ROBERT HUEBERT: Hello, my name is Robert Huebert. I'm a transplant hepatologist at Mayo Clinic in Rochester, Minnesota, and I'm also a physician scientist in the area of liver regenerative medicine. I'm here today with my friend and colleague, George Rakela, who is a hepatologist at Mayo Clinic Arizona and a leader in the field of liver regenerative medicine.

We're here today to talk about a new article that we've written that is to be published soon in *Mayo Clinic Proceedings.* The title of the article is "Cellular Therapy for Liver Disease," and that will be published as part of a series of articles that comprise the symposium on regenerative medicine. In its inception, this was a review article, but over time, I think, it's really become quite a bit more than that. And really we envision it now as more of a roadmap. So it will tell us where the basic science in liver regenerative medicine has progressed to, what clinical applications have been tested thus far, and hopefully provide a vision for the future as to what the opportunities and what the challenges will be.

So the liver is a remarkable organ. It it's really the most naturally regenerative organ in the body. A human being can tolerate 70% resection of the liver, and some animals can tolerate up to 90% resection of the liver. And it will grow back and regenerate on its own to precisely the correct volume and then stop regenerating. And so we've taken advantage of this in medicine with liver resections, with living-donor liver transplantation, and it's also a platform for us to learn about regenerative medicine in general and apply those concepts to other organs.

In the setting of chronic liver disease, this very precise program becomes subverted. And instead of efficient regeneration, you get a process of chronic wound healing that is inefficient and drives fibrosis and chronic disease progression. So there's really a need to develop new regenerative medicine therapies to treat chronic liver disease.

In the basic science realm, a number of really astonishing advances have been made. There are a number of cell types that have been tested, which Dr. Rakela will talk about in terms of cell transplantation into the liver. Perhaps the most astonishing advance has been that of induced pluripotent stem cells.

And as you can see in figure one, the vision would be to start with a patient or an animal model of liver disease and generate skin fibroblasts via skin biopsy. Those cells can then be reprogrammed using the Yamanaka pluripotency factors toward induced pluripotent stem

cells, which are cells that act like embryonic stem cells and can differentiate into multiple different tissue types. And if you expose those to a process of differentiation by exposing them to key morphogens, you can drive differentiation toward hepatocyte-like cells.

And those cells then can be used as an individualized disease model for the patient's disease. They could be tested in vitro to look at new therapies, in terms of efficacy and toxicity, and, perhaps most exciting, is that the cells could be genetically reprogrammed to repair any underlying defects and then transplanted without immunosuppression back to the liver. This is still experimental. But a number of other liver cell types have been tested in terms of cell therapy, and Dr. Rakela will tell us a little bit more about what has been done thus far.

JORGE RAKELA: Yes, I think, with interaction given by Dr. Huebert, we can state that the option that we have right now in clinical practice is liver transplantation, which is replacing a failed liver and provide a new liver to a patient with end-stage acute or chronic liver disease. We have been victims of our own success because, as we have got outstanding results with the liver transplantation with survival at five years, or 70%, the number of patients waiting for liver transplantation liver transplantation has outgrown the availability of the organs that are available through donation. Therefore, the applicability of liver transplantation has been limited. And in some places, up to 20% of patients waiting for liver transplantation may die waiting for the procedure without getting it.

So the interest that we have had with this article was to see where we are in terms of [INAUDIBLE] options, in terms of availability to repair or regenerate a liver. So the concept is beginning to develop about using cell-based therapies. So one of the first attempts to use the primary hepatocytes that are harvested from livers that are not going to be used in liver transplantation-- orthotopic liver transplantation-- have been several review articles. And we cited them in our publication, and there have been over 90 patients who have been treated in different places around the world with some beneficial effect.

The problem is that as we used, again, an allogeneic organ, the patient has to continue to use the immunosuppression. And the second unknown is how long the cells would be functional. And the first unknown is how it's going to be-- what is the interaction between this transplanted primary hepatocytes with the native hepatocytes.

The next approach has been to use the circulating stem cells that we have in our body. Those cells have been used-- cell therapy has been used-- in the setting of bone marrow

transplantation and other formal therapies and now has been used the same approach in patients with end-stage liver disease. And all that has been done in uncontrolled trials as well as limited controlled trials. And we have summarized that in our tables-- one, two, three, and four in our publication.

So the initial trial was with the so-called [INAUDIBLE] time of the stem cells, and that was-- we summarized-- the total of six trials. Two of them were initially uncontrolled trials, showing beneficial effect, and the other four were randomized controlled trials. A few of them showed beneficial effect, and the most recent trial did not show differences between the group of patients who had received the same kind of stem cells versus the ones who received placebo.

Another approach has been to use autologous bone marrow-derived stem cells, and there has been a total of 10 trials-- six of them so called unsorted bone marrow-derived mononuclear cells, three uncontrolled and three controlled. In the controlled trials, two of them showed beneficial effect. One trial did not show beneficial effect. The other four were uncontrolled trials using sorted hematopoietic stem cells, specifically CD34-positive. And this uncontrolled trial showed beneficial effect in those patients receiving this treatment [INAUDIBLE].

More recently, another approach has been to stimulate the [INAUDIBLE] of the [INAUDIBLE] stem cells using a growth factor, like G-CSF, leukocyte colony stimulating factor. A more recent publication coming from India is they treated a total of 47 patients with a placebo-controlled group, and they showed improvement of the MELD score, which is a score that indicates how advanced the liver disease is and also better patient survival.

Again, as we look to analyze all these different approaches with [INAUDIBLE] stem cells and also growth factors. What came to be evident to us is that there are many approaches in terms of what type of cells to use, the concentration of cells, how to administer the cells. Therefore, the comparison, one trial to the other is very difficult.

So we think-- and that is what Dr. Huebert has mentioned about the worries for us in the future and showing how we are going to approach these therapies in the near future-- one obvious approach is to standardize the cell preparations, the number of cells, [INAUDIBLE] and provide to patients and be able to compare with the control group receiving the placebo control group what is the efficacy of this approach in the treatment of patients with end-stage liver disease.

Now in figure two, we have summarized what is coming for the future. So an exciting possibility, which has been outlined by Dr. Huebert, is that from skin fibroblasts from the

patient we can generate induced pluripotent stem cells. And then, with various transcription factors, we can reprogram these cells and produce the hepatocyte-like cells.

And then that opens the possibility to use primary hepatocytes that now are derived from the patient, him or herself, and the other is to a patient with end-stage liver disease. And that will be very exciting as a possibility because this patient probably will not be introduced immunosuppression. Therefore, that is clearly a pathway that is happening for the future for patients with end-stage liver disease. So thank you.

ROBERT HUEBERT: Thank you for watching the video. I hope you read the article as well, and we look forward to your feedback or questions about the article. It should provide you with some sense of where the science has progressed to, what clinical applications have been tested, and potentially what the future holds as well.

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