels, reduce inflammatory response, and improve cognitive abilities in AD mice [49]. Another significant impairment to intellectual development is Rett syndrome, a disease severely affecting children's psychomotor development. Its clinical features include affecting girls, showing autistic behavior, progressive intellectual decline, stereotyped movements, and ataxia, among others. A team successfully created a monkey Rett model using Talen-edited MECP2 mutation technology in 2017, this model has potential value for the study of Rett disease mechanisms and the development of potential therapeutic interventions [50]. It can be imagined that gene-edited NHPs will become a better choice for modeling neurological diseases [51].

Stem cell therapy for Parkinson's disease (PD)

PD is the second most common neurodegenerative disease in the brain, highly heterogeneous, with varying rates and mechanisms of disease onset in different patients. Recent research suggests that transgenic A53T and α-syn aggregation may affect the gut microbiome and metabolites in macaques, identifying five major metabolites with differing compositions related to mitochondrial dysfunction that may be related to the pathogenesis of PD [52]. Current clinical treatments for PD mainly include drugs and surgeries, which can only alleviate the symptoms of PD and cannot replace or regenerate the lost dopaminergic (DA) neurons in the brain. Therefore, researchers are trying to treat PD with cell transplantation. Stem cell therapy involves transplanting cells into lesions or specific parts to regenerate and repair tissues and cure diseases. It has been proven that targeted transplantation of DA neurons can solve these shortcomings of dopaminergic drugs [53]. Induced DA neurons from iPSCs derived from sporadic PD patients can improve the behavior of NHPs with PD [54]. In PD monkey models, transplantation of autologous iPSC-derived DA neurons can improve animal motor function long-term, with transplanted cells able to survive in the body for 2 years and regenerate in the host brain [55].

Whether laboratory-generated DA neurons, even when derived from autologous sources, can evade immune responses remains an unanswered question. Compared to autologous iPSCs grafts, allografts elicited a large amount of immune response without immunosuppressants, including activated T cells, leukocytes, microglia, and astrocytes. This immune and inflammatory response from allografts persisted for 1.5 years, leading to a decrease in the number of tyrosine hydroxylase positive (TH^+) neurons by an order of magnitude [56]. Compared with ESCs and iPSCs, MSCs have strong anti-inflammatory abilities. It has been confirmed

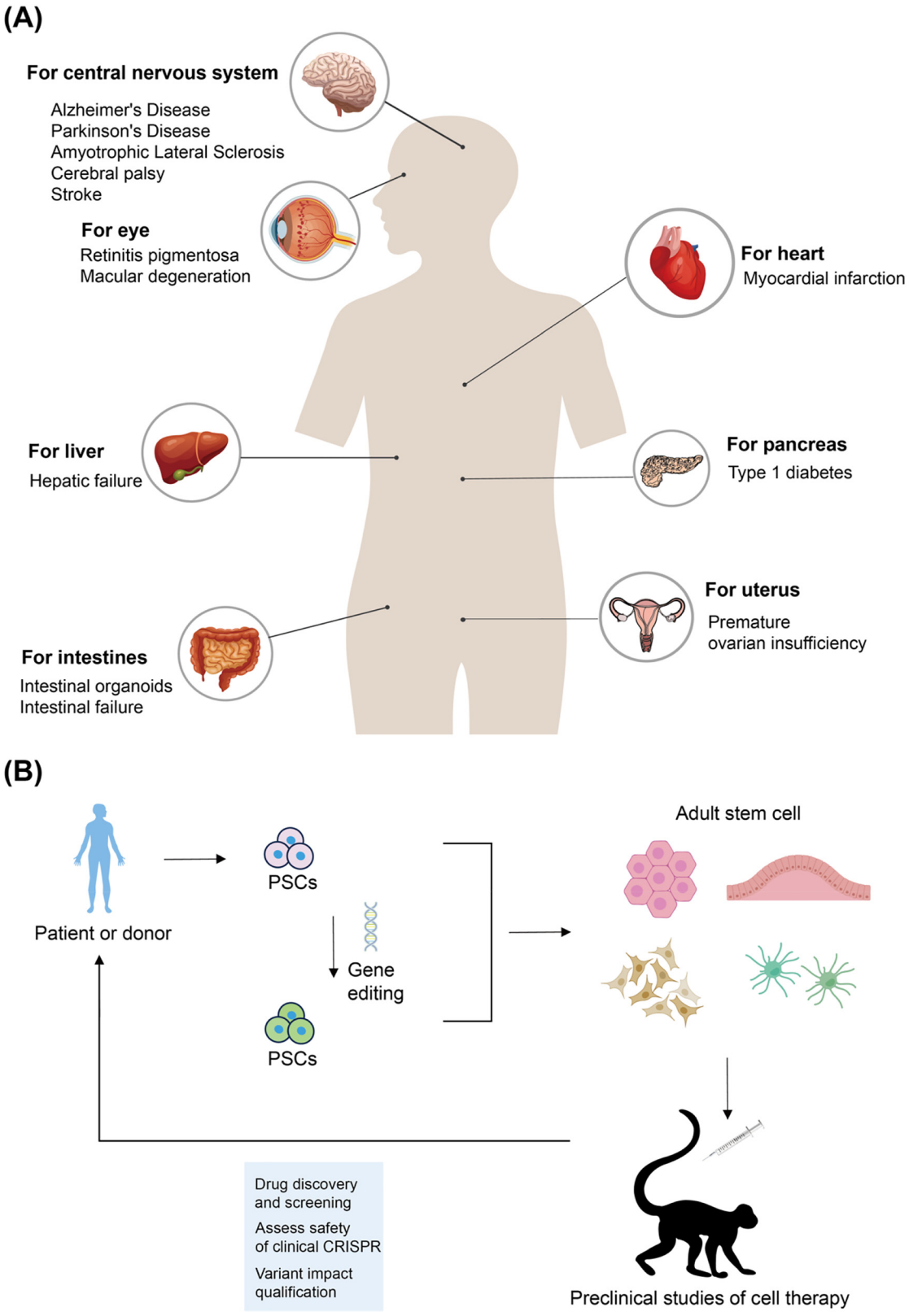

Figure 2: PSCs-based therapies for disease treatment. (A) Shown are cell therapy trials that use PSCs. (B) Pluripotent cells can be differentiated to a desired therapeutic cell type (directed differentiation), which can be studied *in vitro* or used for transplantation into NHP models (preclinical studies) before clinical therapy. The differentiation of disease-specific iPSCs into cell types associated with the disease can recapitulate disease pathology *in vitro*, accelerate the process of drug discovery or identify disease genes. The genetically corrected iPSCs could provide a source of autologous, genetically normal cell types for replacement of tissues directly affected by the ongoing disease process. PSCs, pluripotent stem cells; NHPs, non-human primates; iPSCs, induced pluripotent stem cells.

that dopaminergic MSCs (DOPA-MSCs) maintain their MSCs identity and stable ability to secrete dopamine during passaging. The long-term benefits (reconstruction of midbrain dopamine pathway for up to 51 months) and safety results support the view that developing engineered cell transplantation synthesizing dopamine is an important strategy for treating PD [57]. This research supports the significant therapeutic effects of engineered cell transplantation in various models. Based on current animal trials, this treatment method offers the advantages of ease of operation, rapid action, and efficacy maintenance over a long term. Especially compared to other cell drugs, engineered cells are easier to expand, and the treatment cost is relatively low. Piao and others generated midbrain DA neurons from human ESCs and made large-scale cryopreserved DA progenitor cells for research. After transplantation into mice, rats, and human PD patients, the transplanted cells survived and improved the motor function of PD patients [58]. Zhou's team conducted a safety and effectiveness evaluation of PD treatment with clinically-graded human ESCs-derived DA neurons transplantation in an NHP PD models [59]. Most monkeys showed significant behavioral improvement. Moreover, the slight increase in striatal DA and the significant functional improvement are related, indicating that clinical-grade ESCs can be a reliable cell source for PD treatment. These clinical datas strongly support the initiation of human trials.

Stem cell therapy for stroke

Globally, stroke, colloquially known as a brain attack, is one of the three leading causes of death and injury. It is a disease caused by sudden rupture of brain blood vessels or blockages that prevent blood flow to the brain, leading to injury of brain tissue or cells. It includes both ischemic and hemorrhagic strokes. Currently, the main treatments are aspirin and tissue plasminogen activator, but drug treatment is limited by the time and risk of bleeding [60]. Stem cell transplantation is a potential treatment pathway because stroke causes irreversible neuron damage and neuro tissue damage. Stem cells can compensate for the deficiency of endogenous neurons and increase cell survival rates in inflammatory environments. Treatment of ischemic stroke must be individualized, including heterogeneous treatment, many of which are closely related to the location of ischemic injury, patient age, and the neuron's self-repair ability. The key goal of stroke treatment care is to restore local cerebral blood flow perfusion. Many preclinical animal studies of ischemic stroke have tested the therapeutic effects and protective abilities of transplanted exogenous NSCs. Studies have shown that exogenous NSCs can significantly improve the prognosis of animal models of ischemic stroke, and the occlusion area is significantly reduced [61]. Placenta-derived MSCs protect stroke treatment through the ACE-2/MasR pathway, which represents an innovative and promising target for stroke treatment [60].

Stem cell therapy for multiple sclerosis (MS)

MS is a common central nervous system disease driven by an autoimmune response to myelin, so MS is also an autoimmune systemic disease related to genetic and environmental factors. It usually occurs in young people, and the mechanism of disease progression pathology is not yet clear. Most patients have relapses and remissions, and this relapsing-remitting treatment is mainly through immunoregulatory and immunosuppressive drugs [62]. Studies have shown that autologous hematopoietic stem cells (HSCs) transplantation treatment for MS patients has reduced the mortality rate from 7.3 % in 2000 to 0.2 % in 2016 [63]. Unlike other immunosuppressive treatments, autologous HSCs mainly repair the immune system [64]. Studies have shown that nanovesicles produced by adipose stem cells reduce the activity of central nervous system immune cells, including the reduction of microglial cells and T-cell exudation, and the exosomes produced can carry therapeutic drugs to penetrate the blood-brain barrier, which is good news for MS patients.

A common condition in MS is amyotrophic lateral sclerosis (ALS), colloquially known as Lou Gehrig's disease. It is an invasive and lethal neurodegenerative disease characterized by motor neuron degeneration. It mainly affects the upper and lower motor neurons of the motor cortex and spinal cord. Its symptoms include muscular degeneration, weakness, muscle contraction, and spasm. Due to the gradual loss of motor neurons and skeletal muscle weakness, it progresses to global muscle atrophy and difficulty swallowing, eventually leading to respiratory failure. Patients often die within 3–5 years of onset [65]. Recent research indicates that the expression of the C9orf72 gene G4C2 (GGGGCC) repeat expansion mutation in NHPs can lead to typical ALS symptoms. By examining the expression of Cystatin C and Chitinase-1 in the cerebrospinal fluid related to disease progression, it was found that the aggregation of Poly PR (Pro-Arg) in the nucleus affecting the expression of the MECP2 protein might be a critical pathogenic mechanism [66].

Therapeutic applications in primate animals for liver diseases

Liver transplantation is a viable treatment for end-stage liver disease and acute liver failure. However, the supply of allogeneic liver transplants far exceeds the demand for patients requiring transplantation. Hepatocyte transplantation holds promise for addressing the shortage of donors and is an effective method for liver transplantation treatment. As early as 2008, Ma et al. demonstrated the ability to differentiate monkey ESCs into hepatocyte lineages using specific culture conditions [67]. In 2014, Kuai et al. induced ESCs from rhesus monkeys to differentiate into hepatocytes using cytokines. The differentiated cells exhibit the morphological characteristics, gene expression patterns, and metabolic activity characteristics of normal hepatocytes [68]. Hepatocyte transplantation is considered to provide temporary liver function support during liver regeneration or while awaiting transplantation. Machaidze et al. transplanted microencapsulated pig liver cells into a baboon model of fulminant hepatic failure, demonstrating that microencapsulated pig liver cells provide temporary liver function support for baboons with fulminant hepatic failure [69]. In 2022, Kalsi et al. established a model of acute liver failure in NHPs, which is almost identical to clinical pathology and pathophysiology and results in a 100 % mortality rate. This model allows xenogenic hepatocyte transplantation to be preliminarily tested as a potential treatment method [70].

Therapeutic applications in primate animals for intestinal diseases

In biomedical research, NHPs are valuable animal models that enhance our understanding of human biological responses. 3D organ cultures generate functional gastrointestinal epithelial cells *in vitro* and can be produced from animal and human tissues. Inaba et al. reported the cultivation of intestinal chemosensory cells from macaques using an organic culture system. Macaque intestinal organs can maintain culture for more than 6 months [71].

Therapeutic applications in primate animals for diabetes stem cell treatment

Human PSC-derived islets (hPSC-islets) are a promising cellular resource for the treatment of diabetes [72, 73]. Over the past decade, multiple studies have reported the differentiation of PSCs into pancreatic-related cells and their subsequent transplantation into diabetic mice. These differentiated cells mature in mice, expressing specific markers of normal islet cells and functioning [74–79]. However, rodents differ greatly from humans in both physiological and anatomical structure, particularly the immune system. Therefore, this method of stem cell replacement therapy needs to be evaluated in larger animals, such as NHPs, to provide more clinically relevant references for achieving clinical treatment. Zhu et al. first demonstrated that iPSCs from rhesus monkeys can differentiate into functional insulin-producing cells. These cells, when transplanted into mice, can lower the mice's blood sugar levels, laying the groundwork for studying the efficacy and safety of autologous iPSC-derived insulin-producing cells in the rhesus monkey model for the treatment of type 1 diabetes [80]. Du et al. generated islets from human chemically iPSCs (hCiPSC-islets) and showed that a single intraportal injection of hCiPSC-islets into diabetic NHPs can effectively restore endogenous insulin secretion and improve blood glucose control. All trial subjects exhibited significantly reduced fasting and pre-meal average blood glucose levels, accompanied by the release of C-peptide in response to meals or glucose and overall weight gain [81]. The therapeutic effect of hCiPSC-islets transplantation may be limited by the adaptation process at the transplant site, so Liang et al. in 2023 developed a new transplantation strategy, transplanting hCiPSC-islets into the anterior sheath of the rectus abdominis of eight macaques. The results showed that hPSC-islets survive and gradually mature after transplantation, thus improving the blood glucose control of diabetic primates. Starting from 6 weeks post-transplantation (WPT), C-peptide secretion responds to feeding challenges, with a stimulation index equivalent to primary islets. From the eighth week, the average postprandial C-peptide level reached about 2 ng/mL–1, which is 5 times the peak obtained after portal vein infusion of hPSC-islets and is related to a 44 % reduction in glycated hemoglobin level at 12 weeks [82]. A recent study showed that insulin-producing cells (IPCs) differentiated from human MSCs and transplanted via the portal vein into a non-human primate tree shrew model of diabetes rapidly reduced blood glucose levels to near-normal levels and remained significantly lower than the MSC group or saline group over the subsequent 3 weeks [83]. The omentum is an attractive extrahepatic alternative site for clinical islet transplantation, researchers have explored a method to transplant allogeneic islets to the omentum, and in three types of diabetic NHPs, the omentum is bioengineered with plasma-thrombin biodegradable matrix. Within a week after transplantation, each transplanted NHPs reached normoglycemia and insulin dependence, and remained stable [84].

Primate application in heart disease treatment

PSCs provide a potential solution for the current prevalence of heart failure by providing human cardiac cells to support heart regeneration. Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) transplanted into the myocardial ischemia-reperfusion model of NHPs can cause a large number of infarcted monkey hearts to re-muscularize [85]. In addition, Liu et al. re-muscularized infarcted macaque hearts with hESC-CMs and can durably improve left heart function [86]. At present, induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) are transplanted into infarcted myocardium via direct cell injection or tissue engineering patches. Yang et al. conducted monolayer culture (2D) and engineered heart tissue (EHT) (3D) culture of NHPs iPSC-CMs, and then transplanted them under hypoxic conditions, confirming that the 3D EHT model is more sensitive to ischemic conditions, and similar to *in vivo* natural myocardium in terms of cell-extracellular matrix/cell-cell interaction, energy metabolism, and paracrine signaling [87]. Uyama et al. generated myogenic progenitor cells from three different NHP iPSC lines and demonstrated that the specification and global transcriptional changes during various stages of embryonic development of the myogenic lineage from NHP iPSCs are associated. These progenitor cells were capable of forming myotubes *in vitro* and, importantly, contributed to muscle regeneration *in vivo* [88]. Stauske et al. reprogrammed fibroblasts from cynomolgus monkeys into iPSCs and successfully differentiated NHP-iPSCs into cardiomyocytes using a growth factor/GiWi combination. These iPSC-derived cardiomyocytes exhibited the ability to self-organize into contracting engineered heart muscle (EHM) [89].

Primate application in ophthalmology disease treatment

The macula is an eye structure unique to humans, apes, and monkeys and is the area of sharpest vision. Therefore, NHPs are potentially valuable animal models for studying human macular diseases [90]. In 2015, Shirai et al. first confirmed the ability of human ESC-derived retina (hESC-retina) to form a structurally mature photoreceptor layer after transplantation into nude mice. Then, they established two models of retinal degeneration in monkeys and demonstrated possible integration of hESC-retinal sheets with host bipolar cells in a cobalt chloride-induced retinal degeneration monkey model [91]. In 2021, Liu et al. reported their transplantation of a monolayer of human retinal pigment epithelial cells derived from retinal pigment epithelial stem cells (hRPESC-RPE) beneath the macula of an NHP model for a duration of three months. The transplantation demonstrated the ability to restore and maintain healthy photoreceptors *in vivo* [92].

Primate application in reproductive aspect

Theca stem cells (TSCs) isolated from cynomolgus monkeys and transplanted into monkeys with premature ovarian insufficiency (POI) showed that it can significantly improve hormone levels, rescue follicle development, improve oocyte quality, and increase the oocyte maturation/fertilization rate. It was first shown that autologous TSCs can improve the symptoms of POI in primates, and provide new hope for the development of stem cell therapy for POI [93].

NHPs research in the application of blood diseases

Fetal hemoglobin reactivation (HBF) is a promising approach for treating beta-hemoglobinopathies. Humbert O and colleagues established NHP transplantation model by transplanting BCL11A-edited hematopoietic stem cells into Rhesus monkeys. The HBF production, determined through F-cell staining and gamma-globin expression, showed a slight increase compared to the transplantation control group [94]. Sickle cell disease (SCD) is a life-threatening monogenic disease where a point mutation in the beta-globin sequence (Glu6Val) leads to abnormal sickle-shaped hemoglobin. Various gene-modification strategies can induce gamma-globin expression, which may be beneficial for the treatment of hemoglobin (Hb) diseases. Demirci, S and colleagues established an efficient red cell differentiation culture system in Rhesus monkeys to test the activity of ZFLDB1 constructs on gamma-globin reactivation during erythroid progenitor cell differentiation [95]. AMD3100 (Plerixafor) is an essential component of many clinical and preclinical transplant protocols, facilitating the mobilization and collection of HSCs and progenitor cells from the peripheral blood circulation. Samuelson [96] investigated the mobilization effects of repeated administration of AMD3100 in non-human primate models and humanized mouse models. The results showed effective mobilization following the first injection of AMD3100 in NHPs, but the response was notably poorer for CD34+ and CD90+ cells concentrated in HSCs.

The progress of primordial germ cells differentiation research

Mouse primordial germ cells (PGCs) specialize from the EPI of mouse embryos around E6.5, a process that occurs under the stimulation of Bone Morphogenetic Protein 4 (BMP4) and WNT signals [97]. Transcription factors Blimp1, Tfap2c, and Sox17 play key roles in the specialization process of mouse PGCs [98]. As the mouse embryo develops, mouse PGCs migrate to newly formed genital ridges and proliferate there. Subsequently, the genital ridges differentiate into testes and ovaries in males and females, respectively, and mouse PGCs develop into oogonia, primary oocytes, and secondary oocytes under the encapsulation of gonadal cells [99]. In mice, mouse ESCs derived from the ICM exhibit a naïve pluripotency similar to that of E4.5 EPI, whereas mouse EpiSCs derived from E5.5–6.5 EPI exhibit a primed pluripotency [100]. However, both mouse ESCs and mouse EpiSCs have difficulty differentiating into mouse PGCs [101]. To address this issue, researchers investigated the method of transforming mouse ESCs into mouse epiblast-like cells (EpiLCs), a state between mouse ESCs and mouse EpiSCs. Using activin and FGF stimulation, researchers were able to transform mouse ESCs into mouse EpiLCs with E5.5–6.0 EPI characteristics within two days. After receiving BMP4 and WNT signal stimulation, mouse EpiLCs can efficiently differentiate into mouse PGCLCs. These cells are more similar to E9.5 mouse PGCs at the transcriptional level, and the induction process of mouse PGCLCs mimics many aspects of mouse PGC specialization *in vivo* [102].

The importance of this research lies not only in its ability to mimic the specialization process of mouse PGCs, but also in the fact that researchers have found that they can transplant these mouse PGCLCs into the testes and ovaries of newborn mice, which lack endogenous sperm and egg formation. These mouse PGCLCs can differentiate into mature sperm and eggs capable of producing healthy mice [102]. In addition, the recombinant ovaries (rOvaries) formed by recombining mouse PGCLCs with mouse E12.5 gonadal cells *in vitro* can further develop mouse PGCLCs into MII oocytes under *in vitro* culture, and these fully *in vitro* produced mature oocytes can be fertilized and develop into blastocysts. By transplanting 2-cell stage embryos derived from mouse PGCLCs into mouse uteri, further development can result in mice with reproductive function [102, 103]. Meanwhile, a recent study has effectively demonstrated the *in vitro* reconstruction of the complete male germ cell development process utilizing mouse PSCs [104].

Most importantly, these studies can be successfully replicated using iPSCs, laying the foundation for *in vitro* gametogenesis (IVG), and opening up a new avenue for the treatment of infertility and the study of reproductive biology.

In the mammalian context, such as humans or some rare animals, an important problem arises when using this system for *in vitro* gamete reconstruction: it is difficult to obtain gonadal cells during the fetal period. Thus, a research team reported the derivation of mouse fetal ovarian somatic-like cells (FOSLC) that resemble E12.5 mouse gonadal cells, from the differentiation of mouse ESCs. These mouse FOSLCs function similarly to E12.5 gonadal cells, and when these mouse FOSLCs are recombined with mouse PGCLCs into rOvaries for cultivation, mature oocytes originating from mouse PGCLCs can be obtained, as well as individuals. This provides a new possibility for extending IVG to other species, without the need for individual gonads to obtain gonadal cells to promote mouse PGCLCs' development into late-stage germ cells [105].

The ultimate goal of IVG will be to apply it to other species, such as exploring the mechanisms of human gametogenesis, addressing infertility caused by abnormalities in gametogenesis, or even using IVG-derived gametes as one of the means to treat infertility. IVG might also be helpful in saving some endangered animals. Therefore, it is necessary to translate mouse IVG into a scheme suitable for humans and to promote it in NHPs as one of the pre-clinical verifications of the feasibility and safety of IVG [106].

The origin of human germ cells has always been tricky because it occurs in the early post-implantation period, which is difficult to analyze both ethically and technically. However, research conducted in NHPs such as the cynomolgus monkey or the rhesus monkey provides insights into these two issues. Single-cell transcriptomics reveal that the epiblast cells of the cynomolgus monkey undergo significant transcriptomic changes post-implantation and then maintain a relatively stable transcriptome while producing primitive gut cells (E13). The transcriptome of cynomolgus monkey ESCs is highly similar to the late (E16 or E17) and early (E12 or E13) post-implantation EPI [107]. Histological studies of *in vivo* embryos suggest that cynomolgus monkey PGCs, expressing key PGC markers SOX17, TFAP2C, and BLIMP1, may originate from the amnion, unlike mouse PGCs that originate from the EPI [108]. Methods for inducing human PGCLCs from human PSCs have been reported. Human PSCs cultured under 4i (MEK, GSK3b, p38, and JNK inhibitors) conditions resemble pre-gut epiblast-like cells, and they can be induced to become human PGCLCs in response to BMP signals [109]. Moreover, human PSCs cultured under primed conditions can be processed into mesodermal-like cells through Activin and WNT signals, and then be induced into human PGCLCs through BMP signals [110] (Figure 3). Subsequently, when co-culturing hPGCLCs with mouse E12.5 gonadal cells, fetal germ cells at the retinoic acid response stage were observed [111]. Simultaneously, sperms

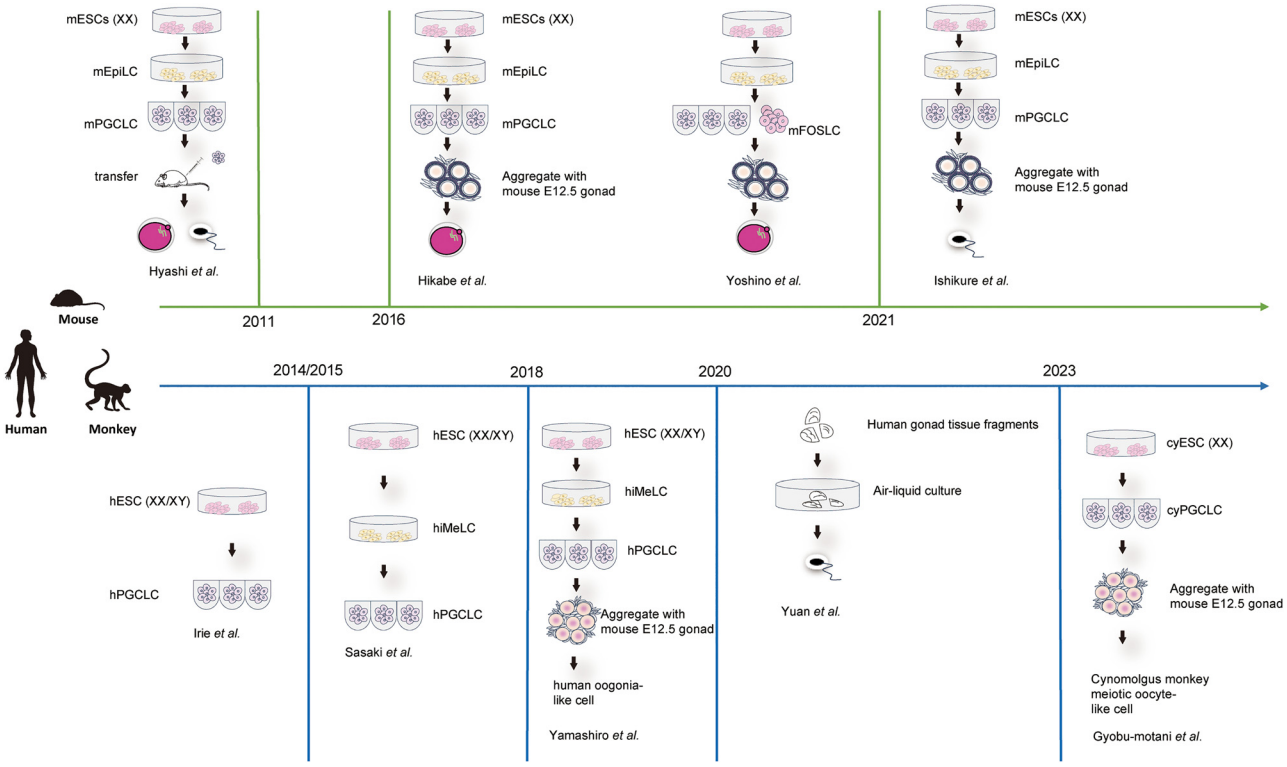

Figure 3: Progress of *in vitro* gametogenesis researchs in mouse, non-human primate and human. A timeline of key IVG research in 2011–2023. IVG, *in vitro* gametogenesis; mESCs, mouse embryonic stem cells; mEpiLC, mouse epiblast-like cells; mPGCLC, mouse primordial germ cells; mFOSLC, mouse fetal ovairian somatic cell-like cells; hESCs, human embryonic stem cells; hPGCLC, human primordial germ cells; hiMeLC, human induced mesoderm-like cells; cyESCs, cynomolgus monkey embryonic stem cells; cyPGCLC, cynomolgus monkey primordial germ cells.

with fertilization capability were successfully obtained through in vitro culture of human testicular tissue [112]. Within these studies, it is important to recognize the species-specific differences that exist between mice and primates. For instance, SOX2 is expressed in mouse PGC (LC) but not in primate PGC (LC), while SOX17 is essential for primate PGC (LC), but not for those in mice or rats. Such differences underscore the value of NHPs as exceptional models for germ cell research.

The path to developing and translating human IVG for clinical applications is undoubtedly challenging due to safety and ethical considerations. Nevertheless, these advancements lay the groundwork for IVG in humans and other species, paving the way for groundbreaking treatments for infertility and significant contributions to conservation efforts.

Stem cell therapy: challenges in efficacy and safety

The primary challenges in the field of stem cell therapy lie in ensuring its efficacy and safety. Two of the most pressing issues are tumorigenicity and immunogenicity [17].

Cells derived from PSCs pose the risk of tumorigenesis or teratoma formation if the cellular products contain undifferentiated or immature cells. The first step in mitigating this risk is the development of precise *in vitro* differentiation techniques. Additionally, to meet the stringent safety standards required for clinical trials, more advanced cell purification processes are necessary. Selecting appropriate cell lines for safe transplantation is also crucial. For instance, if reprogramming factors in iPSCs remain active, there could be an increased risk of tumor formation. Tumorigenicity can also sometimes arise due to genetic mutations incurred during the *in vitro* culturing process of PSCs.

The risk of teratoma formation due to genetic variations is fairly common in cells expanded *in vitro* prior to transplantation. The process of cellular expansion *in vitro* can inadvertently introduce genetic alterations, which may include chromosomal deletions, duplications, or rearrangements. These chromosomal abnormalities can be monitored through karyotype analysis. Cells with such abnormalities are not suitable for cell therapy. Additionally, smaller scale mutations like single nucleotide variations (SNVs) and copy number variations (CNVs) can be detected through next-generation sequencing. ESCs and

iPSCs still exhibit differences in their differentiation capacities and gene expressions [113]. Specifically, iPSCs may not reach a fully dedifferentiated state during the reprogramming process, which could trigger T-cell-dependent immune responses [114]. Through these analyses, we emphasize the need for more in-depth research to resolve these critical issues, thereby advancing the clinical application of stem cell therapies [115].

Immune rejection is undeniably a central issue in cell therapy. Traditionally, allogenic transplant-induced rejection has often been mitigated through the use of immunosuppressive drugs, which may require long-term or even lifelong maintenance. Several potential avenues are available to address this issue. Clinical studies for ischemic stroke have shown that stem cell therapy can improve patient function even without immunosuppressive treatment. This is because immunosuppression may increase the risk of post-stroke infections [116]. Another method to alleviate rejection is considering the compatibility of human leukocyte antigen (HLA) alleles between the transplanted stem cells and the host. This practice has been widely implemented in hematopoietic stem cell transplantation. However, creating a bank of human PSCs that covers thousands of unique HLA haplotypes is not practical in real-world applications.

The use of patient-specific cells with already matched HLA haplotypes opens new possibilities for autologous PSCs therapy. Despite this, the immunogenicity of autologous PSCs remains a matter of debate. Particularly, in patients with familial genetic mutations, it is unclear whether DA neurons generated from patient-derived iPSCs are suitable for treatment.

While studies indicate that DA neurons derived from patients with sporadic PD may be functional in the brain, caution should be exercised when considering iPSCs for cell replacement therapy due to the potential inability to produce healthy DA neurons [117].

By examining these complex factors and potential solutions, we recognize the multifaceted nature of the immune rejection issue, as well as the many challenges that must be addressed before further clinical application of cell therapies [118].

Numerous studies have preliminarily demonstrated the significant safety and efficacy of cell therapy. For instance, in a Phase II clinical trial for treating ALS [119], the intrathecal injection of bone marrow MSCs (BM-MSCs) twice showed potential clinical benefits for at least six months and proved to be safe. The anticipated mechanism of action may involve a shift from pro-inflammatory to anti-inflammatory states facilitated by BM-MSCs, laying the groundwork for future large-scale, randomized, double-blind phase III clinical trials [120].

The field of cell therapy has also seen a transition from using MSCs to utilizing nutrients or extracellular vesicles secreted by MSCs (MSC-EVs). For example, Neurotrophic factors (NTFs) have been proven to extend the lifespan of motor neurons in ALS patients, and their combined delivery has shown significant synergistic effects.

When it comes to treating MS and lyme disease (LD), both intramuscular (IM) and intravenous (IV) injections of human ESCs have resulted in significant improvements in patients' skills, overall stamina, cognitive abilities, and muscle strength [121]. This further confirms the safety and efficacy of the treatment. However, more well-designed clinical trials and long-term follow-ups are still needed for evaluating the long-term effects and safety of human ESC therapy for MS and LD.

For the treatment of pediatric cerebral palsy (CP), the use of human ESCs has significantly improved motor function and cognitive abilities in CP patients without severe adverse reactions, providing ample encouragement for the further application of human ESCs in the field of CP treatment [122]. Although these clinical study results are encouraging, caution must be exercised in interpreting them due to the relatively small sample sizes and non-controlled study designs. For instance, in a study on chronic stroke, only about 4.7 % of patients were included in the research due to highly selective screening criteria [123].

In summary, while preliminary data present a promising outlook for cell therapy (Table 1), more refined, large-scale, and long-term clinical studies are essential for a comprehensive and accurate assessment of its long-term efficacy and safety. NHPs offer an ideal platform for the development and optimization of cell therapy protocols for clinical applications. This includes determining the best sites and methods for cell transplantation, a step crucial before clinical implementation. As research deepens, the choice of cell transplantation site has been identified as a key factor in enhancing treatment efficacy and safety. For instance, in ischemic stroke therapy, researchers have started opting for the striatum rather than the white matter as the target location for cell transplantation. This choice is based on the striatum's closer proximity to subcortical neuronal groups, while white matter injection could further damage axons [116]. In the field of diabetes cell therapy, treatment protocols have evolved as well. The anterior rectus sheath is now considered a more optimal transplantation site as it effectively supports the survival and long-term function of pancreatic islet cells [124]. Moreover, the surgery for transplantation at this site is safer and more convenient, allowing for monitoring and management via medical imaging.

Table 1: Summary the application of stem cell therapy in the treatment of diseases.

Disease	Cell type	Transplanted site	Cell mass	Treatment effect	References
Liver failure	Porcine hepatocytes	Intraperitoneal of baboons	6–10 × 10^8 cells	One animal developed liver failure but survived to 21 days, and three animals recovered completely with normal liver function.	[69]
Type 1 diabetes	hCiPSC-islets	The portal vein	3–6 × 10^8 cells	Fasting and average pre-prandial blood glucose levels significantly decreased in all recipients, accompanied by meal or glucose-responsive C-peptide release and overall increase in body weight.	[81]
Type 1 diabetes	hCiPSC-islets	Underneath the abdominal anterior rectus sheath	40,000–48,000 islet/kg	Fasting and average pre-prandial blood glucose levels significantly decreased in all recipients, accompanied by meal or glucose-responsive C-peptide release and overall increase in body weight.	[82]
Myocardial infarction	hESC-CMs	The infarct region and adjacent border zones	1 × 10^9 cells	hESC-CMs can remuscularize substantial amounts of the infarcted monkey heart.	[85]
Myocardial infarction	Cardiomyocytes	The infarct and peri-infarct region	~7.5 × 10^8 cells	Remuscularization of the infarcted macaque heart with human myocardium provides durable improvement in left ventricular function.	[86]
Retinal degeneration	Retinal pigment epithelium (RPE)	Under the macula	/	The transplant was able to recover *in vivo* and maintained healthy photoreceptors.	[92]
Premature ovarian insufficiency	Thecal stem cells (TSCs)	Ovary	~4 × 10^7 cells	Significantly improves hormone levels, rescues the follicle development, promotes the quality of oocytes and boosts oocyte maturation/fertilization rate.	[93]
AD	BMMSCs	The tail vein	1 × 10^6 cells	A reduction in microglial numbers in the cortex and in microglia size, not observe transplant-related acute toxicity or adverse events.	[125]
AD	BMMSC-EVs	Intracranial	~3 × 10^6 cells	Effective at reducing the Aβ plaque burden and the amount of dystrophic neurites in both the cortex and hippocampus.	[48]
AD	MSCs	Hippocampus, Precuneus	3 × 10^6 cells	Administration of MSCs into the hippocampus and precuneus by stereotactic injection was feasible, safe, and well tolerated.	[126]
AD	hUCB-MSCs	Intracerebroventricular	1 × 10^6 cells	hUCB-MSCs can attenuate Aβ42-induced synaptic dysfunction by regulating TSP-1 release, thus providing a potential alternative therapeutic option for early-stage AD.	[127]
MPTP PD	NPCs	Putamen	3 × 10^4 cells	Successfully induced the development of DA neurons using a feeder-free culture method. Moreover, the cells survived for 6 months in the brain of a primate PD model.	[128]
MPTP PD	DA neurons	Putamen	2 × 10^5 cells	An increase in spontaneous movement of the monkeys after transplantation, the mature dopaminergic neurons extended dense neurites into the host striatum.	[54]
MPTP PD	DA neurons	Putamen	2 × 10^5 cells	iPSC-derived midbrain dopamine neurons for up to 2 years following autologous transplantation, and increased motor activity.	[55]
MPTP PD	DA neurons	Caudate, Putamen	1 × 10^5 cells	Significant long-term improvement in motor and depressive behaviors, supporting the feasibility of autologous cell therapy.	[56]
MPTP PD	DA neurons	Caudate, Putamen	5 × 10^5 cells	Produced variable but apparent behavioral improvement for at least 24 months, a slight DA increase in the striatum correlates with significant functional improvement.	[59]

Table 1: (continued)

Disease	Cell type	Transplanted site	Cell mass	Treatment effect	References
MPP + PD MPTP PD	DOPA-MSCs	Putamen	6×10^6 cells	Provide homotopic reconstruction of midbrain dopamine pathways by restoring striatal dopamine levels, and safely and long-term (up to 51 months) correct motor disorders and nonmotor deficits.	[57]
Ischemic stoke	MSC-EVs	Intravenously, Intraperitoneally	2×10^6 cells	Significantly decreased infarct volume and neuronal injury and reduced neurological deficits.	[129]
Ischemic stoke	hPMSCs	Intraperitoneally	5×10^5 cells	Given immediately after reperfusion in the stroke model.	[60]
Ischemic stoke	NSCs	Putamen	2×10^6 cells 5×10^6 cells 1×10^7 cells 2×10^7 cells	No adverse events and were associated with improved neurological function.	[96]
Ischemic stoke	MSCs	The peri-infract subcortical stoke region	2.5×10^6 cells 5×10^6 cells 1×10^7 cells	Implantation of SB623 cells in patients with stable chronic stroke was safe and was accompanied by improvements in clinical outcomes.	[103]
CP	Human ESCs	Intramuscular, intravenous	7.5×10^5 cells 8×10^6 cells	No serious adverse events were observed, improvement in cognitive skills CP patients.	[102]
MS	HSCs	Intravenously	1×10^9 cells	Modified balance of regulatory and pro-inflammatory lymphocytes.	[64]
MS	Human ESCs	Intramuscular, intravenous	1.6×10^6 cells	Both the patients showed remarkable improvement in their functional skills, overall stamina, cognitive abilities, and muscle strength.	[101]
ALS	MSCs	Intramuscular, intrathecal	1×10^6 cells	IT and IM injection of MSC-NTF cells in patients with ALS is safe and well tolerated over the study follow-up period.	[100]
ALS	BMMSCs	Intrathecal	1×10^6 cells/kg	ALS plausible action mechanism is that BM-MSCs mediate switching from pro-to anti-inflammatory.	[99]

ESCs, embryonic stem cells; hCiPSC-islets, islets from human chemically iPSCs; hESC-CMs, human embryonic stem cell-derived cardiomyocytes; RPE, retinal pigment epithelium; TSCs, thecal stem cells; AD, alzheimer's Disease; MSCs, mesenchymal stem cells or mesenchymal stromal cells; BMMSCs, bone marrow MSCs; EVs, extracellular vesicles; NSCs, neural stem cells; NPCs, neural progenitor cells; hUCB, human umbilical cord blood; DA, dopaminergic; DOPA-MSCs, dopaminergic MSCs; HSCs, hematopoietic stem cells; hPMSCs, human placenta mesenchymal stem cells.

Advancements in MSC therapies

Researchers around the globe are on a quest for stable, safe, and highly accessible sources of stem cells that hold significant promise for regenerative medicine. Cells isolated from mouse bone marrow, which exhibit plastic adherence properties and form spindle-shaped colonies, have been termed colony-forming unit fibroblasts [130]. Owing to their ability to differentiate into specialized cells originating from the mesoderm, these cells have been designated as mesenchymal stem cells or mesenchymal stromal cells. MSCs, also recognized as multipotent stem cells, are found in adult tissues from a variety of sources, including both murine and human. These cells are endowed with unique qualities such as self-renewal, multipotency, ease of accessibility, and the ability to expand in culture *in vitro*. Coupled with their remarkable genomic stability and minimal ethical concerns, MSCs have gained substantial attention in the fields of cell therapy, regenerative medicine, and tissue repair [131].

ESC-derived MSCs

Yan et al. investigated the restorative effects of human ESC-derived MSCs spheres on MS using a primate animal model. First, they established a primate experimental autoimmune encephalomyelitis (EAE) model using a cynomolgus macaque, and then treated the model animals with human-derived MSC in spheres (MSCsp). The results showed that the injection of MSCsp significantly alleviated the clinical symptoms, brain injury, and neural demyelination of

EAE monkeys [132]. In 2019, Jiang et al. tested the efficacy of MSCs derived from human ESCs on spontaneous osteoarthritis in monkeys. They used intra-articular injection of MSCs to treat six macaques with spontaneous osteoarthritis. The results showed that human BM-MSCs spheres and allogeneic BM-MSCs can prevent the further development of macaque osteoarthritis and improve osteoarthritis [133]. In the same year, Guo et al. used a drug-induced acute liver failure (ALF) monkey model to explore the efficacy and safety of hUC-MSC-based ALF treatment. They found in the study that peripheral infusion of hUC-MSCs can effectively inhibit the activation of c-Mos and its IL-6 secretion, thereby preventing the monkeys from developing lethal ALF [134]. Their results suggest that hUC-MSC-based ALF therapy has a promising outlook, but further investigation and validation are needed before it can be applied to clinical practice.

Bone marrow MSCs

Go et al. used bone marrow-derived MSCs to treat aged monkeys with cortical injuries, exploring the myelination conditions of the monkeys after cortical injury with and without MSCs derived extracellular vesicles (MSC-EV) treatment by intravenous. The results showed that EVs could reduce myelin damage and enhance myelin maintenance in the aging brain, providing theoretical support for the use of MSCs in cortical injury repair treatment [135]. Mohsen Emadedin et al. conducted a clinical trial for intra-articular implantation of autologous BM-MSCs for the treatment of knee osteoarthritis (OA). They randomly assigned knee OA patients into two groups: a group receiving intra-articular injection of MSCs (40×10^6 cells), or a group injected with 5 mL of saline (placebo). Follow-up was done 6 months after the operation. At 3- and 6-months post-cell implantation, pain levels and functional improvement of patient-reported outcomes were assessed based on visual analogue scale (VAS), the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and its subscales, walking distance, painless walking distance, standing time and knee flexion, compared with the placebo group [136]. The results showed that patients treated with MSCs had significant improvements in all aspects compared to those treated with placebo. No major adverse events associated with MSC treatment occurred during the period. Anders B Mathiasen et al. conducted a study on the treatment of ischemic heart failure patients with BM-MSCs. Patients were randomly assigned at a 2:1 ratio to receive either intramyocardial injection of MSCs or a placebo injection. The primary endpoint of the study was changes in left ventricular end-systolic volume (LVESV) as measured by magnetic resonance imaging or computed tomography. Patients were followed clinically for 12 months, hospitalization and survival data were retrieved for 4 years. Results showed that at 12 months, the LVESV was significantly reduced in the MSC group compared with the control group. Moreover, a significant reduction in the number of hospitalizations for angina pectoris was observed in the MSC group after 4 years [137]. No side effects were observed during the trial. Marieke C Barnhoorn et al. reported on a long-term evaluation study of the treatment of Crohn's disease anal fistula with bone marrow-derived MSCs, which recorded a 4-year assessment of 21 refractory Crohn's disease anal fistula patients treated with BM-MSC at Leiden University Medical Center from 2012 to 2014. The patients were divided into three groups, each receiving injections of different dosages of BM-MSCs: 1×10^7 (group 1, n=5), 3×10^7 (group 2, n=5), 9×10^7 (group 3, n=5). During the period, the closure of the patients' fistulas was observed, the levels of anti- HLA antibodies were assessed, and the patients underwent pelvic magnetic resonance imaging and rectoscopy. The results showed that among the 15 patients treated with BM-MSCs, 13 (87 %) underwent long-term follow-up. Two were non-MSC-related malignancies, and no serious adverse events related to BM-MSC treatment occurred during the period. In experiment group 2 (n=4), all patients' fistulas were closed 4 years after treatment. Forty-three percent of patients in group 1 (n=4) and group 3 (n=5) had their fistulas closed. Anti-HLA antibodies were not detected in all patients at 24 weeks and 4 years after treatment. Meanwhile, MRI showed that pelvic fistulas significantly narrowed 4 years later [138]. Similarly, in 2023, Catherine Reenaers et al. conducted a clinical trial to assess the safety and clinical outcomes of bone marrow-derived MSCs injection treatment for Crohn's disease anal fistulas, evaluate the magnetic resonance imaging (MRI) evolution of the fistulas, and determine the factors associated with fistula closure [139]. The results also showed the safety and effectiveness of bone marrow-derived MSCs for the treatment of Crohn's disease anal fistulas. Grégoire et al. conducted a phase I/II clinical trial of BM-MSCs treatment for severe COVID-19 patients. They treated 8 severe COVID-19 patients with MSCs therapy. The patients received three intravenous infusions of bone marrow-derived MSCs every three days, and all patients concurrently received standard supportive treatment. These 8 patients were then compared with 24 control group patients, and the results showed that the 28-day and 60-day survival rates of the MSC group were significantly higher than those of the control group and no serious adverse events occurred during the period [140].

Adipose tissue-derived MSCs

Lee et al. conducted a Phase IIb randomized clinical trial to evaluate the efficacy and safety of a single intra-articular injection of adipose-derived MSCs (AD-MSC) in treating patients with knee osteoarthritis. They administered AD-MSC injections to 12 patients (MSC group), while the control group (12 people) was given normal saline injections. After six months of observation, it was found that intra-articular injections of autologous AD-MSC provided satisfactory functional improvement and pain relief for patients with knee osteoarthritis [141]. Meanwhile, no adverse events were caused during the six-month follow-up. However, more comprehensive data would require larger sample sizes and long-term follow-ups. Julien Freitag et al. also conducted a study in 2019 to evaluate the efficacy of autologous AD-MSC in treating pain, function, and disease modification of knee osteoarthritis. They randomly divided 30 symptomatic knee osteoarthritis patients into three groups. Two treatment groups received intra-articular AD-MSC therapy, and no serious adverse events were observed during this period [142]. At the same time, both groups that received AD-MSC showed alleviation and improvement in pain and function when the 12-month follow-up was completed. Moon et al. conducted a study on the potential of AD-MSC for the treatment of diabetic foot ulcers. In their study, 59 patients with diabetic foot ulcers were randomly divided into an AD-MSC treatment group (n=30) and a polyurethane film-treated control group (n=29). AD-MSC hydrogel or polyurethane film was applied to the diabetic wounds weekly. These wounds were tracked and observed for up to 12 weeks. The results showed that the degree of wound closure in the treatment group was greater than the control group at both week 8 and week 12. At the same time, no serious adverse events related to allogeneic AD-MSC treatment were observed during this period. Thus, allogeneic AD-MSC could be effective and safe for the treatment of diabetic foot ulcers [143]. In 2021, Dantas et al. used adipose-derived matrix cells together with cholecalciferol for the treatment of type 1 diabetic patients. They divided the patients into three groups. Group 1 received one dose of allogeneic AD-MSC (1×10^6 cells/kg) and cholecalciferol 2,000 IU/d treatment, Group 2 received cholecalciferol treatment, and Group 3 received standard treatment, each for six months. C-peptide (CP), insulin dose, and HbA1c were measured at the time of injection (T0), 3 months after treatment (T3), and 6 months after treatment (T6). The results showed that allogeneic ASC + daily cholecalciferol treatment without immunosuppression was safe and might have some protective effect on the β-cells of newly onset type 1 diabetes patients [144].

Umbilical cord tissue-derived MSCs (UC-MSCs)

In 2018, Park et al. conducted a Phase Ia clinical trial for the treatment of rheumatoid arthritis (RA) with MSCs derived from umbilical cord blood. 9 patients were divided into 3 groups, each receiving different dosages of hUC-MSCs (2.5×10^7, 5×10^7, and 1×10^8 cells respectively) [145]. Clinical and safety assessments were carried out throughout the study, which included measuring serum cytokines 24 h before and after injection. The results showed no significant toxicity events within 4 weeks of infusion. Decreases in serum erythrocyte sedimentation rate and 28-joint disease activity score (DAS28) were recorded after 4 weeks. The study concluded that short-term safety was assured in this Phase Ia trial using hUC-MSCs infusion for established RA patients. Matas et al. published a study on the treatment of knee osteoarthritis using UC-MSCs. Symptomatic OA patients were randomly assigned to receive hyaluronic acid treatment (HA, n=8), single-dose (2×10^7) UC-MSCs treatment (MSC-1, n=9), or repeated dose UC-MSC treatment ($2 \times 10^7 \times 2$) [146]. The study reported no serious adverse events throughout and patients experienced alleviated pain. Zhao et al. conducted a study on the treatment of late-stage retinitis pigmentosa with UC-MSCs. They injected a dose of 10^8 UC-MSCs intravenously into 32 subjects and followed up after 12 months. No serious local or systemic adverse effects were observed, and most patients reported an improvement in best corrected visual acuity (BCVA) within the first 3 months. The results indicated that intravenous infusion of UC-MSCs might be a promising treatment for late-stage retinitis pigmentosa (RP) patients [147]. Shi et al. reported a study on the improvement of liver function in patients with decompensated liver cirrhosis (DLC) using UC-MSCs. They divided 219 HBV-related DLC patients into a control group (n=111) and a UC-MSC treatment group (n=108), administering three infusions of UC-MSCs every 4 weeks, with standard treatment only for the control group. The study concluded that UC-MSC treatment significantly improved patients' liver function and there were no observed side effects or treatment-related complications [148]. Kim et al. conducted a phase I clinical trial using human UC-MSCs for AD patients. They recruited 9 mild-to-moderate AD dementia patients from the hospital, injecting low doses (1.0×10^7 cells/2 mL) into 3 patients and high doses (3.0×10^7 cells/2 mL) into 6 patients

via an Ommaya reservoir. Patients were followed for 12 weeks and then for an additional 36 months [149]. The results indicated that the method of repeatedly injecting human umbilical cord blood (hUCB)-MSCs into the lateral ventricles using an Ommaya reservoir was feasible, safe, and well-tolerated. Dilogo et al. used UC-MSCs as an adjunctive therapy for novel coronavirus infection patients. In their study, 40 randomly selected severe COVID-19 patients were divided into 2 groups, 20 received a 1×10^6/kg dose of UC-MSCs intravenously and the other 20 were given a 100 mL 0.9 % SS as a control. The results showed that the survival rate in the UC-MSCs group was 2.5 times that of the control group (p=0.047). No significant adverse events occurred during this period, suggesting that intravenous injection of UC-MSCs could play a good role in the adjunctive treatment of severe COVID-19 patients [150]. Zang et al. published a clinical trial to determine the efficacy and safety of UC-MSCs in Chinese adults with type 2 diabetes (T2DM). They divided 91 patients into two groups, one received an intravenous infusion of UC-MSCs once every 4 weeks for a total of three times (n=45), and the control group received a placebo (n=46). The results indicated that the percentage reduction in insulin was significantly higher in the UC-MSCs group and glucose infusion rate (GIR) was significantly increased, suggesting that UC-MSCs transplantation may be a potential treatment for Chinese adults with T2DM [151].

However, like other cells, MSCs also age, which is specifically manifested as a decrease in cell proliferation ability, phenotypic abnormalities, and a decrease in differentiation ability [152]. MSCs derived from iPSCs are expected to overcome the practical limitations of MSCs in clinical applications. Studies have shown that EVs derived from MSCs have the potential to replace MSCs in immunomodulatory therapy. Research by Ye et al. shows that large extracellular vesicles secreted by human iPSC-derived MSCs can improve tendinopathy by regulating macrophage heterogeneity [153]. Moreover, Kim et al. also found that extracellular vesicles produced by iPSC-derived MSCs showed good therapeutic potential for Sjogren's syndrome [154]. Peng et al. demonstrated that small extracellular vesicles secreted by iPSC-derived MSCs improve sepsis-induced pneumonia and lung injury by delivering miR-125 b-5p [155]. It is reported that MSCs are considered a new therapy for inflammatory bowel disease (IBD). A study by Yang et al. showed that in an inflammatory bowel disease model, human-iPSCs-derived MSC promote healing through TNF-α stimulated gene-6 [156]. MSC also have good applications in repair and regenerative medicine. Research by Kim et al. showed that exosomes secreted by iPSCs derived MSC can accelerate skin cell proliferation [157]. Jungbluth et al. demonstrated that human iMSCs combined with calcium phosphate granules (CPG) can promote bone regeneration in minipigs [158]. Xu et al. transplanted human iPSC-derived MSC into white rabbits with cartilage defects, and cartilage-like tissues were observed in the experimental group at 6 weeks, indicating that iPSCs may repair cartilage defects *in vivo* [159]. Recent studies have shown that iPSC-derived MSC alleviate anxiety and neuroinflammation in aging female mice, which may provide a new option for treating hormone disorders and neurological diseases related to perimenopause in the female aging process [160]. In addition to this, clinical research on MSCs derived from iPSC is also underway. Bloor et al. have conducted clinical trials on the production, safety, and efficacy of iPSC-derived MSCs in acute steroid-resistant graft-versus-host disease [125].

In summary, MSCs are a type of adult stem cell with low immunogenicity, high proliferative ability, and multidirectional differentiation potential. They can be isolated from a wide range of tissues, including fat, umbilical cord, placenta, bone marrow, umbilical matrix, etc., and are widely used in disease treatment, injury repair, basic and clinical research (Figure 4). Inevitably, the clinical application of MSCs also faces limitations due to factors such as stability and safety. For instance, the quantity obtainable from a single donor is limited, as is their ability to proliferate and implant in the body. The diversity of *in vitro* culture plans and source heterogeneity also pose challenges. Despite this, their application in the treatment of many diseases has gradually gained recognition. NHPs, as a class of animals highly similar to humans, make for a good animal model choice in the study of human disease treatments based on MSCs. This has significant implications for the clinical translational application of stem cell therapy (Table 2).

Perspective

The future of cell therapy appears promising and multidimensional (Figure 5). The diversity of sources, including adult stem cells, PSCs and others, provides multiple opportunities for the treatment of a variety of diseases, including genetic disorders, autoimmune diseases, and various types of cancer. The remarkable potential of PSCs for differentiation and self-organization has dramatically transformed the landscape of 3D cell culture models. A key advantage of these models is their capacity to emulate *in vivo* microenvironments and cellular interactions, an aspect where conventional 2D cell culture models fall short. The advent of techniques to create embryo-like structures, including blastoids and gastruloids, from PSCs has significantly broadened our experimental access to embryogenesis, thereby offering enormous potential for modeling

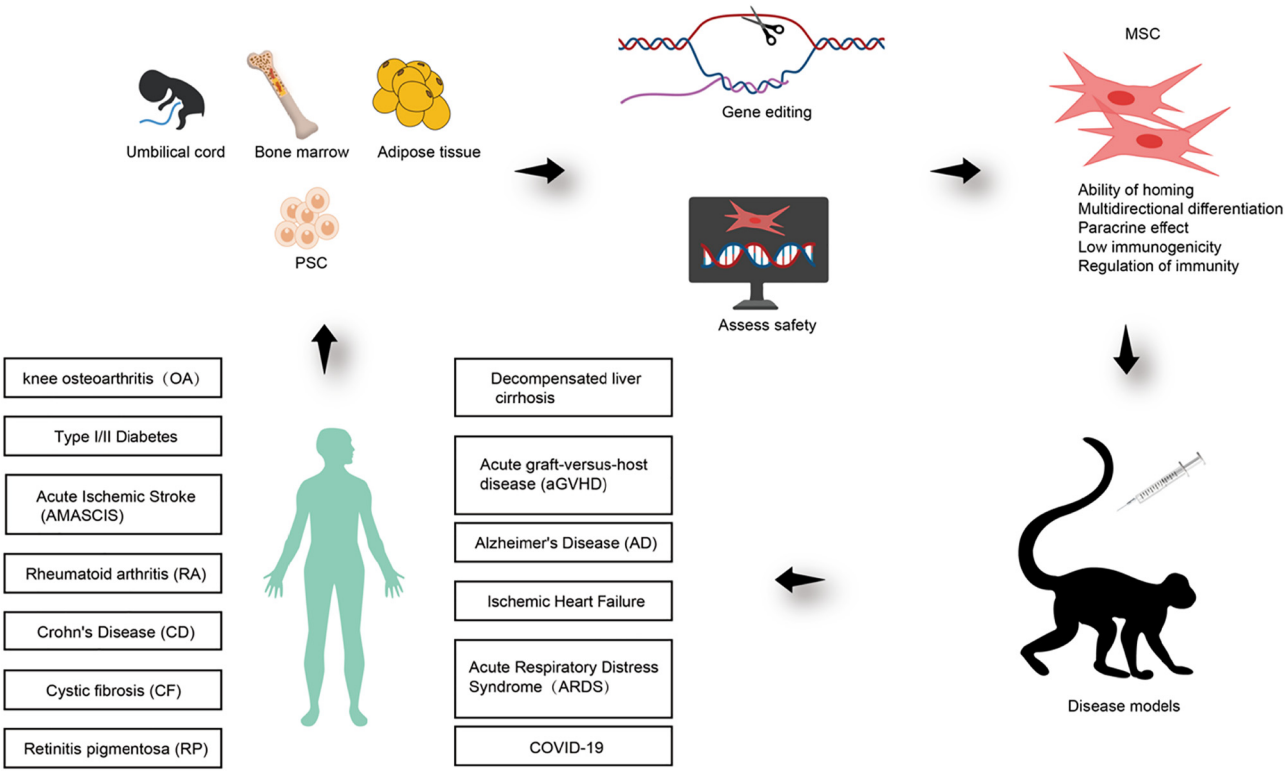

Figure 4: Application of MSCs in NHPs disease models and clinical practice. MSCs have the characteristics of multi-directional differentiation, homing capacity, paracrine properties and low immunogenicity, which can be isolated from tissues or differentiated from PSCs. Validated or gene-edited MSCs can be used in NHPs disease models or clinical disease treatment research. MSCs, mesenchymal stem cells or mesenchymal stromal cells; NHPs, non-human primates; PSCs, pluripotent stem cells.

human development. Furthermore, organoids have provided exciting new opportunities to enhance our understanding of stem cell biology, tissue regeneration, homeostasis, and disease mechanisms. Moreover, the potential of creating chimeras with NHPs and human cells opens up a new dimension in biomedical research. It allows the study of human cells and tissues within the context of a whole organism, thereby providing unprecedented insights into human biology and disease progression. These chimeric models could also be used for testing novel therapeutic interventions including drugs, gene therapies, and regenerative strategies [126]. The integration of gene-editing technologies with cell therapy has created an exciting frontier in the field of medicine. Gene editing, through tools such as CRISPR-Cas9, allows for precise modifications in the genetic material of cells, and this has enormous potential when combined with cell therapies. The most immediate application of gene-editing in cell therapy is in the treatment of genetic disorders. By editing the genetic material of a patient's cells (often stem cells), scientists can theoretically correct genetic defects. Once corrected, these cells can then be reintroduced to the patient, potentially providing a cure. This concept is already being explored in diseases such as sickle cell disease and beta-thalassemia, with encouraging results. With the advancements in biotechnology, we are progressively able to manipulate and control the behavior of cells in the lab. This not only enhances our understanding of the basic cell biology but also allows us to design specific treatments for diseases. The development of cell therapies has led to several successful treatments like CAR-T cell therapies for certain types of cancer.

However, there are still several challenges that need to be overcome, such as issues regarding cell stability, safety, delivery, and possible ethical concerns. Ensuring the controlled behavior of these cells after implantation is crucial to prevent unforeseen complications. Additionally, the process of getting regulatory approval for new therapies is a long and rigorous process, which can slow down the speed of progress.

In the future, NHPs are expected to continue playing a critical role in modeling complex human diseases,

Table 2: Summary the application of MSC therapy in clinical application.

MSC source	Disease	Infusion method	Enrollment number	Cell mass	NCT number	Reference
Adipose derived MSC	Knee osteoarthritis (OA)	Intraarticular injection	12	1×10^8 cells	Unknown	[141]
	OA	Intraarticular injection	30	1×10^8 cells	ACTRN 12614000814673	[142]
	Diabetic foot ulcers	Applied directly to debrided	22	/	NCT02619877	[143]
	Type 1 diabetes	Intravenous injection	7	1×10^6 cells/kg	NCT03920397	[144]
	COVID-19	Aerosol inhalation	7	2×10^8 nano vesicles/day	NCT04276987	[161]
	Acute ischemic stroke (AMASCIS)	Intravenous injection	19	1×10^7 cells/kg	NCT01678534	[162]
	Premature ovarian failure (POF)	Intra-ovarian injection	9	5×10^6, 1×10^7 1.5×10^7 cells	NCT02603744	[163]
	Rheumatoid arthritis (RA)	Intravenous injection	15	2×10^8 cells	NCT03691909	[164]
Umbilical cord derived MSC	RA	Intravenous injection	9	2.5×10^7 cells 5×10^7 cells 1×10^8 cells	NCT02221258	[145]
	OA	Intraarticular injection	18	2×10^7 cells	NCT02580695	[146]
	Retinitis pigmentosa (RP)	Intravenous injection	32	1×10^8 cells	ChiCTR-ONC-16008839	[147]
	Decompensated liver cirrhosis	Intravenous injection	108	5×10^5 cells/kg	NCT01220492	[148]
	Alzheimer's disease (AD)	The ommaya reservoir assisted intraventricular injection	9	1×10^7 cells 3×10^7 cells	NCT02054208 NCT03172117	[149]
	COVID-19	Intravenous injection	20	1×10^6 cells/kg	NCT04457609 NCT04457609	[150]
	COVID-19	Intravenous injection	24	$10 \pm 2 \times 10^7$ cells	NCT04355728	[165]
	Type 2 diabetes	Intravenous injection	45	1×10^6 cells/kg	NCT02302599	[151]
	Acute respiratory distress syndrome (ARDS)	Intravenous injection	9	1×10^6 cells 5×10^6 cells 1×10^7 cells	1066023736	[166]
Bone-marrow derived MSCs	OA	Intraarticular injection	19	4×10^7 cells	NCT01504464	[136]
	Ischemic Heart failure	Intra-myocardial injection	40	$7.75 \pm 6.79 \times 10^7$ cells	NCT00644410	[137]
	Diabetic foot ulcers	Applied directly to debrided	16	1.06×10^7 BM-MSCs/3 cm^2	Unknown	[167]
	Crohn's disease (CD)	Local injection	15	1×10^7 cells 3×10^7 cells 9×10^7 cells	NCT01144962	[138]
	OA	Intraarticular injection	60	1×10^8 cells	NCT02365142	[168]
	COVID-19	Intravenous injection	8	$1.5–3 \times 10^6$ cells/kg	NCT04445454	[140]
	Acute graft-versus-host disease (aGVHD)	Intravenous injection	260	2×10^6 cells/kg	NCT00366145	[169]
	Cystic fibrosis (CF)	Intravenous injection	15	1×10^6, 3×10^6, 5×10^6/kg	NCT02866721	[170]
	Ileal pouch-anal anastomosis (IPAA)	Local injection	22	7.5×10^7 cells	NCT04519684	[171]

especially those that are difficult or impossible to fully reproduce in other animal models. This includes neurodegenerative disorders like PD and AD, metabolic diseases such as diabetes, and various types of cancer. Advancements in gene editing technologies, such as CRISPR/Cas9, will further enhance the utility of NHP models in this area by enabling the creation of genetically-engineered models for specific diseases.

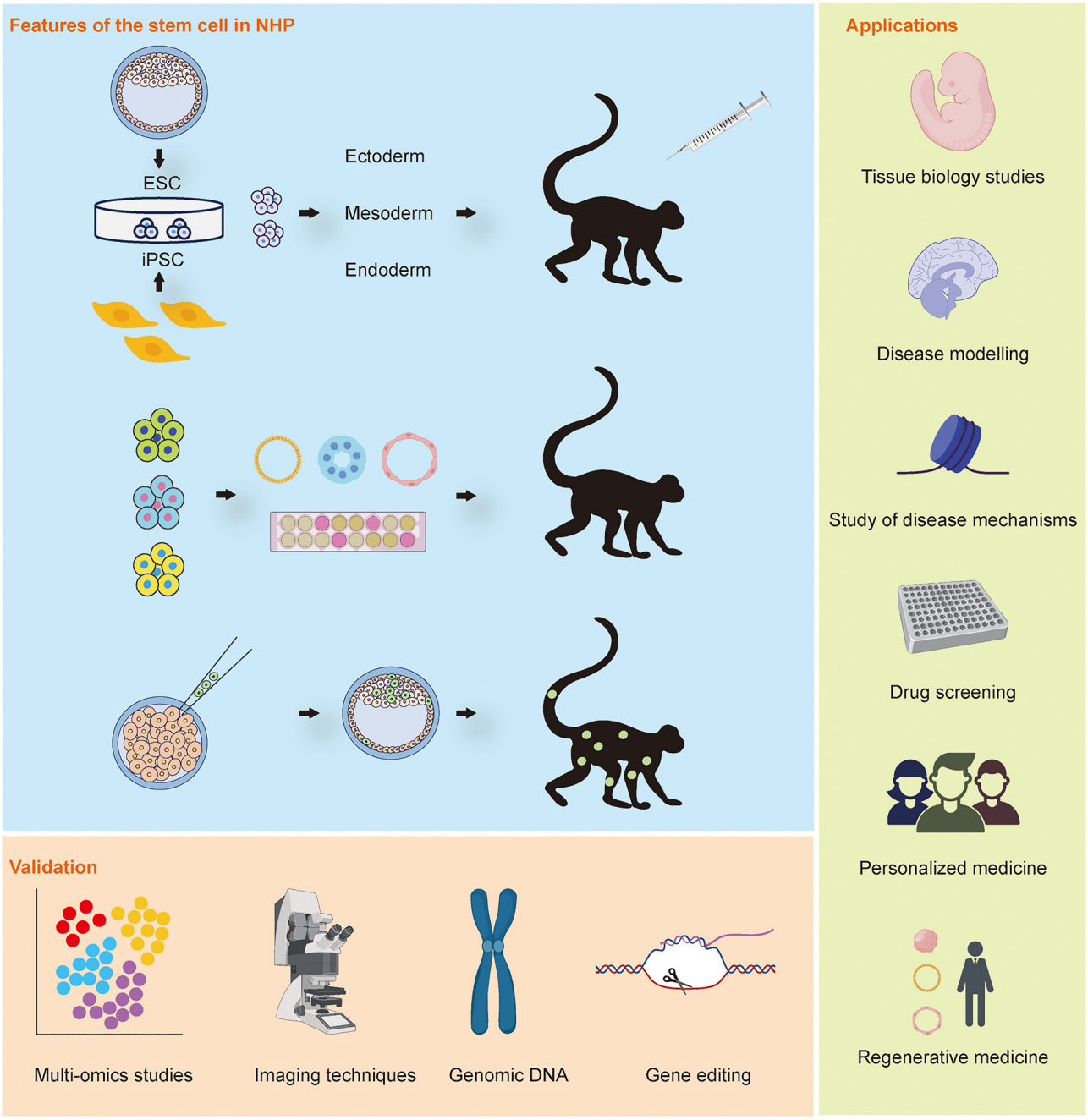

Figure 5: The features, applications, and validation strategies of stem cells in NHPs. Features: differentiation potential of pluripotent stem cells is highly significant for the treatment of animal disease models. These stem cells hold the potential to construct organoids that mimic actual organs and physiological functions. Additionally, their chimeric ability is valuable for studying interspecies chimeras. Applications: Stem cells, owing to their aforementioned characteristics, hold wide prospects in various fields such as tissue biology, disease model construction, disease mechanism research, drug screening, personalized medicine, and regenerative medicine. Validation: A combination of multi-omics analysis, imaging technology, genomic DNA studies, and gene editing technologies can help validate the safety and efficacy of PSCs in various fields. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; NHPs, non-human primates; PSCs, pluripotent stem cells.

NHP models closely mirror human genetics, physiology, and behavior, making them invaluable tools for studying human development and disease pathogenesis. Organoids derived from NHPs can faithfully recapitulate the intricate process of organ development and function. This detailed insight can pave the way for discovering novel preventative measures, diagnostic markers, and therapeutic targets for various diseases. Next, NHP models

combined with organoids can serve as robust platforms for preclinical testing of organoid-based therapies, thus allowing us to evaluate the integration and functionality of transplanted organoids in a relevant physiological context. Then, using NHP models, we can scrutinize the safety profiles of organoid therapies, monitoring for possible adverse effects post-transplantation. Likewise, the therapeutic efficacy of organoid transplants can be evaluated over time, tracking improvements in organ function and overall health.

NHP models undoubtedly possess significant potential in the field of immunotherapy research. They can be employed to test and optimize novel immunotherapies, including immune cell therapies (such as CAR-T cell therapies), antibody therapies, and vaccine therapies. Utilizing these models, researchers are able to assess the efficacy and safety of various therapies and aid in optimizing treatment strategies. In addition, NHP models can simulate a variety of human immune diseases, including autoimmune diseases, infectious diseases, and immunologically related cancers. Through these models, researchers can gain a deep understanding of disease progression and mechanisms, thereby promoting the development of new immunotherapy strategies.

These models also allow for the study of complex physiological and disease processes, which can help in the understanding of human diseases. However, the use of NHPs in research also raises ethical considerations, and it is important to ensure that their use is justified and minimized wherever possible. Overall, the future of cell therapy is full of opportunities and challenges. Continued collaboration between different scientific disciplines will be necessary to continue making advances in this exciting field.

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