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Cell Stem Cell Forum

A Decade of iPS Cells

10 Questions: Clinical Outlook for iPSCs

Cell Stem Cell Editorial Team[1,*]
[1]*Cell Stem Cell*, Cell Press, 50 Hampshire Street, 5th Floor, Cambridge, MA 02139, USA
*Correspondence: stemcell@cell.com
http://dx.doi.org/10.1016/j.stem.2016.01.023

In an interview, we recently asked four leaders in the field to share their insights with us on the clinical outlook for hiPSCs. We present highlights from their responses here.

Induced pluripotent stem cells (iPSCs) have great potential for improving our understanding of human disease and regenerative medicine, but as with any innovation in medicine, there are many hurdles to overcome on the road to the clinic. To get a sense of the challenges the field faces and the opportunities ahead, we asked four leaders in the field, Kevin Eggan (KE), Malin Parmar (MP), Masayo Takahashi (MT), and Shinya Yamanaka (SY), ten questions about the clinical outlook for iPSCs. This article contains excerpts from our email interview, and the full transcripts are available in the Supplemental Information.

CSC: In your mind, what are the most promising therapeutic applications for hiPSCs currently in development?

SY: One promising application is iPSC-based cell therapy. The work led by Dr. Masayo Takahashi at Riken Center for Developmental Biology (CDB) is generating a lot of interest, since it is the first clinical research that uses the transplantation of 100% iPSC-derived cell sheets. At CiRA we are eagerly awaiting similar studies for Parkinson's disease and blood transfusions. These studies require a much larger number of cells than the one by Dr. Takahashi. Her team's work was revolutionary for many reasons, and one is that it reprogrammed the patient's own somatic cells to create retinal cells for treatment. However, autologous transplants are not financially feasible at present. Future studies will use allogeneic transplants. Demonstrating allogeneic transplants of iPSC products has tremendous potential for clinical use.

Another development of strong interest is drug discovery. iPSCs have demonstrated promise for not only drug discovery but also drug repositioning. Drug repositioning would bring drugs out faster for clinical use.

Accordingly, CiRA is working with a number of companies that aim to realize cell therapies and/or drug development using iPSCs.

KE: I would currently break this down along two distinct lines. For use in transplantation medicine, at the moment, a large push is being made in forms of macular degeneration using pigmented epithelial cells made from both iPSCs and human ESCs. With an ongoing clinical trial of human ESC-derived cardiac cells for myocardial infarction, one would think that similar efforts with iPSC-derived cardiac cells can't be far behind. The field of directed differentiation of stem cells has progressed remarkably over the last several years with production of nervous system cell types leading the way. If I had to guess which areas will see a great deal of focus in the near future, I would say Parkinson's disease and the epilepsies. The ease of transplanting blood types for many disorders will make this increasingly attractive as time goes on and more high-quality differentiated cells are made.

Another important utility of iPSCs has become their use in the identification of candidate drugs through mechanistic studies or chemical screening. This strategy has radiated throughout academic and industrial settings. There seem to be many drug candidates moving toward the clinic discovered by this approach. Again the ease of generating large quantities of neuronal and cardiac cell types has put indications impacting these cells in the lead. One example is a compound we found to normalize the physiological properties of iPSC-derived motor neurons from ALS patients. There is currently a clinical trial underway to determine if this drug can rescue known electrical changes in the brains of individuals with this form of neural degeneration.

CSC: Do you think it's valuable to bank iPSCs for clinical purposes? What do you think are the main considerations to keep in mind?

MP: I think it makes sense to bank iPSCs from individuals with specific diseases to create a resource for the scientific community to use for understanding the disease pathology and to develop better differential diagnostics and new treatment strategies.

In terms of generating large banks with the intention for the cells to be used for cell therapy, I am less convinced this is a good strategy. One has to keep in mind that it is a very resource-demanding task to create and maintain such a bank under GMP conditions and that each differentiated therapeutic cell product made from a line in that bank will still have to go through safety and efficacy testing prior to use. If that same amount of resources was put into addressing key issues remaining to enable the use of patients' own cells or cells from matched donors to create therapeutic cells directly without a banking step, personalized cell therapy could become a reality.

SY: Seeing the success of blood banks, it would be wonderful if we could do something similar with iPSCs. CiRA is currently building an iPSC bank—the iPS Cell Stock for Regenerative Medicine—and we distributed one quality-assured iPSC line to a pharmaceutical company and a medical organization last year. Our banking system collects blood or skin cells from healthy donors with HLA homozygous alleles and generates iPSC lines. We predict that ~100 such lines would cover a majority of the Japanese population. Banking iPSCs can save time and cost because the quality of iPSC lines can be assured before they are needed for treatment. In addition, target cells derived from the iPSCs can be provided more quickly to patients than cells made from patient-specific iPSCs.

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Kevin Eggan, *Harvard University* (top left); Malin Parmar, *Lund University* (top right); Masayo Takahashi, *Riken* (bottom left); Shinya Yamanaka, *Kyoto University/ Gladstone Institutes* (bottom right).

Another important consideration is the impact of minor antigens and other immunological mechanisms. We have observed beneficial effects of MHC matching in monkey models, but these need to be proved by clinical studies.

***CSC:* On a related note, what are your thoughts on the value of autologous versus allogeneic approaches? Where do you think the community should be focusing its efforts? What do you think will be more feasible or safe?**

MT: Autologous approaches have a strong advantage compared to allogeneic approaches because they do not require immune suppression, and the first patient enrolled in our clinical trial was treated with autologous cells and showed no immune rejection. However, allogeneic approaches have a significant advantage when it comes to cost.

Overall, I think the community should be focusing at the moment on allogeneic approaches, to develop standard treatments that will be applicable to the many, many desperately waiting patients.

In terms of feasibility and safety, whether autologous or allogeneic approaches should be employed depends on the end products and this should be evaluated on a case-by-case basis. I think it is wrong to think of regenerative medicine in a homogenous way. We should consider each disease and case separately.

MP: For neurological disorders immune rejection is less of a concern than for a number of other diseases. In my opinion, all first-in-human trials have to be conducted with cells that are first put through rigorous safety and efficacy testing prior to use in patients. This can be done with either allogeneic ESC-based or allogeneic iPSC-based grafting. As iPSCs encounter some additional concerns relative to ESCs due to their derivation via reprogramming, I think the most straightforward strategy would be to use ESCs. However, looking into the future, one can envision matched donor cells or personalized treatments, and those would only really be possible via cellular reprograming.

***CSC:* What are the pressing technical challenges that need to be addressed for full clinical potential to be reached?**

KE: While major progress has been made in developing strategies for directed differentiation of many cell types, it is still unclear how many of these will transfer well to culture conditions that comply with clinical requirements. I think that many groups will be surprised by what a challenge this will be. Still, I think that many of these challenges are only technical and that through time and effort they will be overcome. Another major challenge will be around reproducibility. Most differentiation schemes make heterogeneous mixtures of cells whose cellular constituents fluctuate in abundance from run to run. This is sort of like baking a fruitcake and having the abundance of cranberries, raisins, and currents change every time you make it. Better methods will need to be developed in many cases to allow the target cells of interest to be purified for downstream use.

MP: In my field we now have very good protocols for cell differentiation and we know we can generate cells that function on par with human fetal DA neurons from PSCs. The challenges that lie ahead are associated with meeting the regulatory requirements for cell production as well as safety and efficacy testing prior to use in patients. Related to this is the need to develop much better markers that predict the in vivo therapeutic efficacy and authenticity of grafted cells.

***CSC:* Where do you see the biggest gaps in our understanding of the basic science of iPSCs? In other words, what are the basic research questions that we still need to resolve for clinical translation?**

MP: A key issue remaining to be addressed is how we precisely and finely control the identity of the cellular products derived from PSCs so that we can generate cells that are very similar to the cells normally found in our bodies. Related to this is the challenge of determining the identity of cells generated in a dish. In the brain, for example, cell identity is often governed by anatomical location and projections and this is lost in vitro. We therefore need much more refined methods for determining the exact identity and functional potential of cells generated from stem cells.

KE: There are many ways to go with this question; I think that one of the largest holes in our knowledge relating to use of iPSCs in transplantation studies centers around what happens to the cells after transplantation. Where do they go after transplantation? How well do differentiated cells survive, function, and integrate over the very long term after transplant? While many studies of function in vitro have been performed, transplant studies are still scarce in rodent models and rare in large animals. There needs to be considerable progress in that area. Additionally, in many cases a deep understanding of the detailed biology of the human differentiated cells we are trying to make from human iPSCs is lacking. For many scientists there are real challenges in obtaining the primary counterparts of cells that they wish to produce from human iPSCs and too often we rely on analogies with cells readily isolated from rodents. I hope in the future more effort will be applied to compare human iPSC-derived cell types to their actual human counterparts.

***CSC:* What do you view as the major regulatory challenges that the field faces for clinical use of hiPSCs?**

MT: We still need to develop a more sophisticated and comprehensive approach to thinking about and developing cell therapies that more fully considers the treatment as a whole. Regenerative medicine is a medicine that sometimes requires surgery. It is not completed only with cells.

MP: One major difficulty is that the regulatory framework is different in different countries. For example, the FDA and EMA have requirements that vary so much that it is hard to develop cells that can be used on both continents or globally.

Another challenge is that this development takes a long time and while we struggle to bring safe and effective stem cell-based therapies to patients as quickly as possible, the number of clinics offering unproven therapies for commercial gain are increasing, which creates unrealistic expectations in patients and puts them at risk.

SY: The biggest question is how to use deep sequencing technology in evaluating the safety of iPSCs and differentiated cells prior to transplantation. In the clinical trial conducted by Dr. Masayo Takahashi, we performed whole-genome sequencing to analyze the cells at the request of the Japanese government. In the first patient, we detected single nucleotide variations in a few genes, with little evidence of association with cancer formation. We therefore concluded that the iPSC line could be used in the clinical trial. In the second patient, who was male, we found that two genes lost one allele and another gene on the X chromosome had a deletion in its coding region. Although there was little evidence that these three genes were associated with cancer formation, we decided not to use this clone in clinical trials because of the deletions. Whether this decision was appropriate or too strict needs to be openly discussed. Similar deletions may exist in other types of cells being transplanted in other types of cell therapies, where genomic analyses beyond karyotyping have not been applied. Regulatory guidelines need to be set as to how to utilize state-of-the-art technologies to evaluate the safety of cell therapies that use iPSCs and related cells.

***CSC:* At the moment, there is a wide variance in how regenerative medicine and cell therapies are regulated at a governmental level. Are there any systems that you view as being exemplary or having significant weaknesses? For example, do you think the new approval system in Japan will help the translation of hiPSC research?**

SY: Because of recent legal changes, Japan has received a lot of international attention regarding its regulatory mechanism for cell therapies. As a scientist who is dedicated to the clinical use of iPSCs, I am very happy to see a shift that eases this transition. However, I also understand some of the unintended consequences. One issue that has emerged is that by separating itself from policies in other countries, it remains unclear how useful clinical data from Japanese studies will be internationally. My goal is for iPSCs to be used globally, with citizenship having no factor in access. Therefore, perhaps the most pressing matter is that governments around the world cooperate on a global scheme so that studies can access patients independently of location.

KE: From a regulatory perspective, I think we are still in the top half of the first inning. (That is an analogy that may play well in America and Japan, but less well elsewhere.) I think the new governmental approach in Japan is interesting. The regulations seem to aim to do two desirable things. First, they seem designed to close loopholes that were concerning because they enabled fringe or poorly established stem cell therapies to go forward in private clinics with little or limited oversight. Second, they were conceived, as I understand it, to create a landscape that promotes new therapies by bringing certain clinical costs related to their development under the umbrella of their health-care system. It seems to be a bold experiment and everyone will be watching the outcome closely.

The situation in other countries remains less clear for now. I don't think that any country is in the lead and I don't think any one is terribly behind yet, either. It will be interesting to see whether countries that were generally supportive of human ESC research in the early days realize a benefit that crosses over to the application of iPSCs.

***CSC:* Are there lessons from related fields (i.e., bone marrow transfer, CAR T cells, ESCs, gene therapy) that you think the field should consider as clinical translation moves forward?**

MP: There are always lessons to be learned from related fields. Many of the pivotal issues relating to safety and efficacy are very similar for ESCs and iPSCs.

As we move forward, it is also important to learn from past mistakes. In my field one particular lesson to learn is that the cells used in clinical trials need to go through extensive pre-clinical testing in relevant in vivo models prior to use in patients. Today, there is a large focus on safety and a lively debate around the starting cells to use, but what cannot be forgotten prior to clinical use has to be good documentation of the cells' ability to function in the diseased adult CNS. A safe but ineffective cell therapy will not cure anyone.

MT: For safety issues regarding genetic alterations, we can learn from bone marrow transfer or other somatic cell treatments since they may also harbor gene mutations, and the way we think about any safety threats they impose should be the same. These issues are not dependent on the cell sources but on the end products and methods of treatment.

If the end product is meant to have limited survival it is usually safe, as is the case for most of the somatic cell treatments currently available. In addition, we can check the risk with in vivo tumorigenicity tests in animal models. If the graft cells survive and divide in the body for a long time, there is a greater possibility of long-term tumorigenicity risk (even with somatic cells). So we can learn from our experience with bone marrow transfer and gene therapy about the risks of genetic changes.

***CSC:* What do you think is the best way to ensure that patients and the broader public have realistic expectations of hiPSC technology? What do you think is the best way to involve patients in the discussion?**

KE: This is perhaps the most challenging question. Clearly this takes effort. Most scientific discourse happens well above the public level of understanding. It takes a lot of energy to translate recent scientific discoveries into an understandable piece of news or information. It will be key to not "oversell" new developments in the interest of trying to attract funding. At the same time it is important for the public to understand that the field is moving forward now and we are gradually ratcheting up greater and greater assurance that there is real utility in hiPSCs. In my experience, involving patients in research is an

important means of moving the discussion with them forward. Patient advocates and advocacy groups have also proven to be an important force in supporting stem cell research and it will be important to continue to engage them.

MT: Researchers and medical doctors should have a clear perspective first. I often feel that researchers do not have a good understanding of the actual value of their research with regard to clinical treatment and are often overly optimistic about the amount of work involved to go from basic scientific discoveries to a clinical therapy. Without correct knowledge about clinical treatments one should not mention the application of basic research findings. Then they (we) should continue to explain whenever we have the opportunity the reality of the situation, both good and bad, to the public, and especially to patients. We have to keep communicating to make sure we promote understanding and realistic expectations.

***CSC:* As you know, 2016 marks the 10th anniversary of the iPSC discovery. Please share any predictions or hopes you have for where the field will be 10 years from now.**

SY: I am often asked this question. Instead of answering, let me share with you my thoughts from 2006, when we first discovered iPSCs. At that time, we could not make iPSCs safely. Yet after such a short time, we can now make iPSCs from various cell types and use them to make various cell types. More excitingly, the first iPSC-based transplant was conducted in 2014 and showed positive results 1 year later. Drug companies are investing huge sums of money to use these cells to find new drugs. The recent advances in genome editing technology, particularly CRISPR, take the potential of iPSCs to a whole new level. That's a short summary of the things I did not expect 10 years ago.

With that said, it is fair to say there will be many surprising discoveries in the next 10 years using iPSCs. I imagine iPSC-based therapies will be readily available for some diseases. I also hope to see several pharmaceuticals that are developed by using iPSC technology put on the market. With luck, maybe iPSC technology will create new approaches to cure cancers and immunological diseases.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes full interview transcripts and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2016.01.023.

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Supplemental Information

10 Questions: Clinical Outlook for iPSCs

Cell Stem Cell Editorial Team

The full transcripts of the individual interviews appear here.

Kevin Eggan,
Harvard University, USA

CSC: **In your mind, what are the most promising therapeutic applications for hiPSCs currently in development?**

KE: I would currently break this down along two distinct lines. For use in transplantation medicine, at the moment, a large push is being made in forms of Macular degeneration using pigmented epithelial cells made from both iPS cells and with human embryonic stem cells. With an ongoing clinical trial of human ES cell derived cardiac cells for myocardial infarction, one would think that similar efforts with iPS cell derived cardiac cells can't be far behind. The field of directed differentiation of stem cells has progressed remarkably over the last several years with production of nervous system cell types leading the way. If I had to guess which areas will see a great deal of focus in the near future, I would say Parkinson's disease and the Epilepsies. The ease of transplanting blood types for many disorders will make this increasingly attractive as time goes on and more high-quality differentiated cells are made.

Another important utility of iPS cells has become use in the identification of candidate drugs through mechanistic studies or chemical screening. This strategy has become well radiated throughout academic and industrial settings. There seem to be many drug candidates moving towards the clinic discovered by this approach. Again the ease of generating large quantities of neuronal and cardiac cell types has put indications impacting these cells in the lead. One example is a compound we found to normalize the physiological properties of iPS cell-derived motor neurons from ALS patients. There is currently a clinical trial underway to determine if this drug can rescue known electrical changes in the brains of individuals with this form of neural degeneration.

CSC: **Do you think it's valuable to bank iPSCs for clinical purposes? What do you think are the main considerations to keep in mind?**

KE: Like many things, I think the answer is, that it depends. For purposes of transplantation medicine I think it is too early to tell. I think as the first iPS cell-derived therapies reach the marketplace, the value of such banks will become more apparent.

CSC: **On a related note, what are your thoughts on the value of autologous versus allogeneic approaches? Where do you think the community should be focusing its efforts? What do you think will be more feasible or safe?**

KE: I generally take a stepwise approach to solving problems. For transplantation medicine with iPS cells, I think that is a sensible strategy. I feel that beginning with an allogeneic approach and the production of a single batch of cell product that could be tested for safety and then administered to a group of patients to test its efficacy makes sense. If the autologous approach seemed safe and it were efficacious, then the added benefits and complexities of an autologous approach could be more reasonably explored.

CSC: **What are the pressing technical challenges that need to be addressed for full clinical potential to be reached?**

KE: While major progress has been made in developing strategies for directed differentiation of many cell types, it is still unclear how many of these will transfer well to culture conditions that comply with clinical

requirements. I think that many groups will be surprised by what a challenge this will be. Still, I think that many of these challenges are only technical and that through time and effort they will be overcome. Another major challenge will be around reproducibility. Most differentiation schemes make heterogeneous mixtures of cells whose cellular constituents fluctuate in abundance from run to run. This is sort of like baking a fruitcake and having the abundance of cranberries, raisins and currents change every time you make it. Better methods will need to be developed in many cases to allow the target cells of interest to be purified for downstream use.

CSC: **Where do you see the biggest gaps in understanding that we need to focus for basic research**

KE: There are many ways to go with this question, I think that one of the largest holes in our knowledge relative to use of iPSCs in transplantation studies centers around what happens to the cells after transplantation. Where do they go after transplantation? How well do differentiated cells survive, function and integrate over the very long-term following transplant? While many studies of function in vitro have been performed, transplant studies are still scarce in rodent models and rare in large animals. There needs to be considerable progress in the area. Additionally, in many cases a deep understanding of the detailed biology of the human differentiated cells we are trying to make from human iPSCs is lacking. For many scientists there are real challenges in obtaining the primary counterparts of cells that they wish to produce from human iPSCs and too often we rely on analogy with cells readily isolated from rodents. I hope in the future more effort will be applied to compare human iPSC-derived cell types to their actual human counterparts.

CSC: **What do you view as the major regulatory challenges that the field faces for clinical use of hiPSCs?**

KE: I think the biggest challenge remains the unknown. Even at nearly 10, iPS cells are still new compared to many other strategies for developing transplantation products. New discoveries concerning their properties and technical improvements in their production create a still shifting landscape that both scientists and regulators have to navigate. I think that once the FDA approves the first clinical studies using iPS cell-derived products for trial, the landscape will come into better focus.

CSC: **At the moment, there is a wide variance in how regenerative medicine and cell therapies are regulated at a governmental level. Are there any systems that you view as being exemplary or having significant weaknesses? For example, do you think the new approval system in Japan will help translation of hiPSC research?**

KE: From a regulatory perspective, I think we are still in the top half of the first inning. That is an analogy that may play well in America and Japan, but less well elsewhere. I think the new governmental approach in Japan is interesting. The regulations seem to aim to do two desirable things. First, they seem designed to close loopholes that were concerning because they enabled fringe or poorly established stem cell-therapies to go forward in private clinics with little or limited oversight. Second, they were conceived, as I understand it to create a landscape that promotes new therapies by bringing certain clinical costs related to their development under the umbrella of their health-care system. It seems to be a bold experiment and everyone will be watching the outcome closely.

The situation in other countries remains less clear for now. I don't think that any country is in the lead and I don't think any one is terribly behind yet either. It will be interesting to see whether countries that were generally supportive of human embryonic stem cell research in the early days realize a benefit that crosses over to the application of iPSCs.

CSC: Are there lessons from related fields (i.e. bone marrow transfer, CAR T cells, ESCs, gene therapy) that you think the field should consider as clinical translation moves forward?

KE: Most certainly there are lessons to be learned from these therapeutic areas. From gene therapy I think we learned that it is better to proceed slowly and carefully, trying to avoid costly mistakes that can set the field back. The transformational nature of iPS cell technology is evident, but we still have a lot to learn about how to execute in this arena. With respect to CAR T, I think its become clear how hard and costly it is to execute on autologous therapies and combination cell and gene therapies even with a therapeutic that seems extremely promising.

***CSC:* What do you think is the best way to ensure that patients and the broader public have realistic expectations of hiPSC technology? What do you think is the best way to involve patients in the discussion?**

KE: This is perhaps the most challenging question. Clearly this takes effort. Most scientific discourse happens well above the public level of understanding. It takes a lot of energy to translate recent science discoveries into an understandable piece of news or information. It will be key to not "oversell" new developments in the interest of trying to attract funding. At the same time it is important for the public to understand that the field is moving forward now and we are gradually ratcheting up greater and greater assurance that there is real utility in hiPSCs. In my experience, involving patients in research is an important means of moving the discussion with them forward. Patient advocates and advocacy groups have also proven to be an important force in supporting stem cell research and it will be important to continue to engage them.

CSC: As you know, 2016 marks the 10th anniversary of the iPSC discovery. Please share any predictions or hopes you have for where the field will be 10 years from now.

KE: I always find it perilous to get out the crystal ball and take a look. Mostly I feel this way because scientists often tend to be overly conservative. I think it would have been almost impossible to predict ten years ago the changes in our world that were brought about by the growth of the world wide web, mobile devices, the decreasing costs of DNA sequencing and of course by iPS cell reprogramming. I do wonder whether production of iPS cell lines will be a natural thing that happens to everyone admitted to hospital, both to potentially make cells for transplant but also for new forms of cellular diagnostics that never would have been conceived prior to the advent of reprogramming.

Malin Parmar

Lund University, Sweden

***CSC:* In your mind, what are the most promising therapeutic applications for hiPSCs currently in development?**

MP: Right now it is very interesting to follow the progression of the newly initiated studies using stem cells to treat RPE conducted in Japan and UK.
I work in the field of cell based therapies for Parkinson's Disease and this is of course a very interesting field today as pluripotent stem cell-based therapies for PD are inching closer and closer to use in clinical trials

***CSC:* Do you think it's valuable to bank iPSCs for clinical purposes? What do you think are the main considerations to keep in mind?**

MP: I think it makes sense to bank iPS cells from individuals with specific diseases to create a resource for the scientific community to use for understanding the disease pathology and develop better differential diagnostics and new treatment strategies.
In terms of generating large banks with the intention for the cells to be used for cell therapy I am less convinced this is a good strategy. One has to keep in mind that it is a very resource demanding task to create and maintain such a bank under GMP conditions, and that each differentiated therapeutic cell product made from a line in that bank still will have to go through safety and efficacy testing prior to use. If that same amount of resources was put into addressing key issues remaining to enable the use of patients own cells or cells from matched donors, to create therapeutic cells directly without a banking step, personalized cell therapy could become a reality.

CSC: **On a related note, what are your thoughts on the value of autologous versus allogeneic approaches? Where do you think the community should be focusing its efforts? What do you think will be more feasible or safe?**

MP: For neurological disorders immune rejection is less of a concern than for a number of other diseases. In my opinion, all first in human trials have to be conducted with cells that are first put through rigorous safety and efficacy testing prior to use in patients. This can be done with either allogeneic ESC-based or allogeneic iPSC-based grafting. As iPSCs encounter some additional concerns relative to ES cells due to their derivation via reprogramming, I think the most straightforward strategy would be to use ESCs. However, looking into the future, one can envision matched donor cells or personalized treatments, and those would only really be possible via cellular reprograming.

CSC: **What are the pressing technical challenges that need to be addressed for full clinical potential to be reached?**

MP: In my field we now have very good protocols for cell differentiation and we know we can generate cells that function *en par* with human fetal DA neurons from pluripotent stem cells. The challenges that lie ahead are associated with meeting the regulatory requirements for cell production as well as safety and efficacy testing prior to use in patients. Related to this is the need to develop much better markers that predict the in vivo therapeutic efficacy and authenticity of grafted cells

CSC: **Where do you see the biggest gaps in understanding that we need to focus for basic research?**

MP: A key issue remaining to be addressed is how we precisely and finely control the identity of the cellular products derived from PSCs so that we can generate cells that are very similar to the cells normally found in our bodies. Related to this is the challenge of determining the identity of cells generated in a dish. In the brain for example, cell identity is often governed by anatomical location and projections and this is lost *in vitro.* We therefore need much more refined methods for determining the exact identity and functional potential of cells generated from stem cells.

CSC: **What do you view as the major regulatory challenges that the field faces for clinical use of hiPSCs?**

MP: One major difficulty is that the regulatory framework is different in different countries. For example, the FDA and EMA have requirements that vary so much that it is hard to develop cells that can be used on both continents or globally.
Another challenge is that this development takes a long time and while we struggle to bring safe and effective stem cells based therapies to patients as quickly as possible, the number of clinics offering

unproven therapies for commercial gain are increasing which creates unrealistic expectations in patients and puts them at risk.

***CSC:* At the moment, there is a wide variance in how regenerative medicine and cell therapies are regulated at a governmental level. Are there any systems that you view as being exemplary or having significant weaknesses? For example, do you think the new approval system in Japan will help translation of hiPSC research?**

MP: I don't think that there is any system that sticks out as being exceptional. The new system in Japan is designed to enable cells to get to clinic more quickly and if this can be done while also upholding the necessary requirements for safety and efficacy it is an interesting model to look at.

CSC: Are there lessons from related fields (i.e. bone marrow transfer, CAR T cells, ESCs, gene therapy) that you think the field should consider as clinical translation moves forward?

MP: There are always lessons to be learned from related fields. Many of the pivotal issues relating to safety and efficacy are very similar for ESCs and iPSCs.
As we move forward, it is also important to learn from past mistakes. In my field one particular lesson to learn is that the cells used in clinical trials need to go through extensive pre-clinical testing in relevant *in vivo* models prior to use in patients. Today, there is a large focus on safety and a lively debate around the starting cells to use but what cannot be forgotten prior to clinical use has to be good documentation of the cells ability to function in the diseased adult CNS. A safe but ineffective cell therapy will not cure anyone.

***CSC:* What do you think is the best way to ensure that patients and the broader public have realistic expectations of hiPSC technology? What do you think is the best way to involve patients in the discussion?**

MP: I think that the research community has a large role in engaging with the public, with patients and with patient organizations, policymakers etc. Organizations like ISSCR are key mediators of such interactions.

CSC: As you know, 2016 marks the 10th anniversary of the iPSC discovery. Please share any predictions or hopes you have for where the field will be 10 years from now.

MP: The iPSC discovery was a real game changer in the field and has already led to a number of important insights into how diseases develop and arise. The hope to useof using the cells in personalized medicine is still in ahead of us, but I am convinced that in the next 10 years, iPSCs or other types of reprogrammed cells will be used to treat patients.

Masayo Takahashi
Riken, Japan

***CSC:* In your mind, what are the most promising therapeutic applications for hiPSCs currently in development?**

MT: Within the realm of regenerative medicine, retinal pigment epithelium (RPE) for Age-related macular degeneration or other RPE impairment diseases seems the most promising avenue to me, and that's why I am actively pursuing it. Parkinson's disease is also a very interesting area.

***CSC:* Do you think it's valuable to bank iPSCs for clinical purposes? What do you think are the main considerations to keep in mind?**

MT: The strength of immune responses is different from cell type to cell type and also depends on the condition of host tissue. For the RPE treatment we are developing, using HLA matched cells for transplantation will avoid an immune response to the graft almost completely, and in Japan several homozygous HLA lines will cover the half of population. So yes.
But I do not know for other cell types or other countries. Also, it is possible that there are other ways to reduce the immune response other than by HLA matching.

***CSC:* On a related note, what are your thoughts on the value of autologous versus allogeneic approaches? Where do you think the community should be focusing its efforts? What do you think will be more feasible or safe?**

MT: Autologous approached have a strong advantage compared to allogeneic approaches because they do not require immune suppression, and the first patient enrolled in our clinical trial was treated with autologous cells and showed no immune rejection.

However, allogeneic approaches have a significant advantage when it comes to cost.
Overall, I think the community should be focusing at the moment on allogeneic approaches, to develop standard treatments that will be applicable to the many many desperately waiting patients.

In terms of feasibility and safety, whether autologous or allogeneic approaches should be employed depends on the end products and this should be evaluated on a case-by-case basis. I think it is wrong to think of regenerative medicine in a homogenous way. We should consider each disease and case separately.

***CSC:* What are the pressing technical challenges that need to be addressed for full clinical potential to be reached?**

MT: Again it depends on the end products, and at present they are in different stages of development.
But in the end, methods for manufacturing a safe product at mass scale and appropriate transportation will be needed.

***CSC:* Where do you see the biggest gaps in understanding that we need to focus for basic research?**

MT: Again we cannot consider iPSC-derived products as a homogenous entity. Many cell types still need the better differentiation protocols to make mature functional cells or tissue. Other cell types continue to require improved protocols to ensure their safe use, such as improved purification methods or the ability to make cells that do not form tumors in vivo. For photoreceptor transplantation, integration in the host tissue and reconstruction of neural networks are the key issues. For RPE cells, we have overcome these basic challenges to the point where it was appropriate to deliver them to a patient in a clinical trial, and we saw that the grafts survived and maintained their durability for over one year in the eye of the patient. The next important issue to address will be devising ways to reduce an immune response in allogeneic transplantation.

***CSC:* What do you view as the major regulatory challenges that the field faces for clinical use of hiPSCs?**

MT: We still need to develop a more sophisticated and comprehensive approach to thinking about and developing cell therapies that more fully considers the treatment as a whole. Regenerative medicine is a

medicine that sometimes requires surgery. It is not completed only with cells.

***CSC:* At the moment, there is a wide variance in how regenerative medicine and cell therapies are regulated at a governmental level. Are there any systems that you view as being exemplary or having significant weaknesses? For example, do you think the new approval system in Japan will help translation of hiPSC research?**

MT: Yes, the Japanese system is suitable for the emerging new concepts in treatment. When a field is still in its infancy the numbers of cases are small and the effects will be minimal. With the new Japanese approval system we can better evaluate regenerative medicine as treatments, and it may change the concept of regulation. We should work hard to make it successful.

CSC: Are there lessons from related fields (i.e. bone marrow transfer, CAR T cells, ESCs, gene therapy) that you think the field should consider as clinical translation moves forward?

MT: For safety issues regarding genetic alterations, we can learn from bone marrow transfer or other somatic cell treatments since they may also harbor gene mutations and the way we think about any safety threats they impose should be the same. These issues are not dependent on the cell sources but on the end products and methods of treatment.
If the end product is meant to have limited survival it is usually safe, as is the case for most of the somatic cell treatments currently available. In addition, we can check the risk with in vivo tumorigenicity tests in animal models. If the graft cells survive and divide in the body for a long time, there is a greater possibility of long-term tumorigenicity risk (even with somatic cells). So we can learn from our experience with bone marrow transfer and gene therapy about the risks of genetic changes.

***CSC:* What do you think is the best way to ensure that patients and the broader public have realistic expectations of hiPSC technology? What do you think is the best way to involve patients in the discussion?**

MT: Researchers and medical doctors should have a clear perspective first. I often feel that researchers do not have a good understanding of the actual value of their research with regard to clinical treatment and are often overly optimistic about the amount of work involved to go from basic scientific discoveries to a clinical therapy. Without correct knowledge about clinical treatments one should not mention about application of basic research findings. Then they (we) should continue to explain whenever we have the opportunity the reality of the situation, both good and bad, to the public, and especially to patients. We have to keep communicating to make sure we promote understanding and realistic expectations.

CSC: As you know, 2016 marks the 10th anniversary of the iPSC discovery. Please share any predictions or hopes you have for where the field will be 10 years from now.

MT: We have experienced enormous improvement in the field of medicine over the last 20 years.
In another 10 years, I hope that the reach of iPSCs will expand further into more clinical trials (in the form of cell therapy or with drugs discovered using iPSCs) throughout the world and change the medical field dramatically. I think that regenerative medicine will become an important field of medicine as well as the standard treatments of today perhaps in say 20 years. Even more, it would be also the means of the preventive medicine in the future.

Shinya Yamanaka

Kyoto University, Japan
Gladstone Institutes, USA

CSC: **In your mind, what are the most promising therapeutic applications for hiPSCs currently in development?**

SY: One promising application is iPSC-based cell therapy. The work led by Dr. Masayo Takahashi at Riken Center for Developmental Biology (CDB) is generating a lot of interest, since it is the first clinical research that uses the transplantation of 100% iPSC-derived cell sheets. At CiRA we are eagerly awaiting similar studies for Parkinson's disease and blood transfusion. These studies require a much larger number of cells than the one by Dr. Takahashi. Her team's work was revolutionary for many reasons, and one is that it reprogrammed the patient's own somatic cells to create retinal cells for treatment. However, autologous transplants are not financially feasible at present. Future studies will use allogeneic transplants. Demonstrating allogeneic transplants of iPSC products has tremendous potential for clinical use.

Another development of strong interest is drug discovery. iPSCs have demonstrated promise for not only drug discovery but also drug repositioning. Drug repositioning would bring drugs faster to clinical use.

Accordingly, CiRA is working with a number of companies that aim to realize cell therapies and/or drug development using iPSCs.

CSC: **Do you think it's valuable to bank iPSCs for clinical purposes? What do you think are the main considerations to keep in mind?**

SY: Seeing the success of blood banks, it would be wonderful if we could do something similar with iPSCs. CiRA is currently building an iPSC bank - the iPS Cell Stock for Regenerative Medicine - and distributed one quality-assured iPSC line to a pharmaceutical company and a medical organization last year. Our banking system collects blood or skin cells from healthy donors with HLA homozygous alleles and generates iPSC lines. We predict that ~100 such lines would cover a majority of the Japanese population. Banking iPSCs can save time and cost because the quality of iPSC lines can be assured before they are needed for treatment. In addition, target cells derived from the iPSCs can be provided more quickly to patients than making patient-specific iPSCs.

Another important consideration is the impact of minor antigens and other immunological mechanisms. We have observed beneficial effects of MHC matching in monkey models, but these need to be proved by clinical studies.

CSC: **On a related note, what are your thoughts on the value of autologous versus allogeneic approaches? Where do you think the community should be focusing its efforts? What do you think will be more feasible or safe?**

SY: Ideally, autologous transplants are theoretically best in terms of safety, but the feasibility is not there. We don't bank our own blood. Similarly, we should not expect to bank our own cells. Furthermore, while autologous cells may be safest, they won't be of much help for genetic diseases, since the cells will need genome editing before the therapy. While genome editing technology is rapidly improving, it would be much easier to avoid this step altogether. On the other hand, autologous cells would be best for personalized medicine, including drug treatments. But again, the cost at this stage is prohibitive.

As mentioned above, using quality-assured allogeneic cells has advantages in saving time and cost. Autologous transplantation at large scale will only be feasible if we significantly improve the efficacy of iPSC generation.

***CSC:* What are the pressing technical challenges that need to be addressed for full clinical potential to be reached?**

SY: When iPSCs were first made, it seemed like a long way before they could ever reach clinical application, but unbelievably they already are. Many of the problems are the same as any stem cell-based therapy – purification, cell numbers, culturing conditions, etc. Other problems, however, arise from our reprogramming the cell. We need to be extremely careful that the reprogramming is stable and does not result in malignant mutations or other perturbations in the cell state. Most importantly, we need to form a consensus among scientists on safety standards for medical use of iPSCs. The Japanese government has set up a study group to discuss and draw up such standards.

***CSC:* Where do you see the biggest gaps in understanding that we need to focus for basic research?**

SY: In my mind, the biggest factor is how to differentiate iPSCs into functional and mature cells. At present, iPSC-derived cells such as cardiac myocytes and hepatocytes are still immature and more closely resemble fetal cells than adult cells. Nobody has succeeded in differentiating iPSCs to hematopoietic stem cells that are equivalent to those in adult bone marrows. Much more efforts are required to overcome this essential hurdle.

***CSC:* What do you view as the major regulatory challenges that the field faces for clinical use of hiPSCs?**

SY: The biggest question is how to use deep sequencing technology in evaluating the safety of iPSCs and differentiated cells prior to transplantation. In the clinical trial conducted by Dr. Masayo Takahashi, we performed whole genome sequencing to analyze the cells upon request of the Japanese Government. In the first patient, we detected single nucleotide variations in a few genes, with little evidence of association with cancer formation. We therefore concluded that the iPSC line could be used in the clinical trial. In the second patient, who was male, we found that two genes lost one allele and another gene on X chromosome had a deletion in its coding region. Although there was little evidence that these three genes were associated with cancer formation, we decided not to use this clone in clinical trial because of the deletions. Whether this decision was appropriate or too strict needs to be openly discussed. Similar deletions may exist in other types of cells being transplanted in other types of cell therapies, where genomic analyses beyond karyotyping have not been applied. Regulatory guideline needs to be set as to how to utilize state-of-the-art technologies to evaluate the safety of cell therapies that use iPSCs and related cells.

***CSC:* At the moment, there is a wide variance in how regenerative medicine and cell therapies are regulated at a governmental level. Are there any systems that you view as being exemplary or having significant weaknesses? For example, do you think the new approval system in Japan will help translation of hiPSC research?**

SY: Because of recent legal changes, Japan has received a lot of international attention regarding its regulatory mechanism for cell therapies. As a scientist who is dedicated to the clinical use of iPSCs, I am very happy to see a shift that eases this transition. However, I also understand some of the unintended consequences. One issue that has emerged is that by separating itself from policies in other countries, it remains unclear how useful clinical data from Japanese studies will be internationally. My goal is for iPSCs to be used globally, with citizenship having no factor in access. Therefore, perhaps the most pressing matter is that governments around the world cooperate on a global scheme so that studies can access patients independently of location.

CSC: Are there lessons from related fields (i.e. bone marrow transfer, CAR T cells, ESCs, gene therapy) that you think the field should consider as clinical translation moves forward?

SY: One reason Japan's policy regarding stem cell therapies changed was a lack of proper regulation allowed access to dangerous treatments, which resulted in tragedy. Even when good regulation exists, tragedy can set back an experimental therapy for years if not decades, as we saw with gene therapies in the U.S. and Europe. I feel that we need to balance the enthusiasm scientists have for iPSCs with good policy.

We must be careful to temper expectations. For example, regarding the study by Dr. Masayo Takahashi I mentioned above, the public and the government must be informed that this initial transplant is not aiming to cure the patient; it only aims to prove the safety of iPSCs. Clear communication with the public and good policy should allow us to proceed at a good pace.

CSC: **What do you think is the best way to ensure that patients and the broader public have realistic expectations of hiPSC technology? What do you think is the best way to involve patients in the discussion?**

SY: As I mention above, tempering expectation is key. We get many phone calls daily at CiRA with people hoping we can cure a loved one. The public must understand that the experiments being done today are to help people a generation from now. Therefore, institutes should invest beyond the science by building a team of ethicists and communicators as we have done at CiRA. Losing control of the message can seriously harm scientific progress.

CSC: As you know, 2016 marks the 10th anniversary of the iPSC discovery. Please share any predictions or hopes you have for where the field will be 10 years from now.

SY: I am often asked this question. Instead of answering, let me share with you my thoughts from 2006, when we first discovered iPSCs. At that time, we could not make iPSCs safely. Yet in such a short time, we can make iPSCs from various cell types and use them to make various cell types. More excitingly, the first iPSC-based transplant was conducted in 2014 and showed positive results one year later. Drug companies are investing huge sums of money to use these cells to find new drugs. The recent advances in genome editing technology, particularly CRISPR, takes the potential of iPSCs to a whole new level. That's a short summary of the things I did not expect 10 years ago.

With that said, it is fair to say there will be many surprising discoveries in the next 10 years using iPSCs. I imagine iPSC-based therapies will be readily available for some diseases. I also hope to see several pharmaceuticals that are developed by using iPSC technology put on the market. With luck, maybe iPSC technology will create new approaches to cure cancers and immunological diseases.