A gene-therapy technique that burst on the scene with enormous promise 6 years ago has turned out to be inconsistent or impossible to replicate in most labs that have tried it

The Strange Case of Chimeraplasty

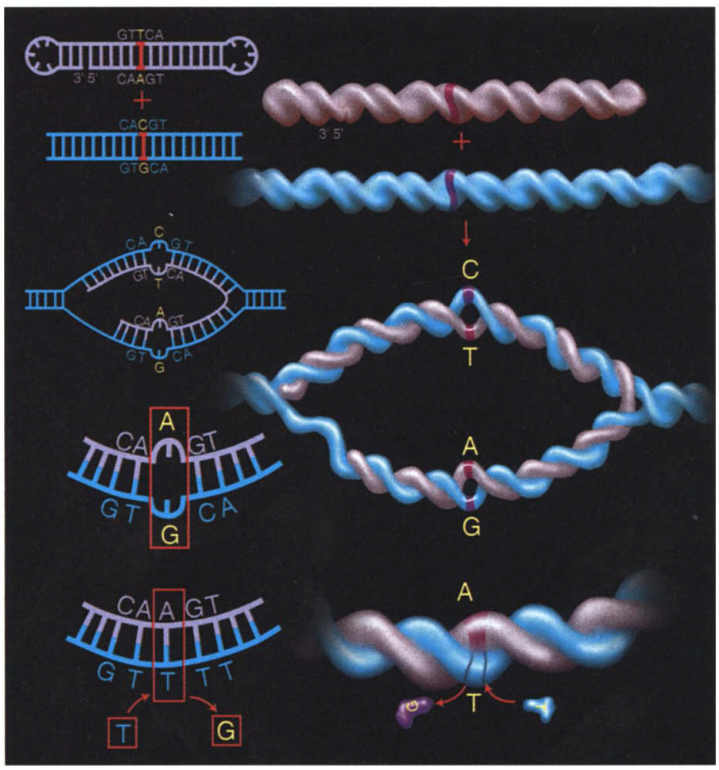
The history of gene therapy is filled with promise, hype, and disappointment. Among the more profound failures is that only a tiny fraction of the genes injected into animals or humans reach their cellular targets. And only a tiny fraction of those that do so actually works. On 6 September 1996, however, *Science* published an article about a technology that promised to change all that (p. 1386).

The article described a radical new technology for correcting genetic defects, one that appeared to be a million-fold more potent than previous approaches were. The implications did not go unnoticed: The publication caught the media's attention, launched research projects around the world, and spawned a gold rush as researchers and entrepreneurs moved to stake their claim to the extraordinary promise of the technology. The result has been a 6-year roller-coaster ride of science at its cutting edge and most controversial.

In traditional gene therapy, researchers stitch a gene into a virus that then shuttles it into target cells. Once inside, if all goes well, the gene integrates into the cellular DNA and begins churning out proteins to replace those missing or defective. In their *Science* paper, researchers at Thomas Jefferson University (TJU) in Philadelphia, led by Eric Kmiec, reported that they had corrected genetic defects without using a virus to do so. Instead, they used a synthetic molecule of RNA and DNA, a chimera that could slip into cells, at least in the test tube, and correct the mutation responsible for sickle cell anemia. The technology, which Kmiec called chimeraplasty, appeared to be astoundingly efficient. If it performed as the data implied, correcting the sickle cell mutation in 50% of cells, it could revolutionize gene therapy. It would also have a dramatic impact on genomics, where it could be used as a powerful tool to elucidate the function of genes.

The promise was such that gene-therapy pioneer Michael Blaese quit his position running the Clinical Gene Therapy Branch of the U.S. National Institutes of Health (NIH) to become chief scientific officer of Kimeragen, the company founded by Kmiec to commercialize his discovery. By 1999, Kimeragen was talking with the U.S. Food and Drug Administration about using chimeraplasty to treat Crigler-Najjar disease, a rare genetic disorder, and *Science* itself reported that the technology had passed the all-important hurdle of scientific acceptance (16 July 1999, p. 316). "The beauty of chimeraplasty is that it appears to be a universal process," Blaese told *Science*. In February 2000, Kimeragen merged with Valigene, a French biotech firm, to form ValiGen, which had as CEO Douglas Watson, the former head of Novartis, and a scientific advisory board that included J. Craig Venter and Nobel laureate Hamilton Smith of Celera Genomics in Rockville, Maryland.

But those heady days are over. In October 2001, Watson resigned; ValiGen closed its Princeton, New Jersey, laboratory, the site of nearly all its chimeraplasty research; and Blaese and his researchers were laid off. According to its representatives, the company was undergoing bankruptcy reorganization in France this summer and has sold the license for chimeraplasty to a small company in San Diego, California, to pursue the technology in plants. "I am still a believer in gene-repair technology," says Blaese, "but the efficiency that was widely touted has been very difficult to reproduce."



Spelling correction. In the above diagram, chimeraplasty replaces an incorrect C-G base pair with A-T. A double-stranded RNA-DNA oligo has a sequence that complements that of the target gene except at the C-G mutation (top). The oligo inserts itself between the DNA strands in the target gene, which bulge at the mismatched bases (middle). DNA repair enzymes then replace the incorrect bases with complements to those of the oligo (bottom). The oligo later decays, leaving the corrected target gene.

ILLUSTRATION: C. SLAYDEN

Chimeraplasty has always been considerably less promising and more controversial than media accounts have suggested. Many gene-therapy researchers expressed initial skepticism simply because the results were remarkable and the data less than iron-clad. For some, this skepticism deepened as critics uncovered what they considered to be serious flaws in both the *Science* paper and another key paper Kmiec published in the *Proceedings of the National Academy of Sciences* (*PNAS*).

The procedure itself has turned out to be fickle at best. Although at least nine laboratories scattered around the world have published reports confirming some ability of chimeraplasty to effect gene conversion, two of those have since moved on to other research projects, and dozens of others—including some of the most experienced in the world in

gene repair—have tried to replicate the experiments and failed. Only three of these negative results have been published, but word of their existence spread through the community. "We live and die on reproducibility," says Harry Orr, director of the Institute of Human Genetics at the University of Minnesota, Twin Cities. "And the scientist in me says to be very dubious of something that cannot be uniformly reproduced."

The ongoing controversy illustrates how publication in a prominent journal, followed by a few confirmations against a much larger but unpublished background of failures, can give life to a remarkable claim. When both journals and journalists attend to the positive signal and ignore the negative background, the result can be a distorted view of reality that can take years to clarify.

Correcting mistakes

For Eric Kmiec, chimeraplasty represented his reemergence after a decade of struggle to rebuild a career that was launched with enormous promise and then descended into controversy. In the 1980s, Kmiec accomplished the noteworthy feat of publishing eight articles in *Cell* based on his graduate and postdoctoral research. The central findings of the first four, however, published with his doctoral adviser William Holloman, now at Weill Medical College of Cornell University, have never been independently replicated; those of the fifth and sixth, also published with Holloman, were publicly refuted. The remaining two *Cell* papers, published as a postdoc with biologist Abraham Worcel of the University of Rochester in New York, were retracted in 1988 by Worcel, whose own lab failed to replicate the results after they were challenged by outside researchers. Kmiec, who has continued to stand by his early papers, struggled for the better part of a decade to build his career before reemerging in 1996 with chimeraplasty.

Kmiec says the idea for chimeraplasty grew out of his graduate studies on homologous recombination, the process in which chromosomes exchange or "recombine" DNA. Over the years, researchers had tried to enlist homologous recombination for gene therapy or genomics. Although they've had some success, the "conversion efficiency" has remained so low—converting genes in perhaps one in every 1000 or every 10,000 targeted cells—that the techniques have seen limited use. In the late 1980s, for instance, University of Rochester biologist Fred Sherman demonstrated that small, single strands of DNA could induce specific changes in the genomic DNA of yeast via homologous recombination. But the efficiency rate was excruciatingly low—"10 to a minus big number," says Sherman.

In 1993, Kmiec explained in a 1999 issue of *American Scientist*, his research convinced him that RNA could facilitate recombination reactions. Instead of relying on a single strand of DNA, Kmiec decided to add a second strand consisting of five nucleotides of DNA sandwiched between two longer stretches of RNA that were intended to provide stability to the molecule. The two strands would then be joined together at the ends into a racetrack shape, to avoid dangling nucleotides that might be attacked and degraded by cellular enzymes (see diagram, p. 2116).

Chimeraplasty proponent. Eric Kmiec conceived the notion of using chimeric RNA-DNA molecules to correct single-nucleotide mutations.

Kmiec theorized that chimeraplasty might correct genetic defects by artificially inserting an error that homologous recombination would naturally correct. The first step required the synthesis of a short, artificial string of nucleotides—made from the building blocks of DNA, adenine (A), thymine (T), guanine (G), and cytosine (C)—that would be flanked by the RNA. This RNA-DNA "oligonucleotide," or RDO, would be designed to seek out the genetic region of interest. Specifically, nucleotides bind to their complement (A with T, G with C), so that an RDO that has, say, a string of A's will seek out and bind to a complementary string of T's.

As Kmiec conceived it, chimeraplasty would repair a genetic defect by tricking the error-correcting mechanisms of homologous recombination into fixing the error introduced by his RDO. Imagine a stretch of gene that should read AAAAA, but instead reads AATAA. Kmiec reasoned that a complementary RDO of TTTTT would bind to the target sequence, bulging out at the site of the mismatch—where there were T's in each strand—and thus alerting the cell's suite of DNA repair enzymes. These would then remove the "bad" nucleotide from the defective gene and replace it with the correct complement to the one on the RDO.

After some encouraging initial results, says Kmiec, he founded Kimeragen in 1994 to pursue the technology, although "with essentially no money." Kimeragen borrowed research money from TJU, with the expectation that the company would pay back one-quarter of the total ($400,000, according to a TJU press release) every 3 months from investor financing. But that financing was slow to come. Kmiec says he "was constantly at the dean's office or in the tech-transfer office" trying to convince the TJU administrators that Kimeragen would meet its payments.

This left the research to Kyonggeun Yoon, a chemist whom Kmiec hired from industry. As Yoon recalls, Kmiec told her they would give his idea 3 to 6 months and "then make a decision to kill it or go on."

Yoon tried Kmiec's RDOs on a variety of cell lines with no success. She then tried an assay that relied on the properties of an enzyme called alkaline phosphatase. If a cell contains "active" alkaline phosphatase proteins, it will turn red when the proper stain is applied. Yoon's idea was to alter a single nucleotide in the alkaline phosphatase gene, leading to an inactive enzyme. Using a plasmid (a circle of bacterial DNA) to carry this defective gene, she would "transfect" it into mammalian cells that otherwise lacked alkaline phosphatase entirely. She would then transfect the cells with RDOs designed to correct the defect. The next day, she would apply the stain and look for the red color that meant the RDOs had corrected at least one of the defective alkaline phosphatase genes and that the genes were producing active enzyme.

For 3 months, Yoon recalls, the RDOs resolutely failed. Then one morning, she arrived at the lab to find that a third of the cells in her latest experiment had turned red. "I couldn't believe my eyes," she says. "I told my husband, 'Either something happened, or I'm hallucinating.'"

In the summer of 1995, Yoon and Kmiec wrote a paper and submitted it to *Science*, which rejected it, Yoon says. She and Kmiec then submitted an article to *PNAS*, where it was published in March 1996. It claimed that their RDOs had corrected single-point genetic defects "with a frequency approaching 30%."

CREDIT: THE REVIEW

Pioneering Papers Under the Microscope

The two papers that launched chimeraplasty in 1996, published in the *Proceedings of the National Academy of Sciences* (*PNAS*) and *Science*, have attracted some withering scrutiny.

In their *PNAS* paper, Eric Kmiec and Kyonggeun Yoon, both then at Thomas Jefferson University in Philadelphia, had stitched an alkaline phosphatase gene containing a single-point mutation into a plasmid and "transfected" it into mammalian cells that otherwise lacked alkaline phosphatase genes. They then transfected the cells with chimeric molecules called RDOs designed to correct the point mutation (see main text). If the correction occurred and the cells started producing active alkaline phosphatase, the enzyme should cause a special dye to turn red.

Yoon and Kmiec reported that "approximately one in three" cells had turned red. To corroborate this result, Yoon extracted the plasmid DNA from the cells and put it into bacteria, which grew into colonies. Then she screened more than 400 of these colonies to assess how many included the corrected alkaline phosphatase gene. Kmiec and Yoon reported that the answer was again roughly one in three. This gave them the confidence to state that their experiments "established clearly that sequence correction by the chimeric oligonucleotide occurred in mammalian cells."

Critics noted, however, that the two tests measured entirely different parameters: The first counted the fraction of cells that contained at least a single healthy gene, whereas the second counted the fraction of corrected plasmid genes themselves in the cells. In interviews with *Science*, researchers who work with similar technologies said they could think of no scientific reason why those numbers should be identical; indeed, they said, if the RDOs had accomplished what Kmiec and Yoon had claimed, then those numbers should have differed by several orders of magnitude. For example, Phil Felgner, who invented the reagent used by Kmiec and Yoon and is now the chief scientific officer of Gene Therapy Systems in San Diego, California, notes that hundreds of thousands of plasmids carrying the defective gene would enter into *each* cell under the conditions reported in the *PNAS* paper, and only a tiny percentage of those—less than 1%—would make it into the cell nucleus, where gene correction by the RDOs could have occurred. So for each cell that contained at least one corrected gene—the minimum necessary to turn a cell red—there could have been several hundred thousand uncorrected genes from plasmids that either never made it to the nucleus or weren't corrected when they did get there.

Experts on the technology, consulted by *Science*, suggested two possible explanations for how both tests could result in identical numbers. The first—which Yoon also ventured as a possibility when *Science* asked her about the criticisms—is that cellular enzymes might have degraded most of the plasmids that didn't make it into the nucleus. That would greatly reduce the number of uncorrected copies of alkaline phosphatase genes that the bacteria would take up in Yoon's confirmatory test. But the experts consulted by *Science* say degradation of the plasmids would be unlikely to happen in the 30 hours reported by Yoon and Kmiec in their paper. And even if such a mass degradation did occur quickly, it would still be a considerable coincidence that the proportion of cells that turned red equaled the proportion of corrected alkaline phosphatase genes that Yoon found in the bacterial colonies.

The other possible explanation was contamination: 30% of the cells may have been contaminated with plasmids that already contained the correct form of DNA. If so, however, the control experiments, which showed no correction of the defective gene, were not contaminated. Yoon told *Science* that contamination was possible. "It would not be hard [to do]," she said, adding that her colleagues had worried about this possibility. She did not believe this had happened.

Correction of the Mutation Responsible for Sickle Cell Anemia by an RNA-DNA Oligonucleotide

Allyson Cole-Strauss,* Kyonggeun Yoon,* Yufei Xiang, Bruce C. Byrne, Michael C. Rice, Jeff Gryn, William K. Holloman, Eric B. Kmiec†

Targeted gene correction of episomal DNA in mammalian cells mediated by a chimeric RNA·DNA oligonucleotide

Initial evidence. Two 1996 papers provided evidence that chimeraplasty is highly effective in correcting point mutations.

CREDITS: (TOP TO BOTTOM) A. COLE-STRAUSS *ET AL.*, *SCIENCE* 273:1386 (1996); K. YOON *ET AL.*, *PNAS* 93, 2071 (1996)

Six months later, Kmiec published even more dramatic evidence in *Science*. In November 1995, Yoon had left Kmiec's lab to take a faculty position at TJU. This left Allyson Cole-Strauss, Kmiec's technician and a co-author on the *PNAS* paper, to carry out the research. (Cole-Strauss did not return numerous phone calls from *Science*.) Cole-Strauss, Kmiec, and their co-authors reported that RDOs designed to correct the β-globin mutation responsible for sickle cell anemia appeared to work successfully in 50% of the targeted cells in test tube experiments.

The article was cautiously written, but at those efficiencies or anything close, it was "the answer to everybody's prayers," says John Wilson, a gene-therapy researcher at Baylor University in Houston, Texas, who later became a member of Kimeragen's scientific advisory board. Despite *Science*'s subsequent publication of two letters strongly critical of Kmiec's sickle cell article (see sidebar above), researchers worldwide considered the efficiencies reported by Kmiec to be reason enough to pursue the technology.

Chain reaction

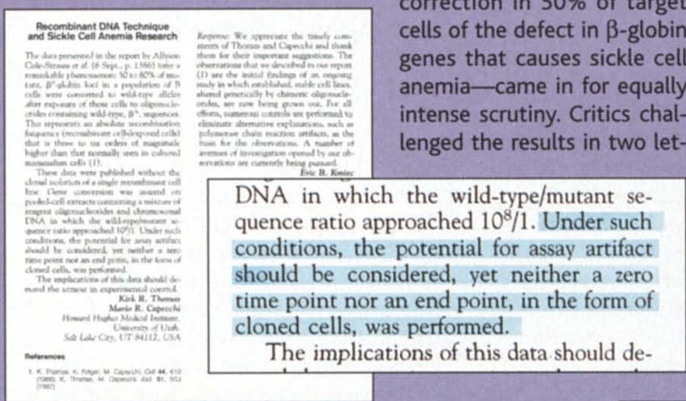
The publication of Kmiec's *PNAS* and *Science* articles had a dramatic effect on his career. Since leaving Rochester a decade earlier, Kmiec's research had subsisted on grants from the American Cancer Society and the Council for Tobacco Research, a funding organization financed by the cigarette industry. In April 1996, based on the results published in *PNAS*, Kmiec received his first NIH support since his postdoc years—a 3-year grant for $432,000 to pursue "New Gene Therapy for Connective Tissue Diseases." In September 1998, he received $850,000 from NIH for 3 years to study "Genetic Repair of the Sickle Cell Anemia Mutation." And since June 2000, he has received almost $1 million more in NIH funding to study the mechanisms of his gene-correction technology.

Kimeragen also benefited, luring Blaese from NIH to be chief scientific officer of the company and raising between $10 million and $20 million in venture capital. In 1998, Kmiec and Kimeragen parted ways, after Kmiec and the company management, by all accounts, clashed over numerous issues.

The two papers also prompted researchers around the world to try chimeraplasty, given what Blaese called "the enormous promise" if it worked. Last October, at the annual meeting of the American Society of Human Genetics held in Baltimore, Kmiec reported that in the 6

When *Science* asked Kmiec about these criticisms, he responded in an e-mail that he does not accept that a large number of plasmids would necessarily enter each cell. He added that the technology was then in its earliest stage of development, and so "one cannot expect that the data would all be explainable by simple answers."

The *Science* paper—in which Kmiec and his colleagues reported correction in 50% of target cells of the defect in β-globin genes that causes sickle cell anemia—came in for equally intense scrutiny. Critics challenged the results in two letters to *Science*. The first, published on 7 March 1997 (p. 1404), was from gene-therapy pioneer Mario Capecchi and his colleague Kirk Thomas of the University of Utah, Salt Lake City. They suggested that Kmiec and his coauthor, Allyson Cole-Strauss, might have inadvertently picked up correct DNA sequences from their RDOs rather than the experimental cells in their tests to determine what fraction had been corrected.


Recombinant DNA Technique and Sickle Cell Anemia Research

DNA in which the wild-type/mutant sequence ratio approached $10^8/1$. Under such conditions, the potential for assay artifact should be considered, yet neither a zero time point nor an end point, in the form of cloned cells, was performed.

The implications of this data should de-

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Eric B. Kmiec

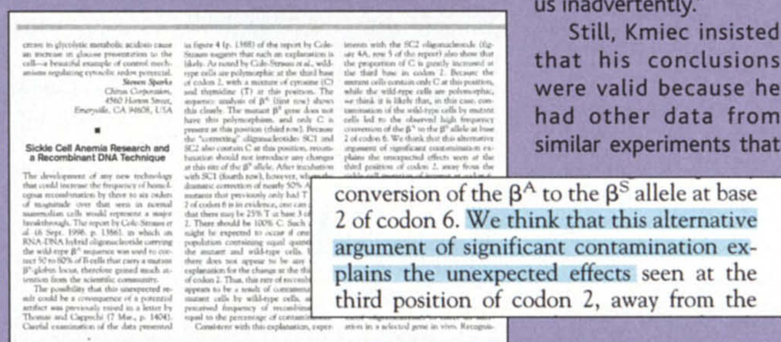
Sharp rebuttal. Two letters to *Science* pointed to potential flaws in the original publication.

CREDITS: (TOP TO BOTTOM) SCIENCE 275, 1404 (1997); SCIENCE 277, 460 (1997)

The second letter was co-authored by three veteran genetic-recombination researchers—Andrzej Stasiak of the University of Lausanne, Switzerland, Stephen West of the Imperial Cancer Research Fund in the United Kingdom, and Edward Egelman, then of the University of Minnesota Medical School. They suggested that data in the *Science* article pointed toward contamination.

Kmiec and Cole-Strauss had sequenced the β-globin genes around the original target mutation and published these sequences in the article. In two of their experiments, these sequences included a second transformation—a dozen nucleotides away from the target—where cytosines had apparently changed to thymines. Stasiak, West, and Egelman pointed out that the RDOs could not have caused this transformation because they only had cytosine at this position. Rather, they suggested, the probable explanation was that the sequencing had been done on a mixture of cells that included wild-type, healthy β-globin genes that happened to be "polymorphic" at this second position; some had cytosine and some thymine. Kmiec and Cole-Strauss's data indicated that their wild-type cells had just that polymorphic mixture. The sequencing data from the control experiments, however, showed no signs of this second transformation.

Science published their letter on 25 July 1997 (p. 460), along with a response from Kmiec stating that contamination of the sort described would not generate the pattern of data he had published. In July 2001, however, Kmiec conceded to *Science* that such contamination was a likely explanation for the published results. "Of course Stasiak was right," he said in a telephone interview, suggesting that the sickle cells used in those experiments "probably had various types of other cell types in them or were contaminated by us inadvertently."


Steven Sparks
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Sickle Cell Anemia Research and a Recombinant DNA Technique

conversion of the β^A to the β^S allele at base 2 of codon 6. We think that this alternative argument of significant contamination explains the unexpected effects seen at the third position of codon 2, away from the

Still, Kmiec insisted that his conclusions were valid because he had other data from similar experiments that could not be explained by contamination. And in an e-mail to *Science* this fall, Kmiec said that tests on the cells had found no evidence of contamination.

Whatever the correct explanation, says West, the chimeraplasty experiments reported in *Science* were "very bad science." After writing the letter and reading Kmiec's response, he says, "we decided that was enough of it. We have better things to do than wave our hands, and we got on with our own work."

–G.T.

years since his *Science* publication, more than 30 published papers have reported some success with chimeraplasty. These include work done in bacteria, plants, mice, rats, and a single dog. They reported that the RDOs could trigger gene repair in these systems with efficiencies ranging from 0.0002% to near 50%. By this autumn, nine laboratories had reported some positive result, including Kmiec's at the University of Delaware, Newark, where he moved in 1999, and Yoon's at TJU.

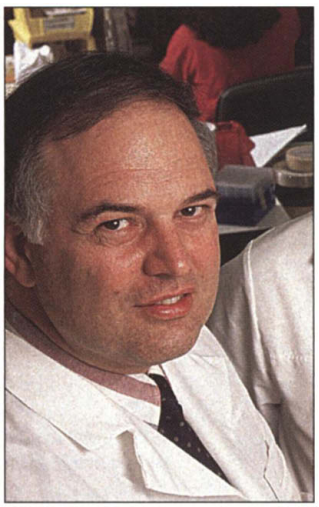
The strongest corroborative evidence has come from Clifford Steer, a University of Minnesota, Twin Cities, medical doctor and liver disease specialist. Before chimeraplasty, Steer told *Science*, he had never worked in either gene repair or gene therapy. He says he was collaborating with Kmiec on other research when he saw the "scathing" letters to *Science* on the sickle cell experiments and recalls saying to Kmiec, "if you have any of those chimeric [RDOs] around, why don't you send me one and we'll try testing it in our lab. If we're successful, at least you can tell the general public or the scientific community that another lab independent of yours was able to reproduce the work." Kmiec sent him the RDOs and within 3 weeks, Steer told *Science*, he demonstrated that chimeraplasty worked. The subsequent paper was published in *Hepatology*, coauthored by Kmiec. (Steer's brother was an original investor in Kimeragen and on Kimeragen's board of directors, but Steer says that had no influence on his decision to work with Kmiec or pursue the research.)

Since his first paper with Kmiec, Steer has reported that his RDOs work with astonishing efficiency. In 1999, for instance, Steer reported in *The Journal of Biological Chemistry* that his RDOs could induce with 48% efficiency a specific mutation in the factor IX gene responsible for hemophilia in the liver of live rats.

Steer says the key to his success is a modified version of a gene-delivery technology that uses polymers known as polyethyleneimines (PEIs) to help plasmids slip into cells. He has reported that his modified PEIs can deliver RDOs and reporter genes to 100% of liver cells in live animals. Researchers such as George Wu of the University of Connecticut at Storrs, Jean-Paul Behr of the University of Strasbourg, France, and Ernst Wagner of the University of Munich, Germany, who work with PEI and similar gene-delivery formulations and who pioneered the technology, told *Science* that the best they've ever achieved with similar sys-

tems is below 1%. Steer's results "boggle the mind," says one gene-repair expert.

Steer says he welcomes researchers to visit his lab and learn his modified PEI techniques. But he told *Science* that he knew of no independent researchers who had reproduced his experiments. At least three other labs took up his offer and saw convincing demonstrations that the technology worked in Steer's lab. However, they still failed to reproduce his findings at their own laboratories. Geneticist Thomas Jensen of Denmark's University of Aarhus says his student spent over a month with Steer in the summer of 2000 and then tried for over a year to replicate the experiments. Although this lab seemed to get some positive results, Jensen told *Science*, the conversion efficiency was so low that "it is difficult to measure." Wu says his lab "spent a fair amount of time and money" in this pursuit but failed.



Early backer. Michael Blaese joined chimeraplasty company.

Even Kmiec's researchers have been unable to achieve similar results. "We're concerned that Cliff stands out there by himself," says Howard Gamper, who works with Kmiec at Delaware. "No one has reproduced his work at the efficiencies he reports. This lab has not, and we're not aware of anyone else [who] has had success at that level."

The difficulty in reproducing chimeraplasty techniques has not been limited to Steer's liver-cell system. In a letter published in the June 2001 issue of *Nature Biotechnology*, Jim Owens and colleagues at the University of London and the Royal Free and University College Medical School in London reported that they had managed to correct defective apolipoprotein E genes in four different cell types with an efficiency above 25%. Since then, however, as Owens told *Science* last week, their chimeraplasty experiments have failed persistently. Owens referred to these relentless negative results as the "somewhat sorry situation in our laboratory." He suggested that the problem might lie with poor-quality RDOs and reagents; his lab is now trying to check that possibility.

Most researchers who tried chimeraplasty failed from the beginning. *Science* spoke to researchers from over 30 laboratories that had tried the RDOs and failed to produce evidence that they could target and correct dysfunctional genes, either in vitro or in vivo. Researchers at biotech companies such as Epoch Biosciences, Isis Pharmaceuticals, Millennium Pharmaceuticals, and Lexicon Genetics all failed to get chimeraplasty to work in their labs. Experienced gene-targeting researchers at MIT's Whitehead Institute, NIH (including in Blaese's own laboratory), Maine's Jackson Laboratory, and Sweden's Karolinska Institute also saw no effects. Even members of Kimeragen's own scientific advisory panel, such as Baylor's John Wilson, tried it and failed. "Under our conditions," Wilson says, "we found no correction above background."

As of last winter, three laboratories had published their negative results, including one from the University of Groningen, the Netherlands, led by Gerrit van der Steege, who saw the technique work in Yoon's TJU laboratory but was unable to replicate it in his Groningen lab. Writing in *Nature Biotechnology* in April 2001, van der Steege and his colleagues described their "persistent failure" and "complete lack of success" with the RDOs.

The great majority of researchers interviewed by *Science* say they find the negative results, even though unpublished, more persuasive than the positive ones because they come from independent labs with considerably more experience in gene repair and gene therapy than those that succeeded have. "The people I trusted, the ones I polled who are really good," says Neal Copeland, for instance, director of the Mammalian Genetics Laboratory at the National Cancer Institute, "invested a lot of time, and none of them got it to work."

Kmiec and other proponents of chimeraplasty disagree. "The 'lab-to-lab irreproducibility,' " Kmiec explained in an e-mail to *Science*, is "overemphasized, and appears to be the consequence of different factors, including incomplete synthesis of the RDO, or a lower frequency of nuclear delivery, or the metabolic state of the cell." He says the failure of groups such as van der Steege's to get the technique to work in their labs "means only that the same cells can respond differently during each attempt or that differences in equipment, supplies, and even the water can influence the results that are observed."

An orphaned technology?

In the past 2 years, the story has taken a peculiar twist. Although Kmiec says, "I believe the chimeraplasty technique is growing in robustness and has never had more potential," he is now focusing on alternatives. Kmiec says he has turned to single DNA strands because he couldn't afford to buy double-stranded RDOs. Even Steer told *Science* that he has switched to single-stranded DNA because it is "easier to make" and "a lot less expensive."

Indeed, both Kmiec and Yoon have reported that DNA single strands, of the kind Rochester's Sherman used in yeast, work better than the double-stranded RNA-DNA chimeras do in some experiments. In November 2000, Kmiec reported in *Nucleic Acids Research* (*NAR*) that single DNA strands repair genes with less than 0.02% efficiency in vitro in a cell-free extract, and that this efficiency was three to four times higher than the RDOs. "In that paper," says first author Gamper, "we're saying that maybe these chimeric RDOs are not so magical." Kmiec said in an interview in November 2000 that this work implies that RDOs are not necessary to achieve gene repair and that they are difficult to work with, in any event. In October 2001, he reported in *NAR* that single DNA strands effected gene conversion in yeast with an efficiency of 0.016%, whereas the RDOs achieved 0.0002% efficiency.

To date, the bulk of the research suggests that when either RDOs or single-stranded DNA work at all, they do so at an efficiency rate 1/100 to 1/100,000 of that originally reported and compatible with that of other gene-targeting techniques that rely on homologous recombination. This is also the efficiency reported by researchers who worked with RDOs in plants. "It has now taken 5 years to go from 50% gene correction in human cells to 0.0002% correction in yeast," says Andrzej Stasiak, a genetic-recombination expert at the University of Lausanne, Switzerland, "and gene targeting in yeast is really easy, so their current, improved method is unlikely to attract a lot of attention."

The concept still has proponents at half a dozen laboratories, from which positive results occasionally emerge. Kmiec and other chimeraplasty proponents consider these results compelling evidence of what Kmiec calls "the successful application of chimera-based gene repair" and "the normal evolution of scientific knowledge."

But this argument is not winning many converts. "Once there is some lack of credibility, one has to present a better case," says Steve Kowalczykowski, a genetic-recombination expert at the University of California, Davis, who was a member of Kimeragen's scientific advisory board. "One more ordinary paper is not convincing. The burden of proof becomes greater."

After 6 years of research, chimeraplasty still lacks unambiguous data and universal reproducibility. Barring a dramatic turn of events, it seems likely that the technology will pass the way of other potential breakthroughs that garnered their 15 minutes of fame and then vanished slowly into the literature.

—GARY TAUBES

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