

Gene Targeting: Potential Treatment or Cure for OI

David Russell, M.D., Ph.D., Professor of Medicine and Biochemistry at the University of Washington School of Medicine in Seattle, published an article in 2004 in *Science*, a peer-reviewed journal. The article described how his work in gene targeting could potentially treat or cure OI. The following interview describes Dr. Russell's progress since 2004.

OI Foundation: We know gene targeting has great potential to eventually cure OI. What is gene targeting?

Dr. Russell: Gene targeting is the process of introducing genetic changes at a specific chromosomal gene in a cell. Using mesenchymal stem cells (MSCs), we performed gene targeting to knock out the mutant collagen gene early in the cell's collagen-making process, so the targeted cell functions normally, producing good collagen. When the targeting is done at the Type I collagen genes (*COL1A1* and *COL1A2*) that cause OI, the cells produce normal collagen and normal bone.

OI Foundation: How did you develop the gene targeting technique?

Dr. Russell: Our lab developed the gene targeting technique as a way to precisely manipulate human chromosomes. We weren't working on OI at all, but as we developed this technique, we discussed it with Peter Byers, M.D., (a member of the Foundation's Medical Advisory Council) who works right down the hall. It turns out that OI is the perfect candidate for treatment through gene targeting because in most cases, the OI mutation is dominant, so it can't be treated by adding more of the normal gene. Rather, we need to disrupt the mutant gene. So we collaborated with Dr. Byers' lab to target collagen genes in cells from people with OI.

We also reviewed the early mesenchymal stem cell (MSC) transplantation studies that were performed on children with OI by Ed Horwitz, M.D., Ph.D., at St. Jude's Hospital in the late 1990s. We felt that our technique could build on that data and improve the results.

We started by obtaining pieces of bone from teens and kids with OI who were undergoing surgery. Using mesenchymal stem cells from these bone fragments, we were able to successfully target the mutant collagen and knock it out.

One of the exciting things about this technique is that we can use cells from the patient's own body (autologous cells). This means we will target a person's own cells and implant the corrected cells back into that person. So there won't be any problems with autoimmune reactions, like those that occurred in the St. Jude's studies.

OI Foundation: What's happened since you published the *Science* paper in 2004?

Dr. Russell: The *Science* paper focused on one of the genes encoding Type I collagen (*COL1A1*). Since then, we've also been able to successfully target the other Type I collagen gene, *COL1A2*. After targeting, both types of OI cells make normal collagen and they also still make bone.

We've also made improvements in the technique to improve accuracy and efficiency, and we think the *COL1A2* gene will be the better one to use for the first trials, because if you have a total knockout of the mutation in the *COL1A2* gene, then you have normal bone.

Right now we've been able to take OI cells, target them, and show that they are producing normal collagen. But we don't know how long this normal collagen production will last. We also can't yet prove that we've made normal bone that is also better bone. So we searched for bigger animal models that could provide larger bones to study.

OI Foundation: The Foundation's advocacy efforts resulted in a \$10 million OI research grant program at the National Institutes of Health (NIH). Your initial work was funded by this grant program. What's happening now?

Dr. Russell: Fortunately, the NIH renewed our grant, which was essential for getting this project going, so we are able to continue our work.



Dr. David Russell and fellow researchers at the University of Washington School of Medicine in Seattle.

OI Foundation: Now that you've developed a successful technique for knocking out the OI mutation in *COL1A1* and *COL1A2* genes, what's the next step?

Dr. Russell: This is an exciting time because we are moving out of the test tube and into living bone. The next step is to develop techniques for returning targeted cells into living bones. We are collaborating with Alicia Bertone, D.V.M., Ph.D., at Ohio State University. She is a Professor of Veterinary Science and a veterinary surgeon. Using autologous cells, we are testing three different gene delivery methods in rabbits: surgical insertion, intravenous injection, and pretreating the bone to improve engraftment. We are using rabbits because we can't do the necessary types of surgical manipulations in mice, and they are a commonly used animal model for orthopedic research.

Of course, we adhere to the Animal Welfare Act, National Research Council *Guide for the Care and Use of Laboratory Animals*, and all appropriate U.S. Department of Agriculture and National Institutes of Health regulations and standards for animal research.

The rabbits we will use do not have OI. We will start with normal mesenchymal stem cells (MSCs), because the medical field is still learning how to do MSC transplants. We will be looking at the cells to see how long they survive after transplantation, and where they go in the rabbit's bone. We hope this will give us the data we need to scale the technique up to humans. Over the next few years we expect to be able to accumulate all the pieces of data we need to move into human clinical trials.

We expect to have good luck with direct surgical injection because it has been shown to be more efficient in other animal models, but the studies will determine this for sure.

OI Foundation: If direct surgical injection is effective, then how will that apply to people?

Dr. Russell: If the animal studies are successful, the next step is to design a clinical trial (testing in people). We would probably start with the weight bearing bones, and initially just one bone on one side, so we could compare it to the bone on the other side. We expect to be able to treat each bone through the skin (percutaneously) with local anesthesia. If it worked, I think we could go after all the important bones pretty easily, even if it meant a fairly extensive set of injections.

There might be other approaches also. For example, during a rodding surgery, we might "seed" the rod with targeted MSCs to improve healing.

OI Foundation: It's truly exciting to think about being able to inject corrected cells back into the bone to cure OI. But we have to ask, what could go wrong?

Dr. Russell: Based on what we know now, there are very few things that could go wrong and cause harm to people. We will be using a person's own cells and creating a very narrowly defined genetic change. We don't expect any inflammation because there are no new antigens (a substance that stimulates antibodies) involved. One real possibility is that the cells won't survive after injection, but this shouldn't be dangerous.

An issue we have to resolve is the bone turnover rate. Bone turnover can be slow, so it may take awhile to see a therapeutic effect from the corrected genes because we don't know how fast they will turn over and how long they will last. We don't know how big a problem this could be, but there may be ways to solve it by improving bone turnover. Some drugs are available to increase bone turnover. We know that bisphosphonate drugs slow bone turnover, but turnover returns to normal after you stop taking them. We have to resolve how bisphosphonate therapy would affect gene therapy and vice versa.

OI Foundation: What else has to be solved before human trials can begin?

Dr. Russell: We are using a bacterial antibiotic resistance gene now to select for targeted cells, and we will have to get rid of that before human trials. We are trying two different methods to solve this. One is to pop out the gene after the targeting is done, the other is to replace the bacterial gene with a different gene from humans, such as a cell surface marker, that won't be recognized as foreign after transplantation.

OI Foundation: Who will be most likely to benefit from gene targeting?

Dr. Russell: We really can't say at this stage. Adults could benefit, and we will probably start with them because of the ethical issues involved with informed consent. However, kids might do better because of their higher bone turnover and growth rate.

If we find that the targeted cells are remodeling the bone, then perhaps proper exercise and therapy would make the bone remodel into a normal shape. Remodeling could also cause improvements in adults. It is somewhat surprising how little is known about these questions. Scientists really don't know very much about bone turnover and why bones develop as they do. For example, why do you end up with a femur in your leg instead of your arm? I'm sure new research discoveries will shed more light on this.

OI Foundation: It sounds like you're covering all the bases. You've done your research into past experiments, you're collaborating with others, and thinking through potential problems in advance. What do you

need right now to move this research forward faster?

Dr. Russell: Funding for all types of OI research will give us the data we need when we're ready to put it to use. The OI Foundation's annual scientific meetings are helping us to find partners and learn about new possibilities. The Linked Clinical Research Centers are putting a network in place that will allow us to test our methods as they are developed. But when we do move into the clinic, the costs and preparations will increase, and we'll have to raise a lot more money. ■