

## Keynote Address: Cytotherapeutics in Veterinary Medicine

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Dr. Rick Vulliet said the history of therapeutic drug development for humans hit a wall around the turn of the century with the inability to cure diseases associated with aging, chronic degenerative disease, and diseases associated with cell loss.

Today, stem cell research holds out the prospect of curing conditions like cardiovascular disease, autoimmune deficiencies, diabetes, osteoporosis, and cancer. Each of these diseases takes a major toll on human populations, Dr. Vulliet said, and many of them have canine equivalents.

Encouraging as it would be to claim that cell loss diseases could be cured by stem cells, however, “the reality is still pretty foggy,” he said. “We have cured thousands and thousands of mutant mice, we’ve made progress in laboratory rodents, but the question is whether we’re ready to move into naturally occurring diseases in real patients, real dogs with real diseases.” While he said he believes they are, he said there is still no guarantee that the techniques will work.

Dr. Vulliet said the first stem cell with the ability to grow all the tissues of the body was a fertilized rat zygote, making *in vitro* fertilization one in a series of treatments that eventually migrated over from veterinary medicine to human health. The first successful human stem cell study demonstrated that muscle, nerve, pancreatic, and other cells could be grown in culture under special conditions.

From that point, ethical concerns began to proliferate, amid concerns about applications that bordered on science fiction. “No serious stem cell researcher supports reproductive cloning, and yet that’s where most of the ethical stuff comes from,” Dr. Vulliet said. When one private company grew a human clone to the stage of about six cells, the work “was soundly denounced by the scientific community.” By contrast, strong support exists for therapeutic cloning of specific cells for specific purposes.

Unfortunately, the majority of attention and resources has been devoted to the ethical debate, rather than to actual stem cell research. “Our job is to work out what we can do,” Dr. Vulliet said. “Then we’ll figure out whether we should do it or not.”

Beyond fertilized zygotes and embryonic tissues, there are several sources of cells that can change from one tissue type, or phenotype, to another. Dr. Vulliet said his work has focused on canine, equine, feline, and rodent bone marrow. “They all seem to grow very well in the same conditions, with similar morphology and response.” The cells can grow

into bone, chondrocytes, myocytes, tendon, ligament, and possibly nerve cells, a degree of versatility that makes stem cells a promising research focus for many breeds.

In 2001, a research team at the US National Institutes of Health (NIH) induced the equivalent of a myocardial infarction in rats, injected stem cells, and found that the cells grew in the infarcted tissue. The study could not be replicated, but researchers in Germany are still conducting follow-up clinical trials. The results have suggested a possible direction for research on heart disease in Doberman Pinschers.

Dr. Vulliet's specific research target was dilated cardiomyopathy (DCM), a heart condition common among Dobermans. In DCM, the heart no longer contracts the way it should, and loses the ability to pump blood. Humans with the same disease are placed on the transplant list, where 16,000 are currently waiting for procedures that take place at a rate of 4,000 per year.

"No one talks about the other 12,000, but I suspect we know what happens to them," Dr. Vulliet said. The NIH should be concerned, he said, "and they should be funding Doberman studies. We're working on it."

An initial series of safety studies revealed a variety of problems with myocardial stem cell treatment, including arrhythmias, micro-infarctions, and clogged arteries where the cells were introduced. Dr. Vulliet said growing techniques, handling methods, and routes of administration have all been revamped in the hope of developing a protocol that can be used on an animal patient.

Unexpectedly, the trans-differentiation the stem cells exhibited *in vitro* did not occur in the initial trials. Benefits may have resulted from improved blood flow through the heart, activation of the immune system, donations of mitochondria, or cell fusions, but "the bottom line is that we don't know how the cells work at this point. It's a very active area of research, not only in dogs, but in humans" and at this point mostly in rodents.

One recent paper reported a statistically significant improvement in rodent heart function following the introduction of stem cells from human bone marrow. The benefit persisted after the cells disappeared, however, suggesting that the cells themselves were not the source of the benefit.

In another NIH study, researchers investigated the effects of adult bone marrow stem cells on spinal cord injury in rats by dropping a precisely calibrated weight on the rat's exposed spinal cord. Seven days later, they added adult stem cells to the injury site and found significant improvement in recovery of the spinal cord to the injury. This improvement was supported by histological techniques as well as functional improvements. The rats showed improved function, likely because the stem cells stabilized existing cells and helped more of them survive the injury. Dr. Vulliet said a similar approach might work with degenerative myelopathy (DM) in German Shepherds, although "you've got some logic leaps" in taking a treatment for acute injury in a rat and applying it to chronic injury in a dog. To owners who volunteer their dogs

for a study, the message will be that “you’re participating in research. We don’t know if these things work. We really want to find out.”

The rodent spinal cord study has been replicated successfully at several laboratories, and Dr. Vulliet said this suggests it would be appropriate to attempt similar studies on dogs with DM. Some veterinarians have objected that dog studies are premature without better data, “but I also know what’s going to happen if we do nothing. That’s your choice as owners.”

Researchers have also injected stem cells into the tracheas of rodents with idiopathic pulmonary fibrosis (IPF) to reduce collagen deposition and scarring of lung tissue following treatments with bleomycin, an antibiotic and anti-cancer drug. Here again, the evidence is limited to laboratory tests on rodents, but “if you have an animal with IPF and you go to your vet, we can only give you symptomatic relief. There’s nothing to treat the disease.” The disease causes 40,000 human deaths per year in America, and the only treatment option is a lung transplant.

Dr. Vulliet also presented results on stem cell treatments for lipoprotein lipase (LPL) deficiency, a genetic mutation that appears in Miniature Schnauzers and Beagles, in felines, and in one in a million humans. The condition defines about 80 different mutations that all occur in the same enzyme.

In diagnostic tests, the blood serum drawn from an LPL-deficient is milky white, when it would normally be straw-colored. Two days after treatment with about 50 million bone marrow stem cells, the opacity of the blood serum declined for about four or five days. After a second treatment, it dropped farther and stayed down for longer. The cells were administered through a standard injection to the jugular vein and involved very little pain to the animals. “In the global scheme of things, compared to dying, I think it was relatively minor,” he said.

Lung tests showed an increase in lipase activity after the first treatment, and a larger increase after the second dose. Most astonishing of all was the behavior change in a cat that Dr. Vulliet described as “the second-dumbest I’d ever worked with.” Before treatment, the cat “just wanted to sit in your lap and shed,” with no behavior and no curiosity. After receiving 100 million stem cells, she began grooming herself, gained some weight, and showed far more complex behavior.

Already, Dr. Vulliet said, the discovery of plastic cells is rewriting pathology textbooks. Until now, physicians thought the week after an injury was characterized by inflammation, cell death, and an influx of inflammatory cells. The new theory is that an influx of regenerative cells begins after a week or two, and is followed by repair and scar formation. In some canine diseases, such as IPF in West Highland White Terriers, there is likely an exaggerated inflammatory response, followed by decreased regenerative cells and more scarring. The condition might now be addressed by infusing regenerative cells to reduce scar formation. “This is in contrast to what I was taught in pathology 25 or 30 years ago, but we really don’t know how this will play out.”

With limited information available, “anyone who donates their dog to this study is a hero,” Dr. Vulliet said. The other option is to wait 15 years for a standard human drug to be approved, and then look for canine applications. “With a real dog with a real disease right now, what are you going to do?”

He noted a study of humans with Parkinson’s disease in which the test subjects showed excellent results after a year, but fell behind the control group by the end of the second year. The results led the NIH and the US Food & Drug Administration to declare a moratorium on future human stem cell studies, leaving canine and other animal studies as an option the NIH should support.

### *Discussion*

Dr. Butherus said CHF hopes to set up three regional stem cell therapy centers serving most major communities in the continental United States, where owners can bring their dogs to have bone marrow extracted and stem cells implanted. The overall approach reflects the Foundation’s three-pronged commitment to prevention, treatment, and cure. Dr. Vulliet said the regional approach was designed to minimize travel distances for the animals. “Many of you think shipping dogs is no big deal,” he said, but “it’s hard enough with a healthy dog, much less with a sick dog.”

Citing Albert Einstein’s observation that “imagination is better than knowledge,” a participant urged CHF to fund creative approaches. “For the money lenders, for research, please look at people who have imagination, not at the status quo of what’s accepted in science,” he said. “There will be no breakthroughs with that.”

A participant suggested that a continuous procedure might be needed to extend the temporary improvements in the Parkinson’s study.

Another participant said any request to owners to ship their dogs would “run smack into the human-animal bond. I might bring you my dog, but I’m not willing to let you have my dog.” Dr. Vulliet said it might be possible some day to have local veterinarians ship bone marrow by courier for extraction, then send stem cells back for implantation. “I can’t do that right now because I really don’t know what I’m doing. I couldn’t afford to send you a batch of cells, have them die in transit, and get a false negative.”

In response to another series of questions, he said stem cell implantation rarely leads to immune reactions.