Prof. Shinya Yamanaka:

Thank you very much Professor Kadowaki for your kind introduction and invitation. It is a great honor to be here today.

I think many of you know that I am now working on stem cells, but my research career actually began 20 years ago with work on atherosclerosis. I did my postdoc training in Dr. Tom Innerarity's lab at Gladstone Institutes of Cardiovascular Diseases in San Francisco. I was testing his hypothesis that by overexpressing APOBEC1, an enzyme responsible for ApoB mRNA editing in the liver, we should be able to lower plasma cholesterol and therefore prevent atherosclerosis.

To test this hypothesis, I generated transgenic mice overexpressing APOBEC1 in a liver-specific manner by using the ApoE promoter. I was able to establish several lines. One day, my technician ran to me and announced, "Your mice are pregnant!" I thought, "That's strange", because my mice were all male. I went to the animal facility and checked. The mice were indeed still male, but they did look pregnant. I sacrificed some of the mice and found huge tumors, or hepatocellular carcinoma. This finding proved that APOBEC1 is a very active oncogene and can never be used for gene therapy.

From this result, I learned three important lessons in science. First, research leads to unexpected findings. Second, before considering any therapies, drugs or procedures directly on humans, we must first test them in animals. Third, all hypotheses need to be proven by experiments - even if they come from our boss.

About APOBEC1 itself, I really wanted to understand why it causes tumors. So I decided to work on tumorigenicity and was able to identify a new target of APOBEC1, NAT1. Until then, ApoB mRNA was the only known target. From this finding, I made my own hypothesis that some tumor suppressor gene is responsible for the APOBEC1-mediated tumor development.

To test my hypothesis, I generated NAT1 knockout mice. These mice led to an even more surprisingly result than my "pregnant" male mice. I found that NAT1 is essential for the pluripotency of ES cells. Thus began my interest in ES cells. ES cells were first generated from mouse embryos in 1981 and have two important properties. The first is their capability to divide and renew for a long period of time. This allows us to expand ES cells without limit. The other is pluripotency. ES cells can differentiate into many types of somatic and also germ cells. Exploiting these two properties, we can prepare many types of mouse cells, including germ cells in large quantities. ES cell technology has proven to be extremely useful in biomedical research.

In 1996, I decided to go back to Japan to work on this project. Although I thought my research on mouse ES cells was very interesting and exciting, many of my colleagues disagreed. I was often told by colleagues, "Stop working on these strange mouse cells, and work on something more related to human diseases." I brought three mice from Gladstone Institutes to Osaka, but after a few months the number of mice became 20 and after a year it increased to 300. I say this, because I had no staff to assist me in caring for these critters.

Too many mice and experimental results caused me to suffer from PAD, or Post-America Depression. I was seriously thinking about quitting science and returning to the clinic as a doctor. However, two events happened in my life as this time cured me of PAD. I mentioned above that mouse ES cells were created in 1981. But it was not until 1998 when the generation of human ES cells was first reported by Dr. James Thomson of the U.S.

Human ES cells share the two same important properties of mouse ES cells. Therefore, we can differentiate human ES cells into many types of human cells.

Switching my research from mouse ES cells to human ES cells allowed me to continue my interest in pluripotency, but also to study human diseases. ES cells have promise to help patients suffering from various diseases and injuries such as Parkinson's disease, spinal cord injury, cardiac failure, diabetes, and so on by transplanting the many types of cells in which they can be derived. Yet human ES cells can only be acquired by destroying human embryos, which has created great controversy.

So human ES cells were the first event that helped me recover from PAD. The second was my promotion. In late 1999, I got an appointment at Nara Institute of Science and Technology (NAIST), which made me an associate professor and more importantly gave me my own laboratory for the first time. This institute has a beautiful campus, lots of good equipment and many talented faculty members and graduate students.

It was there that I decided to set a long-term goal for my research, and my life really, which is to apply human ES cells to medicine. However, this required me and my team to overcome many physical and immunological hurdles in human ES cells. “Wouldn’t it be better if we could reprogram somatic cells such as skin cells and blood cells into human stem cells?”, I thought to myself. More precisely, I wanted to identify the factors that can reprogram somatic cells back into the embryonic state.

To make a long story short, we did this in mouse cells and reported it in 2006. We found that by introducing four specific transcriptional factors into mouse skin cells, we could reprogram the cells into an ES-like state. I named these iPS cells, or induced pluripotent stem cells. In the following year, we and other groups were able to recapitulate the same reprogramming procedure in human skin cells and proved human iPS cells can be generated with the same four factors.

I want to briefly describe how we identified these four factors, including the people who were most involved. The first step was to classify 24 candidate factors into five or six groups. These three young members from my lab, Kazutoshi Takahashi, Yoshimi Tokuzawa, and Tomoko Ichisaka, along with many other students were involved in this first step.

The second important step was that we were able to establish a very simple, but very sensitive assay system to evaluate the 24 candidates. Yoshimi and Tomoko also contributed a lot to ths step. Finally, Kazu used this sensitive assay system to identify the 4 factors. I cannot express how thankful I am really to these three for our accomplishment.

Now we can generate iPS cells from many types of somatic cells. For example, we can generate iPS cells from peripheral blood samples. Using just 5 or 10 ml of peripheral blood and the four factors, we can make blood cell types, such as T cells, into iPS cells. Like ES cells, iPS cells can be expanded into large quantities. Moreover, by treating iPS cells with growth factors, cytokines, and other chemicals, we can induce differentiation into many different types of somatic cells. For example, we can generate beating cardiac myocytes. Thus, iPS cell technology allows us to take skin cells, blood cells, or (in theory) any type of cell in the body and make it into cardiac myocytes.

The implications of this technology are immense, especially for medical treatment, which is where I ultimately want to take it. There are two major applications of iPS cells in medicine. The first one is *in vivo* applications such as cell therapy. Research is showing that transplanting iPS cell-derived somatic cells can have tremendous benefits for patient care. Last year the first

application of iPS-based cell therapy was used to treat a patient suffering from macular degeneration. Dr. Masayo Takahashi of RIKEN transplanted sheets of retinal pigment epithelial cells derived from iPS cells. It has only been a few months since the patient received this treatment, but success here will help initiate clinical research on macular degeneration. Admittedly, I am very nervous about the results.

In our institute, Dr. Jun Takahashi has been working to apply iPS-based cell therapy to Parkinson's disease. He has developed a very specific and robust procedure for dopaminergic neuron differentiation from human iPS cells and ES cells and is testing these dopaminergic neurons in a monkey model of Parkinson’s disease. He hopes to translate this research into human clinical research within a few years. I think it is fair to see that he and his wife, Dr. Masayo Takahashi, make a great couple in stem cell therapy in Japan.

Another colleague of mine, Dr. Koji Eto, has been working on blood differentiation from iPS cells. He can now make functional platelets and erythrocytes from human iPS cells. Unlike the above studies, we can evaluate platelets and erythrocytes before blood transfusion and therefore confirm their safety. In addition, platelets and erythrocytes are anucleate, so we do not have to worry about tumor formation, which is probably the biggest hurdle of iPS cell-based therapy. There is an urgency for iPS cell-based blood technology. The Japanese Red Cross Society predicts that within 10 to 15 years there will be a severe shortage of blood donors in Japan.

iPS cell-based beta cells should be able to help the many patients suffering from both type 1 and type 2 diabetes. We have two groups working on beta cells at CiRA, Professor Kenji Osafune and Professor Yoshiya Kawaguchi. They are taking two different approaches. Dr. Osafune has been using a conventional 2D culture approach and is searching for chemicals and growth factors that promote beta cell transition from iPS cells. Dr. Kawaguchi, who is also a surgeon and expert on islet transplantation, has been working on 3D culture. Instead of making beta cells in culture, he is now trying to generate gut organs from which he is also trying to generate pancreatic tissues. These two approaches are very different, but complementary to each other. I am optimistic that in the very near future we will be able to prepare beta cells for islet transplantation from iPS cells.

At the University of Tokyo, Dr. Hiromitsu Nakauchi is taking a third approach and has already succeeded in making rat pancreas in mice and mouse pancreas in rat. He used PDX1 knockout mouse blastocysts, which cannot make functional pancreas. However, PDX1 knockout mouse blastocysts could be rescued by injecting rat pancreatic cells into them. He is applying this technology to humans. The hope here is to generate human pancreas or human kidney by a similar approach.

One great attribute about iPS cells is that they can be made from patient cells, which should remove certain ethical controversies and lower if not eliminate the risk of immune rejection. However, preparing iPS cells from a patient is very costly. Also it takes about five to six months to expand iPS cells and induce their differentiation, an unreasonable time for patients suffering from acute diseases and injuries such as acute liver failure and spinal cord injury. Therefore, we are now preparing an iPS cell stock project in Kyoto. In this strategy, we make iPS cells using cells from healthy volunteers or from cord blood samples instead of the patient. Since we make iPS cells in advance, we can perform vigorous quality control and can select the best and safest iPS cells, which can later be used for iPS cell-based therapy.

However, in this case we must consider immunogenicity. This iPS cell stock is not for autologous transplantation. Thus, we have to match HLA to minimize immune rejection, which is not an easy task. Consider 10 patients who have different HLA haplotypes. If

we want to prepare iPS cell stocks that can match these 10 patients, we need to establish iPS cell lines for each. This is neither time nor cost effective. However, preparing cell lines from HLA homozygous donors can significantly reduce this effort. In fact, we have calculated based on the HLA haplotype database that all we need is 140 HLA homozygous donors to cover more than 90% of the Japanese population. We aim to prepare iPS cell stocks from these 140 donors by 2020.

However, first we must identify HLA homozygous donors, which is why we are collaborating with the Japanese Red Cross Society and cord blood banks. In Kyoto University Hospital, we already have set up an iPS cell clinic where we obtain informed consent from the donors and collect blood samples. At CiRA, we are generating iPS cells in our GMP facility. The goal here is to make these iPS cell stocks available for preclinical and clinical trials.

Let me move on to the pharmaceutical applications of iPS cells. These include the study of toxicology. It is too often discovered that drugs can have a deleterious effect on the body, thus leading to their withdrawal from the market. In particular, the cardiac cycle can be disturbed. Because human cardiac myocytes are not readily available for testing a drug's toxicity, pharmaceutical companies instead use cancer cells that have encoded into them cardiac potassium channel genes. This system is far from ideal and results in many false positives and also false negatives. On the other hand, human iPS cells can be used to generate a large number of beating cardiac myocytes, offering perhaps a better model for toxicology. Many scientists including my colleagues are applying multielectrode arrays to these myocytes and recording ECG in petri dishes. We expect this approach to better predict negative effects on the heart.

Other pharmaceutical applications include the making of disease models and drug screening. Let me use ALS as an example. ALS is an intractable autoimmune disease. It's most famous patient, Lou Gehrig, the famous baseball player, passed away before his 40^{th} birthday due to the disease more than 70 years ago. Yet we still have very poor understanding of ALS. Ironically, we can treat mice that suffer from ALS well, but not humans. The reason is that medicines found to work on ALS mice have been found ineffective on humans. What we need is to test drugs on the neurons from ALS patients, but it is almost impossible to obtain these cells from living patients.

iPS cells, however, may solve this problem, because all we need is a small skin sample or blood sample. Dr. Haruhisa Inoue at our institute has been working on this goal. He has generated iPS cells from many control individuals and ALS patients. Interestingly, the iPS cells between these two groups did not differ. Yet when he differentiated the iPS cells into motor neurons, he observed many differences. One typical difference is morphology. Healthy motor neurons have long neurites, but he showed that motor neurons derived from ALS-iPS cells have shorter neurites. Using an automated system, he is now conducting drug screening and has already collected 50,000 drug candidates. One candidate compound is anacardic acid, which can recover ALS motor neurons to normal neurite lengths. The finding of this candidate, if not impossible, was much accelerated by using iPS cells.

That then is a summary of the type of goals we have at CiRA. I like to think of CiRA as a unique research institute, because making great discoveries is not the final goal of our scientists. The real goal is to bring this technology to patients and to realize the clinical applications of iPS cells. That is why we named the institute *Center for iPS cell Research and Application*. All the more than 300 people in this building are working to use iPS cells to help patients in the near future. Thank you very much for your attention.

Prof. Kadowaki:

Thank you very much, Professor Yamanaka, for your beautiful lecture. Professor Yamanaka talked about the current status of iPS cell research and also he talked about near-future promise of application of iPS cells in degenerative medicine, drug development, and disease modeling in many diseases including, cardiovascular diseases and diabetes.

In his Nobel lecture, Professor Yamanaka emphasized that he always worked very hard especially when he obtained unexpected results. He also told in his Nobel lecture that generation of iPS cells was an exceptional experience in his scientific career from medical school, and his career has been full of failures. So I would like to emphasize to the audience, especially to young researchers, please do not be discouraged when you obtain unexpected results or when you experience a difficult work, which is one of the reasons that Professor Yamanaka discovered iPS cells.

So with this, I would like to close this session again by thanking Professor Yamanaka for his really beautiful and wonderful lecture. Thank you very much.