CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

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PRESIDENT'S UPDATE ON ADVANCES IN STEM CELL SCIENCE

Highlights of recently published papers from CIRM grantees and other leading research teams around the world—November 2011

More Pro and Con Data on Heart Salvage and Repair

This update focuses on papers from peer reviewed journals, however a number of reports on unpublished studies on heart therapies appeared in the press this month after presentations at the American Heart Association annual meeting. Much of the data coming out at the AHA was encouraging for cell-based therapies, but it should be viewed as preliminary until peer reviewed and published. If you want to see the slides from those presentations as well as from discussants on the presentations visit the AHA meeting web site here, including a presentation by CIRM grantee Eduardo Marban:
http://my.americanheart.org/professional/Sessions/ScientificSessions/ScienceNews/SS11-Late-Breaking-Clinical-Trials_UCM_432888_Article.jsp#.TsbSzPEVFUg

One of the most promising AHA presentations by Roberto Bolli and his team at the University of Louisville was published online in *The Lancet* Vol. 378 (9806) the week of the meeting (November 14). That same week the *Journal of the American Medical Association* published a report by a multicenter team led by Lemuel Moye' at the University of Texas and it provided pretty definitive negative results for one type of cell therapy post heart attack.

Bolli led a study dubbed SCIPIO that was a well-controlled look at whether a patient's own cardiac stem cells can be used to reverse heart failure months after it was diagnosed. Patients who had bypass surgery to try to improve cardiac function were assigned to treatment or control groups an average of four months after surgery. Treated patients had a biopsy to retrieve heart tissue and the team sorted the cells from that sample to isolate those cells positive for c-kit, a marker for cardiac stem cells. Those cells were expanded in the lab and re-infused into a cardiac blood vessel. Four months later the treated patients had significant improvement in heart tests, known as left ventricular function, and the controls did not. And in a subset of patients the benefit was even more pronounced one year later.

The *JAMA* study called LateTIME used the cell type more commonly used in prior heart therapy studies. These cells, called mononuclear cells, are found in bone marrow and tend to be a mixture of cell types. This team infused a patient's own marrow cells into the cardiac blood vessels two to three weeks after a heart attack and compared these patients to ones given a placebo infusion. They found no benefit unlike some studies, which had found some modest benefit, generally when given sooner after the attack. However, those benefits in many studies were short-lived. The same team now plans to repeat the trial in patients three and seven days after an attack.

This definitively negative trail is a significant benefit to the field. The fact that this type of cell delivered in this time frame did not benefit the patients will help focus the design of future trials. The field clearly needs more research into mechanisms of action to define best cell type and timing and location of delivery.

Another Complex Tissue in a Dish, This Time the Pituitary Gland

Yoshiki Sasai of Japan's RIKEN Center published a study in *Nature* online November 9 in which he and his team were able to grow pituitary glands in culture starting with mouse embryonic stem cells. These lab-grown glands restored pituitary function when transplanted into pituitary deficient mice. Hormones produced by the pituitary gland control reproduction, growth and metabolism.

The same team reported growing an optical cup in a dish earlier this year. The pituitary is a bit more complex. It can't form without signals from the neighboring region in the brain, the hypothalamus. So the team had to coax the embryonic cells into cells like those in the hypothalamus as well as progenitors to the pituitary in the same three-dimensional cell culture. Through many repeated experiments they found the right set of conditions—involving two growth factors and a drug—to get the pituitary to self-assemble. The drug turns on the production of the Sonic Hedgehog protein, which is known to have important roles in organizing the developing fetus.

Once assembled the cells were able to secrete the appropriate hormones and when they were transplanted into mice with pituitary defects, they were able to restore glucocorticoid hormones and reverse behavioral symptoms such as lethargy.

The team plans to repeat the experiment with human stem cells.

Neurons Grown from Embryonic Cells Integrate and Function in Brain

Papers published online in *Nature* November 7 by Lorenz Studer's group at Memorial Sloan Kettering and in the Proceedings of the *National Academy of Sciences* November 21 by Su-Chun Zhang at the University of Wisconsin both got neurons grown from human embryonic stem cells (hESC) to integrate and function in mouse brains.

Studer's group specifically grew dopamine-producing neurons, the type lost in Parkinson's disease. While many groups have been able to coax hESCs into becoming dopamine-producing neurons, they have generally had very little success in getting them to efficiently engraft in animal models. The team postulated that they succeeded because of the way they derived the precursor cells. They started by driving the hESCs to become like an area of the brain during development known as the midbrain floor plate. To do this they too, turned to activators of Sonic Hedghog and a second development gene, WNT. After 25 days they were able to get engraftable dopamine neurons that could be maintained in a dish for several months. In models for Parkinson's disease in mice, rats and monkeys, these engrafted neurons produced improvements in forelimb use and in akinesia, the inability to start moving that is common in Parkinson's disease.

The Wisconsin team grew neurons that could be transplanted into the hippocampus of mice, the section of the brain that deals with processing and storing memories. The cells were modified so that they could be stimulated by light, which allowed the researchers to verify the human cells had integrated into the mouse tissue both in culture and in the animals. When stimulated by light the cells showed the rhythmic firing behavior that is characteristic of mouse hippocampal cells.

Genetically Modified Stem Cells Treat Hemophilia in Sheep

Christopher Porada and his team at Wake Forest report in *Experimental Hematology* Vol. 39 (1124-1135) that they were able to reverse the effects of hemophilia in sheep. The team used mesenchymal stem cells from the father that had been genetically modified to produce Factor VIII, the clotting factor missing in hemophilia. The paper will be published in the December issue but is online now.

The sheep model for hemophilia develops spontaneous bleeds including bleeds into joints causing defects in posture and gait in the animals. The researchers used a lenti virus vector to carry the pig gene for clotting Factor VIII into the mesenchymal stem cells (MSC) from the father of the effected lamb. After they transplanted those modified cells into the peritoneal space the bleeds stopped and the animals regained normal posture and gait. Postmortem exams showed that the cells had engrafted in many tissues, but particularly in joints that had been impacted by the bleeds. It looked like the MSCs homed to sites of ongoing inflammation.

Team Creates Safety Switch to Get Rid of Errant Cell Transplants

A group of researchers from various Texas institutions led by Baylor College of Medicine's Malcolm Brenner published results in the November 3 *New England Journal of Medicine* Vol. 365;18 (1673-1683) that showed they were able to stop Graft Versus Host Disease (GVHD) in children being treated for leukemia. The team modified the bone marrow transplants the children received by genetically modifying the T cells in the donor marrow so that they could be destroyed if GVHD developed.

Leukemia patients receiving stem cell transplants from donor bone marrow to reconstitute their immune system after extreme chemotherapy are susceptible to a horrible side effect known as Graft Versus Host Disease, which is, like it sounds; when the grafted cells from the donor start to attack the patients own tissues causing whole layers of cells to come off. The children in this study were receiving cells from only partially matched donors and therefore were among the most vulnerable to GVHD.

The genetic trick employed by the Texans was to first sort out the T cells in the marrow and add a gene to the donor T cells using a plasmid, a ring of DNA. The gene they choose, iCasp9, can trigger what is known as programmed cell death or apoptosis, but only when it is activated by a particular compound, an otherwise inert drug AP1903. These cells were given to five children being treated for relapsed acute leukemia. Four of the patients developed GVHD and after they were given a single dose of the drug more than 90 percent of the donor T cells were eliminated within 30 minutes.

Houston's Bellicum Pharmaceuticals plans to launch a full clinical trial for GVHD using the system in 2012. If this pilot data pans out, the system could greatly improve the safety of these cell therapies for cancer patients. But, it also offers clues as to how to reduce the risk of rogue cells in other cell therapies.