Feature 2 New Developments in Regenerative Medicine and Innovative Drugs Using Human iPS Cells

On October 8, 2012, the Nobel Committee announced that the 2012 Nobel Prize in Physiology or Medicine would be awarded to Shinya Yamanaka, M.D., Ph.D., a professor at Kyoto University and Director of the Center for iPS Cell Research and Application (hereinafter referred to as "CiRA"). He is the 19th Japanese winner of the Nobel Prize, and the 2nd Japanese in the category of physiology or medicine since Susumu Tonegawa, Director of the RIKEN Brain Science Institute, who was the winner of 25 years ago. The prize was awarded for his achievement of the discovery that matured (differentiated) cells can be reprogrammed to have pluripotency. This achievement has overturned conventional wisdom and has caused the world to recognize that "the destiny of a cell can be changed."

Professor Yamanaka receiving a medal and a certificate from King Carl XVI Gustaf

Professor Yamanaka's motive for starting basic research began while he was a clinician and arose from his desire to find cures for patients with incurable diseases. He said, after receiving the Nobel Prize, "I will keep the medal carefully, but will never see it again. I will continuously and steadily do what I should do as a scientist." This shows that his will to clarify the fundamentals of diseases as well as the basic principles of his research field has never changed, even after receiving the Nobel Prize. Indeed he has achieved great success in the establishment of human iPS cells, but his new challenge is driven by his regret that he has "never saved even a single patient yet."

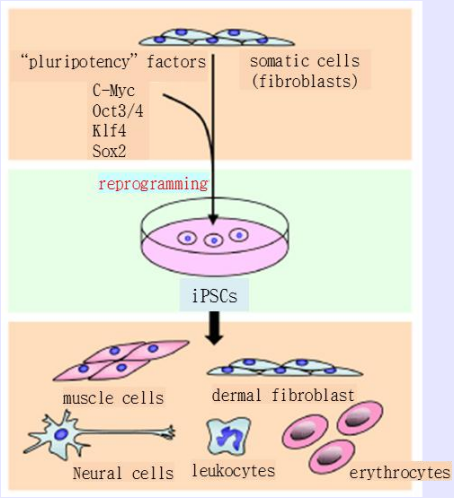
1 What is an iPS Cell?

(1) What is an iPS Cell?

iPS cells (induced pluripotent stem cells) are stem cells[1] which have an artificially induced ability to differentiate into almost any type of cell within the body. In concrete terms, when culturing somatic cells for several weeks after conducting the transfer of only four specific types of genes (Yamanaka Factors[2]) from among approximately 20,000 of human genes existing in the human somatic cells (including blood and skin cells), these cells are then initialized (reprogrammed)[3] to transform into pluripotent stem cells. An iPS cell is defined as a pluripotent stem cell which has the ability to differentiate into the cells of diverse tissues and organs, and to grow almost infinitely (Figure 1).

This method of iPS cell generation is a technological epoch that is relatively easy to engineer, and one which provides high reproducibility, making it a major breakthrough in stem-cell research.

Figure 1 / Achieving Induced Pluripotency



Source: Ministry of Education, Culture, Sports, Science and Technology (MEXT) iPS Cell Research Network

(2) Development of Pluripotent Stem-Cell Research and the Characteristics of iPS Cells

Regenerative medicine aims to restore the functions of damaged tissues and organs by transplanting cells, cultured in–vitro, into damaged or malfunctioning organs and tissue, and its research had been conducted before the establishment of iPS cells.

The major types of stem cells currently being researched for the realization of regenerative medicine are as follows: somatic stem cells[4], embryonic stem cells (hereinafter referred to as "ES cells"), and iPS cells (Table 2).

1 Cells having both the ability to differentiate into plural systems of cells (pluripotency) and the ability to maintain pluripotency even after cell division (replication competence).

2 Oct3/4, Sox2, Klf4, and c-Myc, these four types of genes form the transcription factors and are referred to as "Yamanaka Factors" as identified by Professor Yamanaka's team.

3 To reset matured (differentiated) cells into an immature state. It represents the ability to differentiate into almost any type of cell within the human body.

4 Also referred to as "adult stem cells" or "tissue stem cells." They are undifferentiated cells found inside the body of living organisms, and they divide infinitely, or replicate, and can generate any type of cells that make up the organs, and they potentially have the ability to regenerate whole organs from a few cells.

Table 2 / Characteristic Features of the Main Types of Stem Cells Used in Regenerative Medicine

		Stem Cell Type		
		Somatic stem cells (Adult stem cells)	Embryonic stem cells (ES cells)	Induced pluripotent stem cells (iPS cells)
	Derivation	Exists in body	Derived by destroying the embryo	Created from adult stem cells
Characteristic Features	Differentiation Potential	Limited	Pluripotent	Pluripotent
	Are there bio-ethical issues related to use of human embryos?	No	Yes	No
	Is rejection a possibility?	Only when using the cells of others	Yes, but not when using ES cells[1] derived from adult stem cells	Only when using the cells of others
	Are there problems with medical application?	Difficulties increasing and maintaining outside the body	Possibility of tumors	Possibility of tumors

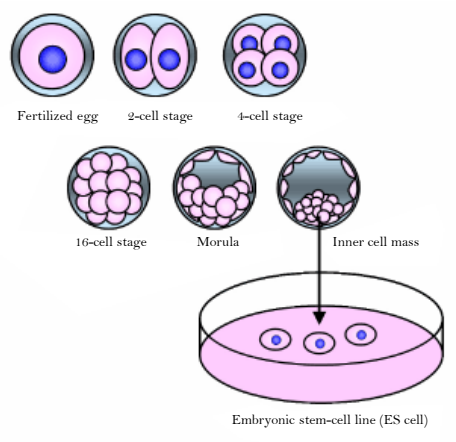
Source: Created by MEXT

All of these stem-cell types are in the process of research and development toward their practical application, and they have the following characteristics and issues.

Somatic stem cells, which originate in a living body, are free from both ethical issues and the risk of rejection when regenerating tissues using a patient's own cells; thus, they are expected to be an important supply source for cell transplantation. On the other hand, how to keep replication ability is a major issue when somatic stem cells are growing outside of living organisms.

Theoretically, ES cells and iPS cells can grow almost infinitely when they exist outside of living organisms by maintaining the pluripotency to differentiate into any type of tissue; thus, their potential application to regenerative medicine is drawing attention.

Figure 3 / ES Cell Generation Process



Source: Created by MEXT "The iPS Cells Research Network, iPS Cells Story No.5"

Human embryonic stem (ES) cells are generated by using fertilized eggs or embryos which are unused, or which would be disposed of after in infertility treatments (Figure 3); hence, they raise a bioethical issue as to whether they can be generated without destroying fertilized eggs or embryos that could potentially become human if they were returned to the womb. Furthermore, the genetic information inherited from the fertilized eggs might cause rejection due to the reaction of the immune systems of transplant recipients, yet it has been reported[2] that ES cells were successfully generated from

1 An ES cell is produced by the process of fusing a somatic cell nucleus with a denucleated egg cell in order to create a cloned embryo by the somatic cell nuclear transfer (SCNT) technique, and by forming a blastocyst outside the body of living organisms. It's possible to generate ES cells with identical genetic information and with no risk of rejection during transplant when human cloned embryos are created from the patient's own somatic cells.

2 Tachibana M., *et al.*,(2013)
Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer
Cell **153**, 1-11
http://dx.doi.org/10.1016/j.cell.2013.05.006

a human somatic cell nuclear transfer blastocyst formed by a somatic cell nuclear transfer (SCNT) technique in May 2013; thus, the possibility of their application to regenerative medicine is being explored.

iPS cells have overcome the major hurdles faced by ES cells and have blazed a trail toward the realization of regenerative medicine. As iPS cells use somatic cells, such as blood or skin cells, instead of fertilized eggs and embryos, so there are no bioethical issues that arise from their use. Furthermore, when generated from a patient's own cells, iPS cells are supposed to be free from the risk of rejection.

On the other hand, the common issue facing both iPS cells and ES cells is that there is a risk of tumors forming if cells that failed to differentiate remain in the transplanted cells; thus, studies to reduce such risk are continuously being conducted.

(3) Clarification of the Reprogramming Mechanism of iPS Cells

While research about the application of iPS cells to medicine are ongoing, the technological developments based upon the outcomes of basic research have also been promoted in parallel, and the following findings have been obtained so far: the establishment of a safer method for generating iPS cells that will lower the risk of tumors, the establishment of a method for inducing differentiation into target cells, and the development of a system to remove undifferentiated iPS cells.

In 2006, Professor Yamanaka's team identified four types of genes (Oct3/4, Sox2, Klf4, c-Myc), and clarified a method for inducing the reprogramming of somatic cells, but it has not yet been clarified as to which mechanism reprograms cells that have already differentiated. In the future, the clarification of the mechanism is expected to enable a safer and more efficient establishment of iPS cells; hence, the scientific community is looking forward to the progress of this research.

It is also important to accumulate fundamental knowledge about pluripotent stem cells, not only knowledge about iPS cells, but also about ES cells.

Column Feature

Other New Approach than iPS Cells

iPS cells have opened new pathways in the research of regenerative medicine and drug-discovery, but not everything has been clarified about the operations required for their application to transplant medicine, and there are still many hurdles to overcome; for example, reducing the risk of tumors, and establishing techniques to induce differentiation into target cells. In order to address these issues, the research of direct conversion, which seeks the realization of regenerative medicine without the intermediary use of iPS cells, is also ongoing.

Direct conversion is a technique of direct transformation into target cells without the intermediary use of iPS cells. In January 2010, Doctor Marius Werning and his team at Stanford University discovered that mouse fibroblasts that received a transfer of three types of genes (Asc1, Brn2, Myt1) directly transformed into neurons, and they released a report[1] about direct conversion into an entirely different lineage for the first time.

1 Vierbuchen T., *et al.*,(2010)
Direct conversion of fibroblasts to functional neurons by defined factors
Nature **463**(7284):1035-41
http://dx.doi.org/10.1038/nature08797. Epub 2010 Jan 27

2 Present State and Issues of Regenerative Medicine and Drug-Discovery Research

There could be a great opportunity for the broad medical application of iPS cells' ability to differentiate into almost any type of cell throughout the body and for the application of their almost infinite growth. Throughout the world, they are expected to become a pioneering technology for regenerative medicine, drug-discovery, disease-research, and gene therapy.

The entire scope of applications involving iPS cells to improve medical care is described below.

(1) The Present State of Regenerative Medicine and the Issues Facing It

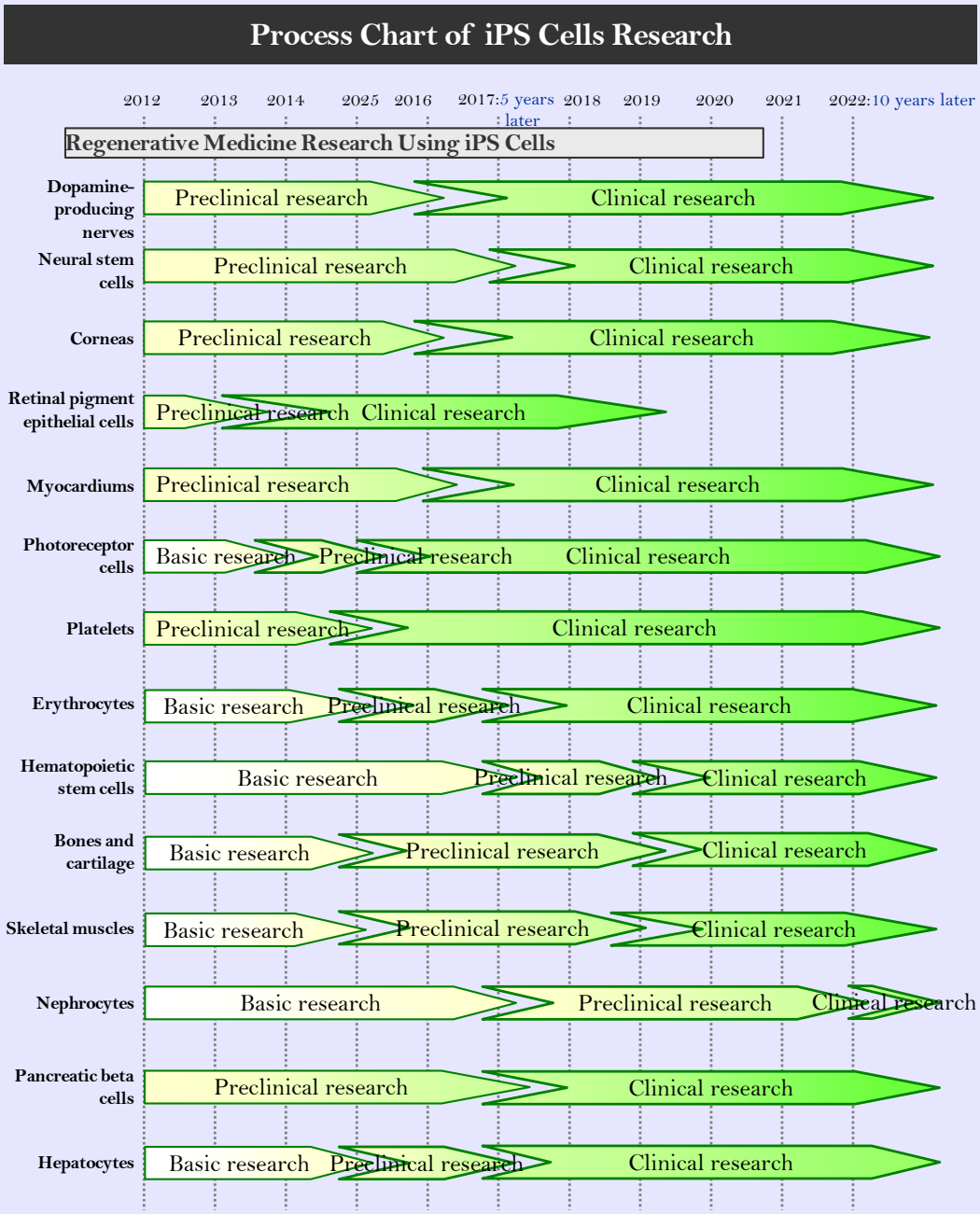
1) Present Approaches in Regenerative Medicine

Regenerative medicine aims to partially compensate for the lost functions of organs and tissues damaged by injuries and diseases by transplanting cells cultured in vitro, for which above-mentioned stem cells are used.

The present state of regenerative-medicine research in Japan that uses somatic cells or somatic stem cells include the following: 1) Clinical research: Approximately 70 examples of treatment for heart failure, hepatic cirrhosis, corneal injuries, cerebral infarctions, etc. (as of the end of April, 2013); 2) Clinical trials: Eight examples of product development for thermal injuries and cartilage deficiency (as of the end of April, 2013) were conducted or being conducted and two of those examples were already covered by insurance.

Regarding iPS cells, RIKEN and the Institute of Biomedical Research and Innovation (IBRI, operated by the Foundation for Biomedical Research and Innovation) have applied to the Ministry of Health, Labour, and Welfare (MHLW) in order to conduct clinical research on age-related macular degeneration using iPS cell-derived retinal pigment epithelial cells (to be described later). Clinical research is also expected to start on the following diseases: (in 2016) heart failure, platelet thrombocytopenia, and retinitis pigmentosa; (in 2017) spinal cord injuries and Parkinson's disease (decided by the Council for Science and Technology (CST), the Subdivision on R&D Planning and Evaluation, Life Science Committee, Stem Cell, Reproduction Medicine Strategy Taskforce, on February 1, 2013). (Figure 4)

Figure 4 / Process Chart of Regenerative Medicine Research Using iPS Cells



Source: Created by MEXT Council for Science and Technology (CST) the Subdivision on R&D Planning and Evaluation Life Science Committee Stem Cell, Reproduction Medicine Strategy Taskforce

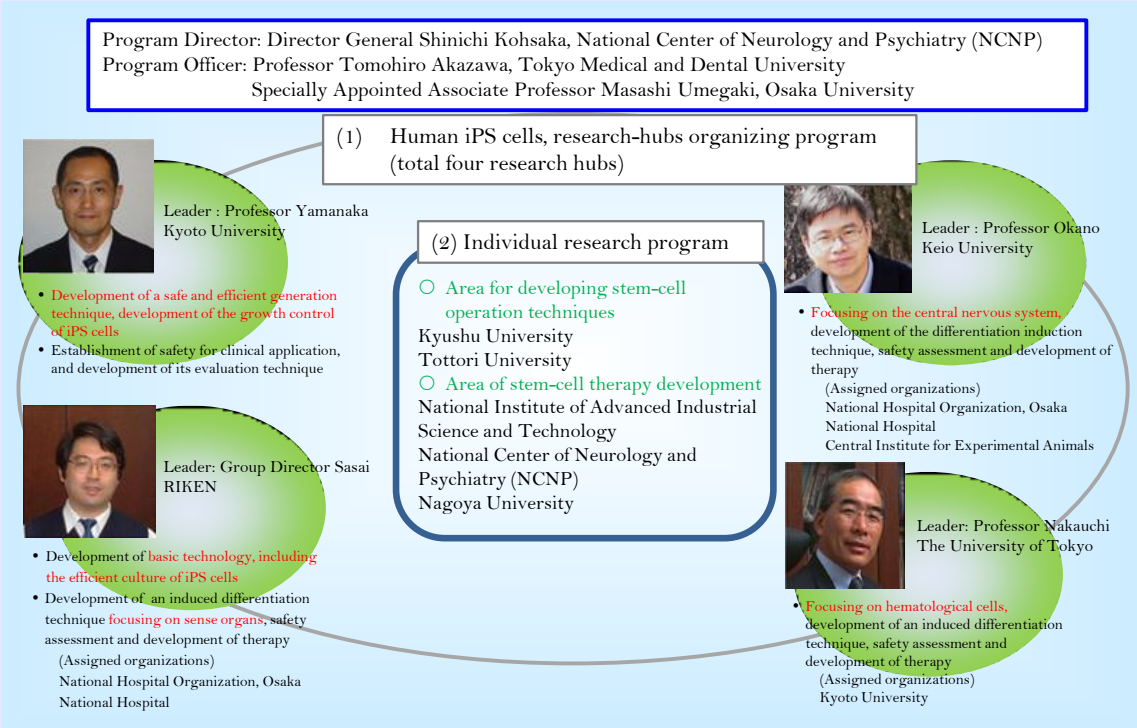
2) Benefits of Research in Regenerative Medicine

To really utilize the global benefits of iPS cells, MEXT has started "the Project for the Realization of Regenerative Medicine (2nd term)" since FY 2008.

As a result of the project, thus far, four domestic hubs have been established for the research of stem cells and regenerative medicine (Kyoto University, Keio University, the University of Tokyo, and RIKEN). Research focusing on iPS cells has been organized, and the general research of stem cells has also been intensively promoted (Figure 5). Consequently, there has been progress toward the practical application of stem cells, including the use of iPS cells in regenerative medicine.

Major benefits of the research conducted as part of this project are as follows.

Figure 5 / Framework of the Project for the Realization of Regenerative Medicine



Source: Created by MEXT, Stem-cell Reproduction Medicine Strategy Taskforce

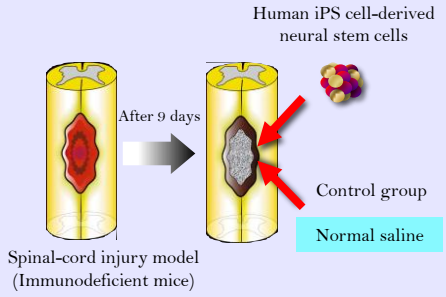
At Kyoto University, Professor Yamanaka's team is working on a study concerning the safe and efficient generation and growth control of iPS cells, the safety of its clinical application, and the evaluation of techniques. Regarding the method for iPS cell generation, safer generation has been enabled by the development of the safe transfer of reprogramming factors preventing gene integration into a genome[1], and the discovery of new reprogramming factors replacing c-Myc, which can act as an oncogene. They have also developed a more prospective method for its clinical application, including the removal of different-species-derived ingredients from a culture solution. Moreover, they have also achieved the following benefits: 1) the establishment of a method for inducing differentiation into the retina, hemocyte system, nervous system, myocardium, etc., 2) the establishment of an evaluation system of genomic

1 The word indicates all of the genes (genetic information) owned by living organisms. Its substance consists of DNA molecules existing in biological cells, which include genes and information controlling the expression of genes.

stability for safety evaluations, and 3) the development of a system for the selective removal of undifferentiated iPS cells. Consequently, the protocols for standardized iPS cell generation are being established.

At Keio University, Professor Hideyuki Okano's team works on a study focusing on the central nervous system, including the development of a differentiation induction technique, a safety assessment and the development of therapy. Furthermore, they are drawing global attention for their preclinical study concerning regenerative medicine in regard to spinal cord injuries, for which modern medicine cannot offer any effective therapeutic methods. In 2009, they conducted a transplant experiment using mice with spinal cord injuries, and they succeeded in curing mice which had previously had paralyzed legs, enabling them to walk, almost properly; this was done by transplanting an iPS cell-derived neural stem-cell mass into the injured part of their spinal cords. (Figure 6)

Figure 6 / Recovery of Motor Function in Mice with Spinal Cord Injuries as a result of the Transplantation of Human iPS Cell-Derived Neural Precursor Cells

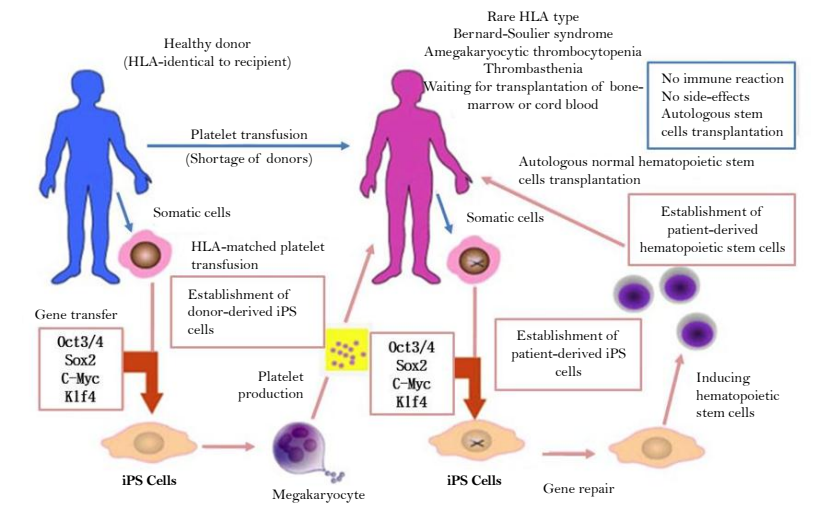


Source: Created by Okano Laboratory, Department of Physiology, Keio University School of Medicine

At the University of Tokyo, Professor Hiromitsu Nakauchi's team is working on a study focusing on hematological cells, including the development of a differentiation induction technique, a safety assessment, and the development of therapy. In 2009, Professor Hiroyuki Eto and his colleagues at CiRA, who also worked on the study with Professor Nakauchi, succeeded in generating megakaryocytes[1] (origin of platelets) from an iPS cell, and further, by generating platelets from the megakaryocytes. Furthermore, they also succeeded in generating hematopoietic stem cells from mice and from human iPS cells, so that a solution is now expected for the rejection issues that occur with bone-marrow or cord blood transplants. (Figure 7)

1 The largest hematopoietic lineage cell of diameter ranging from 35 to 160 micrometers which exists in marrow. They produce platelets.

Figure 7 / Radical Cure of Hereditary Hemorrhagic Disease Using iPS Cells



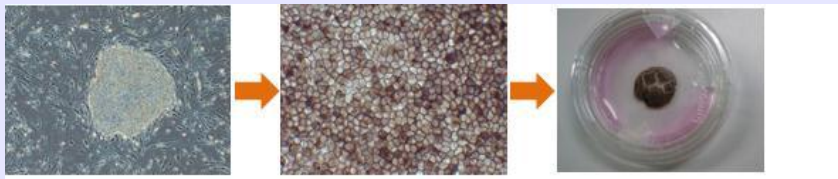
Source: Created by Eto Laboratory, CiRA, Kyoto University

They also successfully generated T-cells[1] from iPS cells, and a new T-cell transplant therapy is under consideration. Specifically, iPS cells are first generated from T-cells that recognize specific antigens[2] (cancer, virus, etc.). As T-cells do not lose their memory (recognition) of the antigens even after being reprogrammed, regenerating T-cells from previously generated iPS cells makes it possible to produce large volumes of T-cells that recognize specific antigens, and the transplant of those T-cells into patients with diseases such as cancer is expected to result in significant therapeutic effects.

At RIKEN, Group Director (hereinafter referred to as "GD") Yoshiki Sasai's team works on the development of basic technology, including the efficient iPS cell culture, the development of differentiation induction techniques focusing on sense organs system, safety assessment and therapy development. Their studies about retinal tissues are some of the most advanced research in regenerative medicine using iPS cells. In February 2013, RIKEN and the Institute of Biomedical Research and Innovation (IBRI, a unit of The Foundation for Biomedical Research and Innovation) applied for approval of their collaborative clinical research project using iPS cell-derived retinal pigment epithelial cells[3] in relation to eye disease called age-related macular degeneration[4], and they obtained conditional approval by the Ethics Committee of RIKEN and the Regenerative Medicine Reviewing Committee of IBRI, respectively. (Figure 8)

1 A type of lymphocyte that precursor cells produce in marrow, and which differentiates and matures after selection in the thymus. T-cell owns distinguishing T-cell receptor on cell surface.
2 A substance existing on the surface of foreign substances, and a target that allows antibodies to recognize and destroy foreign substances.
3 Epithelial cells that exist between the retina and choroid and supply pigment to photoreceptor cells.
4 A disease that lowers vision due to retinal defects with aging.

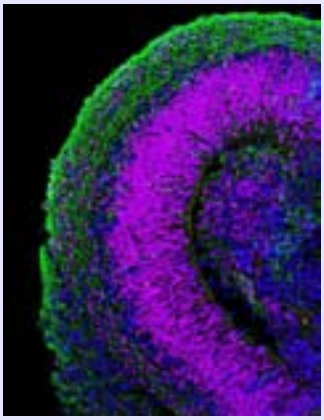
Figure 8 / Generation of iPS Cell-Derived Retinal Pigment Epithelial (RPE) Sheet



Source: Press release by RIKEN

It will be decided whether or not to implement the project after an examination by MHLW. If a green light is promptly given by the examination, the world's first clinical trial using iPS cells is expected to be implemented as early as FY 2013. The retinal pigment epithelium has a brown color and can be easily distinguished from undifferentiated cells, which allows easy removal in case of tumorigenic transformation while also ensuring safety. In the clinical trial, they plan to proceed in the following manner: generate iPS cells from a patient's own cells; differentiate them into pigment epithelial cells that support retinal functions; make sheets that are several millimeters square in size and transplant them; and conduct the safety assessment.

Figure 9 / Brain Tissues Generated from Human ES Cells



Courtesy of the RIKEN Center for Developmental Biology

Sasai's team has also tackled the technical development of culturing cells under free conditions, and has developed the technique to culture ES cell masses under floating conditions in 2005. In 2008, by using this technique, they succeeded in growing the ES cells of both humans and mice into 4-layer structured brain tissues which are quite similar to those of a fetus (Figure 9). In 2011, they also succeeded in the 3-dimensional generation of pituitary gland[1] tissues, which secrete vital hormones, from the ES cells of mice.

3) Evaluation of Regenerative Medicine Research and Future Direction

Owing to the cooperative and intensive research conducted by the four research hubs for stem cells and regenerative medicine focusing on iPS cells throughout "The Project for Realization of Regenerative Medicine (2nd term)," progress has been made to come within the range of regenerative medical applications of stem cells, including iPS cells and their practical application. At the same time, the following iPS cells-related issues are also being addressed: safety enhancement, generation methods for the improvement of standardization (including time reduction and efficiency improvement), and the need for improvements in differentiation-induction methods. Consequently, it has been commonly recognized as one of the measures needed to overcome the above-mentioned issues and the development of

1 An organ existing in the body of vertebrate animals that works as an endocrine organ secreting many hormones. Also known as a hypophysis.

fundamental technology for the clinical application of stem cells, including iPS cells, such as the clarification of the iPS cells reprogramming mechanism, is essential. Furthermore, those iPS cells-related studies of generation methods, differentiation induction methods, and safety verification, are all in progress, hence both preclinical and clinical research need to be accelerated. For that to happen, the following issues need to be addressed: support in handling regulation-related issues for clinical research, techniques for safety assessment, and organizing a framework to ensure the implementation of both clinical research and clinical trials. Moreover, practical therapies need to include the following operations: iPS cell generation from self-cells; differentiation induction into target cells; and safety assessment, all of which are supposed to take several months or more until the cells become available for transplant, which may lead to a lack of availability or to an increase in cost in urgent cases. Thus, when considering clinical applications and industrialization, it is necessary to construct an "iPS Cells Stock for Regenerative Medicine" which can store high-quality iPS cells generated in advance from donated cells having a low possibility of immune rejection when transplanted, in order to provide timely transplant therapies.

The ex-post evaluation report of "The Project for the Realization of Regenerative Medicine (2nd Term)" published in August 2012 states, "Fruits toward the realization of regenerative medicine have been continuously produced throughout this project, and the publication of those findings through news releases and outreach activities has greatly attracted the public's interest in the regenerative medicine field. This is expected to become a base for expanding people's interest into the entire field of life sciences, and is not limited to just the regenerative medicine field." The report also says "It is necessary to accelerate further research and development by leveraging the findings acquired through the project in order to make the practical application of regenerative medicine beneficial to the public," praising the attitude of Professor Yamanaka, who published the research findings, carefully chose his words at the press conference, so as to avoid raising excessive expectations among patients.

(2) Present State of Drug-Discovery and Disease Modeling and the Issues Related to Them

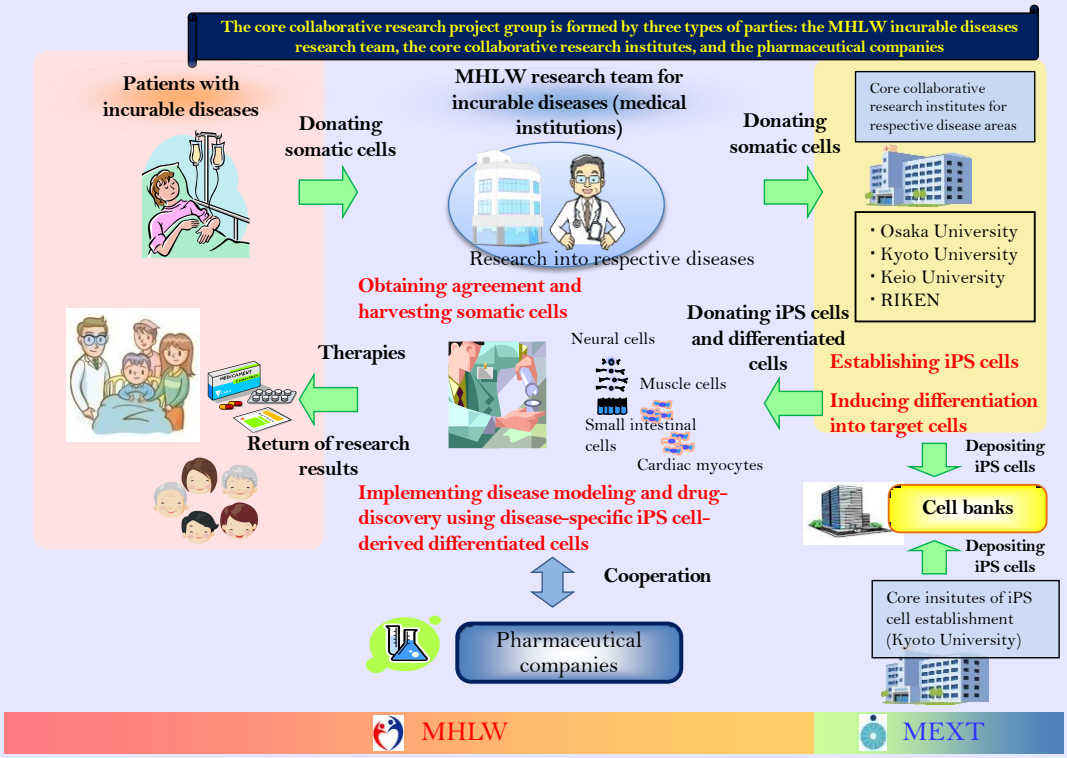
1) Present Approaches in Drug-Discovery and Disease Modeling

Another medical application of iPS cells is their usage as a fundamental technology for the clarification of disease causes and for target selection in drug-discovery. Concretely, researchers generate pathological tissues from patient-derived iPS cells (hereinafter referred to as "disease-specific iPS cells") outside the body, and then use them to test side effects during the early stages of development for new drugs or for target selection in drug-discovery. Up until now, drug-discovery has faced difficulties in targeting diseases that do not allow cells to be non-invasively harvested from diseased tissue or that have insufficient functional analysis due to a lack of proper disease modeling, but the application of disease-specific iPS cells has made it possible to address pathologic clarifications and therapy developments targeting those diseases. This kind of research and development does not involve cell transplant to human bodies, hence its practical application can be realized earlier than that of regenerative medicine, which is increasingly attracting expectations. Research institutions, pharmaceutical companies, and others are now addressing the development and organization of disease-specific iPS cell banks and the development of systems for evaluating candidate drug compounds.

Since February 2013, MEXT and MHLW have jointly been conducting "Research into Incurable

Diseases using Disease-Specific iPS Cells[1]." (Figure 10) This is a project to promote research into the development of therapeutic methods, including the clarification of disease mechanisms and the screening of drug-discovery candidate substances, by using cells that are inductively differentiated from disease-specific iPS cells, and is being conducted through the cooperation of the following groups: 1) the core collaborative research institutes selected by MEXT, such as universities that own facilities and have techniques for regulating a large amount of high-quality iPS cells; 2) the research team of the "Intractable Diseases Conquest Research Program," a project conducted by MHLW; and 3) pharmaceutical companies. According to the report entitled "International Trends and Future Research Developments Concerning iPS Cells" published by the Center for Research and Development Strategy at the Japan Science and Technology Agency (hereinafter referred to as "CRDS") in October 2011, life innovations that are to be brought about by stem cells, including iPS cells, will be organized in two main streams: "development into drug-discovery" and "development into medicine." iPS cells have the power to promote innovations, both in the drug-discovery field and in the medical field. The report describes the "development into drug-discovery" as an attempt to use iPS cells as tools for research into drug effects and into target disease models in order to increase the probabilities of successful drug-discovery, and will trend toward new drug exploration in cooperation with pharmaceutical companies.

Figure 10 / Overview of "Research into Incurable Diseases Using Disease-Specific iPS Cells"



Source: Press release by MHLW

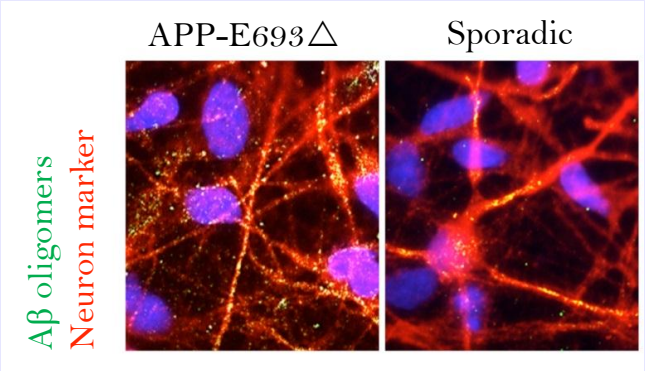
1 The core institutes and the promoted research categories decided at the moment are as follows: RIKEN (nerve), Keio University (nerve), Osaka University (circulatory system), and Kyoto University (bone/cartilage/muscle, and blood).

2) Benefits of Research in Drug-Discovery and Disease Modeling

The fruits of research into incurable diseases and drug-discovery using disease-specific iPS cells are as follows. In August 2012, Assistant Professor Haruhisa Inoue of CiRA and his colleagues constructed a model to reproduce pathological conditions of amyotrophic lateral sclerosis[1] (hereinafter referred to as "ALS") at cellular levels by using patient-derived, disease-specific iPS cells and they clarified a part of the ALS pathological conditions that had been unknown before that. Furthermore, they discovered new drug seeds[2] for ALS through their disease modeling.

Also in February 2013, the research team of Assistant Professor Inoue and the team of Professor Nobuhisa Iwata at Nagasaki University generated disease-specific iPS cells from different types of Alzheimer's disease (hereinafter referred to as "AD") patients and clarified the inherent pathological conditions for each type of AD[3]. (Figure 11)

Figure 11 / Aβ Accumulated in Neurons



Remark 1. The neurons inductively differentiated in vitro from the iPS cells-derived AD patients (juvenile: APP-E693Δ, advanced-age-onset type: sporadic) to reproduce the pathological conditions

2. The green parts show the accumulated Aβ oligomers, blue parts show the cell nucleus, and red parts show the neurons. The parts where Aβ exists appear yellow (due to the mix of green and red). The Aβ oligomers are not detected in the control (healthy persons) neurons.

Source: Press release by CiRA

AD is the most common disease in senile dementia and one of its pathological characteristics is a protein accumulation in the brain, called senile plaque. A main component of senile plaque is amyloid β[4] (hereinafter referred to as "Aβ"). Excessive Aβ accumulation was thought to be deeply involved in the onset of AD, but how it is linked to pathological conditions in human brain cells was not well known. The study has clarified cases in which Aβ oligomers[5] accumulated in the neurons astrocytes[6] of two respective types of AD patients--the juvenile-onset type (familial); and the advanced-age-onset type

1 A disease involving progressive muscle atrophy that disables muscles throughout the body and in many cases leads to death by respiratory muscle paralysis. Defects in motor neurons are known as the cause, but there is no established effective therapy yet, and in Japan it is designated as one of the intractable diseases.

2 Substances or methods of approach that can serve as clues for drug development.

3 In the study, they generated iPS cells from the skin of patients with gene mutations in their amyloid precursor protein (APP), which is a gene responsible for juvenile (familial) AD. They also used the skin of patients with non-familial and advanced-age-onset type (sporadic) AD, respectively, and inductively differentiated them into cerebral neurons. As a result of the analysis, they found that a mutation called APP-E693Δ causes Aβ to form aggregates called oligomers which accumulate in cells leading to endoplasmic reticulum stress and oxidative stress, both of which are factors in promoting cell death. However, docosahexaenoic acid (DHA) alleviated those stress responses in the cells and suppressed cell death. They also found that Aβ oligomers in cells and cellular stress are also observed in some advanced-age-onset type (sporadic) AD patients as well as in APP-E693Δ mutation.
Kondo T.,*et al.*,(2013)
Modeling Alzheimers Disease with iPSCs Reveals Stress Phenotypes Associated with Intracellular Aβ and Differential Drug Responsiveness
Cell Stem Cell **12**, 487-496
http://dx.doi.org/10.1016/j.stem.2013.01.009

4 Amyloid-β (Aβ) is a peptide of 40-42 (43) amino acids that is cleaved from the amyloid precursor protein (APP: amyloid β protein precursor) by the function of enzymesß- and γ-secretase.

5 In general, a polymer formed by relatively small numbers of monomers.

6 One of the glial cells existing in central nervous systems. Also known as astroglia.

(sporadic)--causing various kinds of cellular stress. In addition to the pathologic clarification and the drug-discovery research using iPS cells technology, the study has also shown a path to preemptive medicine, which predicts pathologic conditions in order to provide proper therapies.

3) Evaluation and the Future Direction Concerning Drug-Discovery and Disease Modeling

To take advantage of iPS cells in the drug-discovery process, it is necessary to establish high-quality, disease-specific iPS cell banks and to consider an institutional design to promote its application into the pharmaceutical industry and academia. It is also necessary to standardize the protocols for regulating the differentiation of cells or tissues from disease-specific iPS cells, and to develop a support system including technical guidance, so that the experts throughout respective disease areas can use it.

3 Approach for the Practical Application of Regenerative Medicine and Drug-Discovery

As mentioned above, owing to the discovery of iPS cells establishment, it is rapidly turning into a real possibility that regenerative medicine can be achieved at an early date and that drug-discovery will dramatically accelerate. However, in terms of the ultimate aim, which is therapy, researchers are still at the beginning. For its realization to occur, there are still many issues that need to be overcome in regard to research environments, such as the establishment of safety, fundamental technology including techniques to generate iPS cell-derived target cells or tissues, and the development of a therapy framework that is reasonable in terms of its efficiency and cost.

The approach to realize regenerative medicine is actively progressing worldwide, and once excellent methods and related products are developed, they immediately spread throughout the world. That means, even though Japan can domestically accumulate cutting-edge knowledge, if foreign companies are quicker in providing safe and efficient service or products, they will also dominate the Japanese market, thus preventing the growth of related Japanese industries. On the contrary, if Japan can realize safe and efficient regenerative medicine prior to other countries, Japanese companies will be able to have greater chances of success in the global market. The global competition regarding the development of regenerative medicine has already begun showing signs of intensification. We will hereafter review what approach is to be taken by Japan under the current circumstances.

(1) Global Trend

According to the discussion about the future direction of research on stem cells and regenerative medicine in the meeting: "the Future of Research on Stem Cells and Regenerative Medicine" held by "the Subdivision on R&D Planning and Evaluation, the Council for Science and Technology (CST)" on February 1, 2013, the clinical research using ES cells and mesenchymal stem cells[1] is ongoing, mainly among venture business in Western countries and Korea etc., but, globally, future progress is still being awaited regarding clinical research using iPS cells.

Regarding the budget for regenerative-medicine related research, Japan invested around 10 billion yen of its budget (FY 2012), while the US invested around 90 billion yen of their annual budget just for

1 Somatic stem cells that are derived from mesenchyme. They have the ability to differentiate into cells that belong to the mesenchyme system (bone cells, myocardial cells, chondrocytes, tendon cells, adipocytes, etc.).

NIH[1] alone, and around 300 billion yen of their 10-year budget for CIRM[2], with the state of Massachusetts alone investing around 80 billion yen of that 10-year budget. - This shows that the U.S. Federal Government and its state governments have begun numerous research programs and that the United States is far ahead of other countries in terms of investment. Additionally, CIRM is providing general support for research implementation, not only in terms of funding, but also by supporting regulatory aspects and by organizing collaborative research teams.

Furthermore, NIH decided to entrust the production of iPS cells used in regenerative medicine to Lonza, Switzerland, and to Cellectis, France (October 2012), which shows that their approach to regenerative medicine using iPS cells is also accelerating.

(2) Improvement of the Research Framework for the Practical Application of Regenerative Medicine and Drug-Discovery

Regarding the research on stem cells and regenerative medicine in Japan, owing to the establishment of iPS cells by Professor Yamanaka and his team, the research concerning iPS cells has steadily advanced, and the combined number of published articles and cited articles are high making Japan's research potential the highest in the world. Additionally, regarding the security of intellectual property rights, which is essential for both clinical and industrial applications, the patent on Kyoto University's basic techniques including the establishment of iPS cells has been approved in Japan, the USA, and major European countries, which shows that Japan has an advantage at this moment. However, the global approach to practical application is becoming more active, and the competition for the application of iPS cells to drug-discovery is especially intensifying.

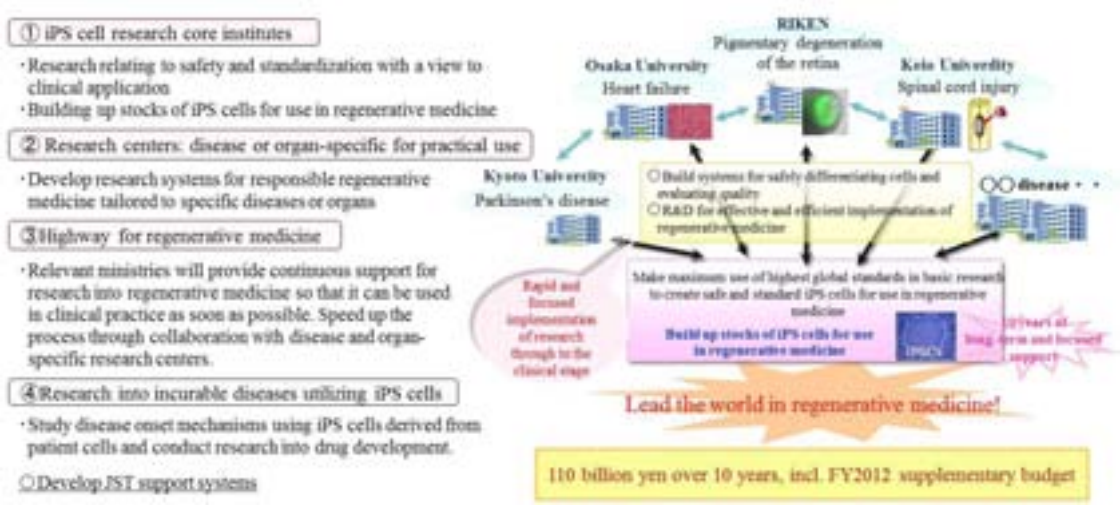
In the future, it is necessary to tackle the practical application of regenerative medicine and drug-discovery by taking advantage of the current advantage held by Japan, and it is also necessary to accelerate a strategic approach driven by collaboration among the three ministries of MEXT, MHLW, and METI, in conjunction with pharmaceutical companies, etc.

- The Research Center Network for the Realization of Regenerative Medicine

In order to realize regenerative medicine using iPS cells etc., before other countries do so, the Japanese government has formulated a program that will operate from 2013 onward: "the Research Center Network for the Realization of Regenerative Medicine" (Figure 12), which will succeed the previous program that has been conducted by MEXT since 2008, "the Project for Realization of Regenerative Medicine (2nd term)." The government has implemented a policy to conduct the new program under a cooperative framework integrating all relevant ministries and agencies, research institutions, universities, and the industrial sector.

1 National Institutes of Health (USA)
2 California Institute for Regenerative Medicine

Figure 12 / Research Center Network for Realization of Regenerative Medicine

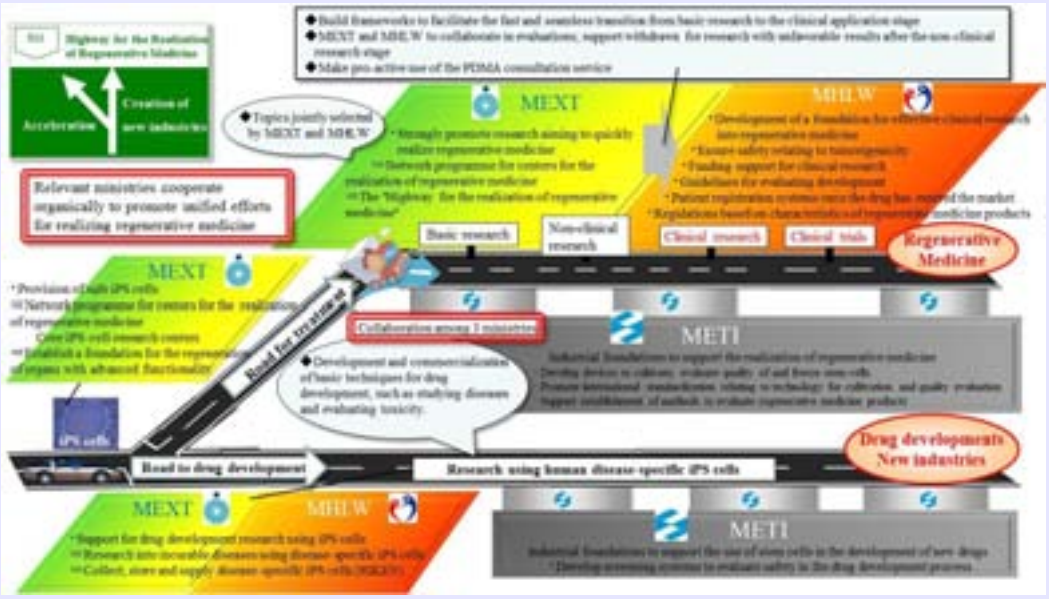


Source: Created by MEXT

"The Research Center Network for the Realization of Regenerative Medicine" aims to accelerate research and development on regenerative medicine and drug-discovery using iPS cells, and it will be involved in the intensifying global competition by optimizing Japan's advantage. In concrete terms, "the iPS-cell-research core institutes" will conduct research on safety and standardization with a view toward clinical application, and it will build up stocks of iPS cells for use in regenerative medicine. Other research are independently working on each practical application categorized by disease and organ and have the responsibility to develop a research system tailored to specific diseases or to specific organs that will be studied in order to realize regenerative medicine. Each research center will set clear targets for practical application of research involving specific diseases or organs, and will conduct research and development by using cells from the above-mentioned "stocks of iPS cells for use in regenerative medicine." At the same time, they will clarify the challenges that need to be overcome in order for practical application to occur, and they will conduct research and development in order to find a solution of those challenges.

Since FY 2011, the Japanese Government has commenced "the Highway Program for the Realization of Regenerative Medicine," through which MEXT, MHLW, and METI are providing cooperative, long-term (10 to 15 years), and consistent support for all research, from basic to clinical. This program (Figure 13) aims to build up a system that enables the quick and seamless transfer of basic research to clinical research, and MEXT and MHLW are cooperatively conducting both the selection and the evaluation of research to which "the grant-in-aid for scientific research" is granted. Aid for research with unfavorable evaluation results will be discontinued at the non-clinical stage as a scheme for ensuring the smooth progress of research.

Figure 13 / Three-ministry Vision for Realization of Regenerative Medicine



Source: Created by MEXT

In parallel with the progress of basic research and clinical application, METI is building up an industrial infrastructure to support the realization of regenerative medicine. Specifically, they are conducting 1) the development of apparatuses that will allow for a series of operations, including the culture of stem cells, their quality evaluation, and their cryopreservation; 2) international standardization concerning the techniques of both the culture and the evaluation of quality; and 3) support for the establishment of methods to evaluate regenerative medical products etc.

In order to accelerate applications to drug-discovery and the creation of new industries, it is necessary to drive pathological research and the development of therapies by using iPS cells generated from the patient-derived somatic cells (or disease-specific iPS cells.) For this purpose, MEXT is building a framework to collect patient-derived somatic cells and to generate disease-specific iPS cells to be used in the clarification of onset mechanisms for diseases, drug-discovery, and the development of advanced medical care and therapies. Furthermore, MEXT is developing a bank of generated disease-specific iPS cells for broad use in drug-discovery and disease modeling. Regarding rare and intractable diseases for which effective therapies are not yet available, MEXT is developing a collaborative approach with MHLW's Intractable Diseases Conquest Research Program titled "Research into Incurable Diseases using Disease-Specific iPS Cells" (refer to the Feature 2-(2)).

METI is developing industrial infrastructure to support the realization of drug-discovery using stem cells, including the development of a screening system for assessing safety in the drug-discovery process. The Japanese government is also conducting "The Project for Accelerating Medical Application of iPS Cells" based on hub institutions for human iPS cells research as established by "the Project for Realization of Regenerative Medicine," and operated under an organic framework consisting of an academic-industrial alliance involving the participation of the major domestic pharmaceutical companies.

This project is actively promoting the application of human iPS cells for pathologic clarification, drug exploration, toxicity testing, and the development of rejection-free cell transplant therapies.

(3) Environmental Improvements for the Acceleration of Practical Applications

1) Present Approach for Environmental Improvements

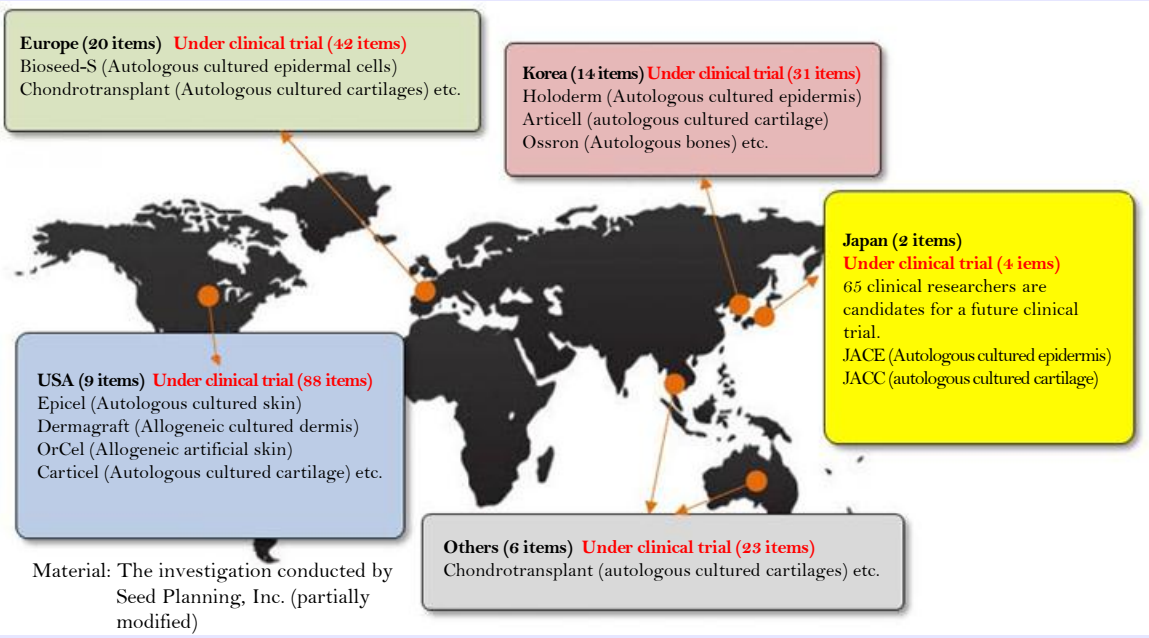
As mentioned previously, Japan has the world's highest level of research on regenerative medicine, but in terms of its practical application, the products launched into the market are less than those of Western countries; thus, Japan does not have an advantage and is now exposed to fierce competition.

So far, in Japan, the following two items have been launched into the market as regenerative medical products which are approved for manufacturing and sales under the Japanese Pharmaceutical Affairs Law (the law number 145 enforced in 1960): the autologous cultured epidermis[1], used for the treatment of severe burn injuries, and the autologous cultured cartilage[2], used for the treatment of damaged knee cartilage (as of the end of April 2013.) On the other hand, Western countries and others are also active in the research and development of regenerative medical products, and global competition for their practical application is intensifying. (Figure 14)

1 It is manufactured based on the method of Professor H. Green, USA, and others, and manufacturing-controlled by the technology of Japan Tissue Engineering Co., Ltd.

2 It is manufactured based on the method of Professor Ochi at Hiroshima University orthopedics, and others, and manufacturing-controlled by the technology of Japan Tissue Engineering Co., Ltd.

Figure 14 / The Global Aspects of Regenerative Medical Products Launched into Market or under Clinical Trial (as of December 2012)



Material: The investigation conducted by Seed Planning, Inc. (partially modified)

Note: The calculation policy concerning the numbers of products launched into the market or under clinical trial

1) The numbers are counted by the country where the product was launched

2) The products launched into the market are defined as pharmaceuticals or medical equipment which have obtained pharmaceutical approval, and manufacturing and sales approval, in each country and which involve cell processing. Homologous or heterologous transplant pieces without involving cell processing, or cell banks (cord blood, hematopoietic stem cells etc.) are not included. The following products are included in the launched products:

- The products approved by the Humanitarian Device Exemption (HDE) program
- The products approved in each country before the implementation of central examination in Europe

Source: Created by MEXT based on "Report Concerning Practical Application and Industrialization of Regenerative Medicine" (February 2013) issued by METI

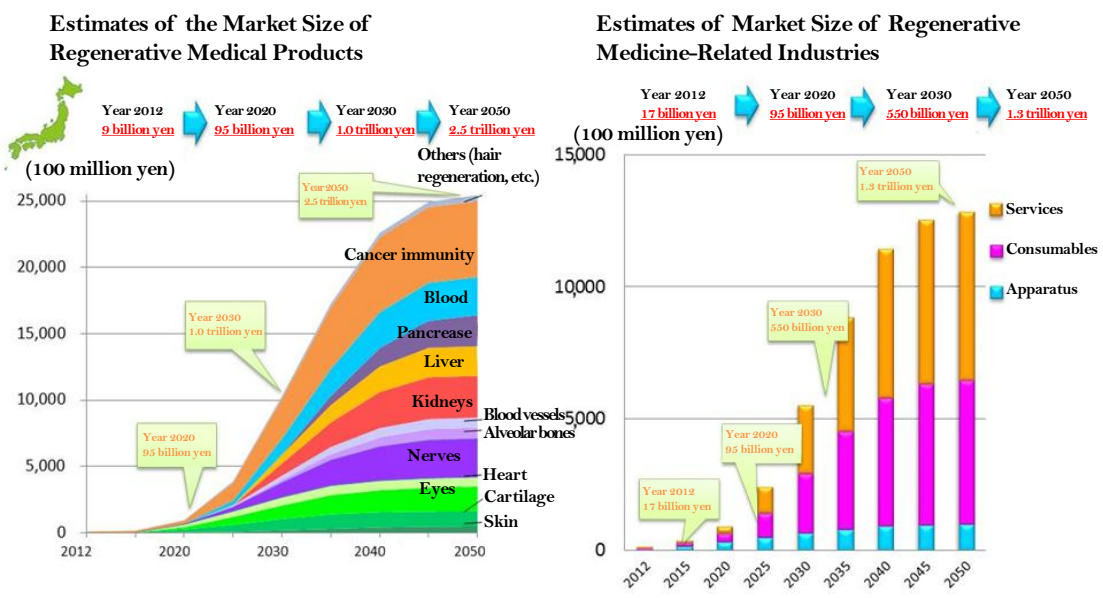
Besides, 70 clinical research trials concerning stem cells were (or are being) conducted (as of the end of April 2013.) The medical activities related to this research include cosmetic surgery, lymphocyte activation therapy and dendritic cell therapy (both for cancer treatment), all of which are domestically conducted as uninsured care. Some of these therapies are conducted at limited medical institutions, aiming for future insurance coverage. Considering that regenerative medicine is conducted for various objects and under various systems, environmental improvement is required so that patients can utilize regenerative medicine quickly, safely, and affordably. Against such a background, METI's "Study Group on the Commercialization and Industrialization of Regenerative Medicine" addressed institutional issues in order to promote the practical application of regenerative medicine, and they studied necessary institutional frameworks and environmental improvements, and finalized "Report Concerning the Practical Application and the Industrialization of Regenerative Medicine"[1] in February 2013.

This report estimates that the practical application and the broad uses of regenerative medicine using iPS cells will expand the domestic market of regenerative medical products from 9 billion yen in 2012 to

1 "the Report Concerning the Practical Application and the Industrialization of Regenerative Medicine" http://www.meti.go.jp/press/2012/02/20130222004/20130222004-2.pdf

1 trillion yen in 2030 and to 2.5 trillion yen in 2050, although the estimation only includes the regenerative medical and cellular therapeutic products manufactured by processing iPS cells (Figure 15). Furthermore, when including related industries, such as reagents, culture media, and automated culture apparatus, the market is estimated to expand from 26 billion yen in 2012 to 1.55 trillion yen in 2030 and 3.8 trillion yen in 2050 (Figure 15). The report also expects the manufacturing costs of regenerative medical products to be reduced by up to 20%, and the costs of clinical trials to be reduced by up to 60% as a result of the "Regenerative Medicine Safety Ensuring Law," which is soon expected to be enacted by the government, and because of the environmental improvements expected in the revision of the Pharmaceutical Affairs Law.

Figure 15 / Estimates of the Domestic Market Size of Regenerative Medical Products and Related Industries



The calculation method for domestic market size
The market size of regenerative medicine = "the number of patients[1]" x "the expenses per one patient[2]"
1: "The number of patients" = "the number of domestic potential patients" x "application ratio of regenerative medicine"
2: "The expenses per one patient" = "the unit price of regenerative medical products" + "the medical expenses for regenerative medicine (technical expenses etc.)"

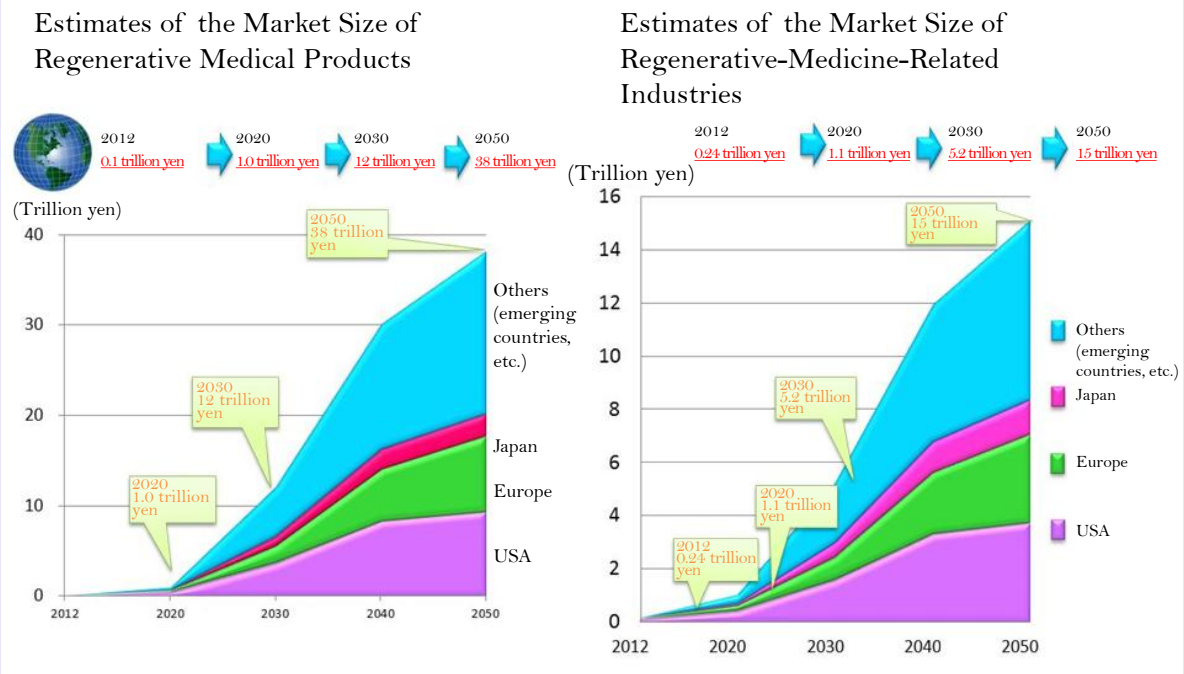
The calculation method of market size for related industries
"The market size" = "the market size of apparatus" + "the market size of consumables" + "the market size of services"

Source: METI "Report Concerning the Practical Application and the Industrialization of Regenerative Medicine" (February 2013)

In forecasting the future global market of regenerative medical products, so far, the United States is the country where most of the products in practical application have been marketed and the U.S. accounts for 77% of the global market, in the future, however, those products will also spread among emerging countries, like China and India, as their population and income increases. Consequently, the global market size is estimated to meet the following numbers: approximately 1.0 trillion Yen in 2020, approximately 12 trillion Yen in 2030, and approximately 38 trillion Yen in 2050 in the long term (Figure 16). Including

related industries, the global market size is estimated to expand at the following rates: by approximately 2.0 trillion Yen in 2020, by approximately 17.2 trillion Yen in 2030, and by approximately 53 trillion Yen in 2050 (Figure 16). Regarding related industries, the United States currently accounts for more than half of the market, but in regard to regenerative medical products, the market in emerging countries, like China and India, is also expected to grow in the future, along with the spread of regenerative medical products and processed items.

Figure 16 / Estimates of the Global Market Size of Regenerative Medical Products and Related Industries



<Calculation method of the global market size>
Market size of respective countries = "Current market size of relevant countries[1]" x "penetration rate of regenerative medicine[2]"
1: "The number of patient" x "expenses per patient (same as the calculation for domestic market)"
2: Estimates based on population, income (price), developed items and existing market, as well as budget for research and development.

<Calculation method of the global market size of related industries>
"Market size" = "market size of apparatuses" + "market size of consumables" + "market size of service"
Source: METI "Report Concerning the Practical Application and the Industrialization of Regenerative Medicine" (February, 2013)

The MHLW have studied the concept of regulations concerning regenerative medicine, and as a result, the bill to amend the Pharmaceutical Affairs Law and the bill on ensuring the safety of regenerative medicine, etc., were respectively submitted to the Diet on May 24, 2013.

The amendment of the Pharmaceutical Affairs Law aims to establish a system based on the characteristics of regenerative medical products in order to hasten their practical application while also ensuring their safety. In concrete terms, the amendment adopts a system which allows granting conditional, time-limited, and especially prompt approval to the heterogeneous regenerative medical products, subject to their presumed effectiveness and confirmed safety. In this case, effectiveness and safety shall be verified after approval. Doctors shall provide patients with proper explanations before

using the products and shall try to obtain agreements on usage of the products from the patients. In addition, post-marketing safety measures shall be taken, such as surveillance on performance, periodic reports about infectious diseases, and record keeping about applied patients and maintenance.

The "bill on ensuring the safety of regenerative medicine, etc." aims to ensure safety by establishing the standards of the medical institutions practicing regenerative medicine and the standards of the cell culturing and processing facilities. In concrete terms, the bill sets procedures, such as submissions of plans categorized into three groups according to the risk of the regenerative medicine practiced, and it also sets the standards and the approval procedures for the cell culturing and processing facilities to ensure safety. Finally, the bill allows medical institutions to outsource cell culturing and processing in order to expedite the operation of regenerative medicine and to reduce its cost.

In the future, Japan aims to capture the overseas market with Japanese original regenerative medical products and related industries through the following approach: 1) system improvement by cooperation among MEXT, MHLW, and METI; 2) the promotion of regenerative medical products, especially those using autologous cells, which have progressed into practical application, and those using pluripotent stem cells including iPS cells, which relatively suit mass-production; and 3) the technical development and standardization of related apparatuses based on the properties of regenerative medicine.

2) Issues on Environmental Improvement

As seen above, what is important in this field is the enhancement of basic research and the consistent promotion of research and its development for practical application. Concerning the improvement of the research environment, the report entitled "The Future of Research on Stem Cells and Reproduction Medicine" (Stem Cell & Reproduction Medicine Strategy Taskforce, Life Science Committee, CST's Subdivision on R&D Planning and Evaluation, MEXT, May 2012) suggested concrete measures to accelerate a strategic approach as follows: the development of a research system; the promotion of regulatory science[1] and its systematic improvements; research support; the development of human resources; and the enhancement of intellectual property. It also expressed policies to solve the issues related to the improvement of the research environment.

"Report Concerning the Practical Application and Industrialization of Regenerative Medicine" published by METI in February 2013 pointed out system requirements, cost, and public understanding as the issues associated with regenerative medicine, and urged the necessity of overcoming those issues to improve the environment while promoting the practical application and industrialization of regenerative medicine.

(i) Issues on Cost Rationalization

In order to spread the practical application of regenerative medicine and drug-discovery, "Report Concerning the Practical Application and Industrialization of Regenerative Medicine" pointed out the following necessities: 1) the need to rationalize costs subject to sufficiently ensured safety; 2) the need to develop safety standards based on the characteristics of regenerative medicine while avoiding the imposition of excessive requirements on facilities and the processes required for the production of regenerative medical products; and 3) the need to establish a reasonable and stable supply of culture media,

1 "A science which implements proper, evidence-based prediction, evaluation, and judgment, and which regulates the achievements of science and technology in its most favorable form to ensure harmony with society, while aiming to make use of the benefits created by science and technology for society." (the Fourth Science and Technology Basic Plan, a cabinet decision on August 19, 2011)

reagents, and culture apparatuses, all of which are necessary for cell processing, in order to practice regenerative medicine smoothly and efficiently. The report also pointed out that Japan has produced a limited supply of those three items, forcing the Japanese scientific community to depend on expensive foreign products and to face the constant risk of a supply disruption. Finally, the reported stated that, to ensure a stable supply of regenerative medical products, the supply system of those items (culture media, reagents, culture apparatuses) needs immediate improvement.

(ii) Issues in Public Understanding

While social expectations for the practical application of regenerative medicine and drug-discovery is rising, the public understanding of the effects and risks concerning regenerative medicine is insufficient and further effort is necessary to improve it. In particular, it is not clear how responsibilities are taken among the parties involved in regenerative medicine —unclear risk allocation. For example, if the expected effects are not obtained, there is no clear means to assess the cause: is it due to patients' conditions, harvested cells, doctors' negligence, or due to regenerative medical products or processed items. Furthermore, in terms of protecting the trial subjects of regenerative medicine, clinical research insurance to cover compensation risk is necessary. Although the four major insurance companies offer such insurance for clinical research using human stem cells, its commercialization has remained partial because it is incompatible with commercial insurance.

When iPS-cell research progresses to clinical application, what is important is whether its effects in clinical research will be obtained, and in this respect, the FDA[1] of the United States is providing consultations at the preclinical research stage with a view toward clinical research. Similarly, Japan recognizes that the aggressive utilization of the Pharmaceuticals and Medical Devices Agency (PMDA)[2] is required to lead to prompt clinical application, and so the CiRA, which is now preparing for the development of iPS cell stocks for regenerative medicine, commenced "the pharmaceutical affairs consultation on R&D strategy with the PMDA" in September 2011. In order to establish iPS cell stocks, compliance with various regulations and related guidelines should be required in addition to coping with technical issues, but an appropriate regulation system for the development of iPS cell stocks has not yet been entirely established because the iPS cell is an unprecedented technology. Thus, in order to deal with this regulatory issue, the CiRA began offering face-to-face advice at "the pharmaceutical affairs consultation on R&D strategy with the PMDA" as its first step for the earliest establishment of iPS cell stock.

(iii) Issues on Research Environment

At the Diet, in February 2013, Professor Yamanaka pointed out the environmental issues on scientific and technological research in Japan. He stated, "While my research institute of approximately 200 members is managed by me, who has never been trained in management, the research institutes in the United States employ professional managers. If a working professional baseball player were assigned to manage his team and he did it well, that would be a miracle indeed." He also discussed the issues associated with scientific and technological innovation at "the 105th Council for Science and Technology

1 The Food and Drug Administration is a U.S. governmental agency that is exclusively responsible for the administration of the approval and supervision of products sold to consumers in their daily lives; for example, foods, pharmaceuticals, cosmetics, animal drugs, toys, etc.

2 The Pharmaceuticals and Medical Devices Agency is providing relief for health injuries caused by pharmaceutical side effects etc., including the examination of pharmaceuticals and medical devices based on the Pharmaceutical Affairs Law, and safety measures to ensure the quality and e safety of pharmaceuticals and medical devices.

Policy (CSTP)" held in November 2012, saying, "Versatile and capable research support personnel are necessary for research and development in addition to researchers; for example, we need experts in intellectual property; experts in regulations; and experts in public relations. We also need high-level research secretaries who are capable of communicating with overseas research institutions and highly skilled technicians, but in reality, such personnel are few at universities today — we need the improvement of such an environment so that scientists can concentrate on their research." To exert Japan's presence in the global competition for research on regenerative medicine and drug-discovery in the future, the continuous development of human resources as well as research support is essential. In addition, it is necessary to share knowledge and know-how with young researchers and to take measures which allow research support personnel (such as technicians with high levels of expertise, and administrative staff dealing with intellectual property and contracts) to work continuously and steadily.

(iv) Issues on Intellectual Property Strategy

Regarding iPS cells — the Japanese original technological epoch, Kyoto University has obtained the basic patent in Japan, Europe, and the United States, and the number of patent applications from Japan is close to that of those from Western countries, which shows that Japan has an advantage. However, when placed in the unpredictable situation of having several applicants competing for the basic patent, Japan needs to strategically acquire intellectual property in order to promote the practical application and industrialization of iPS cells in the future.

Concluding remarks

The research on stem cells including iPS cells is expected not only for the realization of the longevity of a healthy society, but is also expected as the driving force of reform for the economy, and for creating wealth and employment. For the realization of such research to occur, prompt steps for its practical application is necessary, while also paying special attention to the issues of safety and ethics. For this purpose, "the bill concerning the general promotion of measures for the prompt and safe provision of regenerative medicine to the public," which aims at the general promotion of measures from research and development to the practical application of regenerative medicine, was introduced into legislation by Diet members and was passed into law at the 183th Diet on May 10, 2013. As stated in the law, the government is required to steadily conduct the general formulation of measures and their implementation; the enlightenment of the public; and the establishment of a cooperative structure among related ministries and agencies. It is also required to return the benefits of research using stem cells including iPS cells to the public as soon as possible.