CHAPTER

14

Magic Bullets: How Gene-based Therapies Personalize Medicine

OUTLINE

THE READER'S COMPANION: AS YOU READ, YOU SHOULD CONSIDER

- The difference between gene therapy and gene-based therapy.
- How we introduce genes into the human body.
- The advantages and disadvantages of viral vectors.
- Why we test gene therapy in animal models.
- Why we use different approaches to gain-of-function and loss-of-function traits.
- How animal models can advance gene therapy.
- Where you can go to get information about ongoing clinical trials.
- Why some genes are the first chosen for gene therapy trials.
- What is special about gene therapy in the eye and brain.
- Why ADA deficiency was one of the first traits treated with gene therapy.
- What a phase I clinical trial is designed to test.
- The difference between gene therapy and gene-based therapy.
- Why some gene therapy targets downstream pathology instead of primary cause.
- How small RNAs can help suppress RNA levels from gain-of-function alleles.
- Why it may be harder to treat traits that are very sensitive to gene dosage.
- How gene therapy can be used to treat situations that do not involve a mutation.
- How gene therapy can be used to treat cancer.
- How gene therapy can be used to help patients tolerate traditional cancer treatments.
- What role nanotechnology plays in gene therapy.
- How a bacterial recombinase can determine where a gene integrates in the human genome.
- How recombinant proteins can be used to treat genetic traits.
- Why ADA gene therapy stopped for a while, and why it is in progress again.
- What determines which traits are treated.
- What determines who participates in gene therapy clinical trials.

14.1 REPLACING A LOST GENE OR FUNCTION – THE RPE65 STORY

If I have seen further it is by standing upon the shoulders of giants. **—Sir Isaac Newton**

The scene on the screen in the conference room looked just like a home video, a movie showing a beautiful Briard dog named Lancelot walking into a dimly lit room. The speaker presenting the video explained that the last time Lancelot tried to navigate such a room, he could not do it without bumping into things constantly. The room before Lancelot seemed crowded, with disarranged furniture crammed into the space and scattered about. The audience in the conference room watched, spellbound, almost holding their breaths, as Lancelot made his way through the room, carefully avoiding objects as he swung his head around in an odd manner to scan the area ahead of him with one eye. He daintily picked his way through the obstacle course, never touching so much as a table leg. The film stopped. As the lights came up a few quiet spontaneous cheers could be heard over the applause that broke out around the room.

One young man pounded his fist emphatically down onto his knee in time with his head which nodded up and down. Several of the rational, objective researchers in the room had lumps in their throats and tears in their eyes as they listened to the conclusion to the presentation. Gene therapy treatment of Lancelot's right eye when he was four months old had effectively cured a canine model of Leber congenital amaurosis (LCA), a severe form of early childhood blindness that is incurable and may be diagnosed in humans in the first year of life. Those attending the talk had just witnessed a medical miracle: a "blind" dog that could walk through a crowded, unfamiliar room and successfully avoid contact with objects. Lancelot could see with his treated eye!

Lancelot and some of his relatives develop vision problems because of a naturally occurring defect in a gene called RPE65. Since both copies of the gene are defective, the obvious approach to gene therapy was to put a good copy of the RPE65 gene into the cells of Lancelot's eye. The strategy proved valid when the three blind puppies who were treated turned out to be cured, and they stayed cured! Since then many more of Lancelot's relatives have been similarly treated and cured. The movie starring Lancelot has played to audiences of scientists from around the world, and Lancelot has even visited Capitol Hill to attend a congressional briefing on gene therapy. To the scientists in the conference room, the concept of using this approach to cure blind children was emotionally compelling in addition to being scientifically attractive. Since then, the idea of applying such cures to humans has moved beyond the theoretical as the first human RPE65 gene therapy trials have led to improved vision in study participants with LCA. The general approach looked as if it might even be usable for some other recessive forms of inherited retinal degenerations, too.

However, many gene therapy projects have not been so successful. Why can't all of the other diseases in need of gene therapy simply be treated in the same way as the Briard dogs were treated? Not all diseases can be treated this way because there are a broad array of technical and strategic issues to be sorted out that differ from one disease to the next and from one gene to the next. In this chapter, we introduce you to how gene therapy works and to some of the issues that keep gene therapy researchers in their labs burning the midnight oil in search of answers.

FIGURE 14.1 Lancelot, the Briard dog, visits the US Congress to highlight gene therapy research. (Photo credit: Foundation Fighting Blindness.)

After great expense of time and resources on the part of many really, really smart people, we finally know the sequence of the human genome (and many other genomes, as well). The genes have been found (well, many of them, anyway). We are starting to find out what some of the gene products do. Biochemical pathways are coming together that provide us broad conceptual insights into a variety of pathogenic processes. Those of us who consider this a beginning, not an end, now face the critical question: What do we do with all of this knowledge? How do we convert all of these advances into help for people who are not adequately helped by the current state of medical knowledge?

The hope that comes from successful gene hunts points in the direction of gene therapy, the therapeutic use of the discovered genes themselves, and not just the knowledge gained from

finding those genes. Explorations of the idea of human gene therapy began even as the first human genes were being cloned. One of the first genes proposed as a serious target for gene therapy was adenosine deaminase, but the gene therapy field has expanded to the point that the NIH Clinical Trials website lists more than a thousand ongoing gene therapy trials out of more than ninety thousand clinical trials of all kinds. These gene therapy trials target many different diseases. In this chapter we will talk about a variety of gene therapy strategies that are currently being used, others that are being developed, and gene-based therapies that target something about a gene without actually putting a copy of that gene into the body.

For the purposes of this chapter, we start our discussion of gene therapy after the genes in question have already been found. We start with traits for which we know for sure exactly what needs to be added or replaced, information that came out of mapping in families, association studies in populations, and animal model studies including knock-out or knock-in animals that have either lost the gene or gained a specific mutation in the gene. By the time we start working on gene therapy, we know what the gene is, what it does, where it is expressed, and how it fails in the disease situation, and we have an animal model in which to carry out the first rounds of gene therapy. This lets us do a very detailed study of both safety and efficacy before we first try it out on a human being.

In the case of Lancelot, we are dealing with the RPE65 gene. This gene produces a protein that carries out a critical step in the visual cycle pathway, which you may recall from Chapter 9 as the pathway that processes vitamin A, essential for photoreceptor function. The defect in Lancelot's eye is a simple monogenic trait caused when both copies of the RPE65 gene have been lost or become defective. Lancelot the Briard dog provided the proof of principle: that it is possible to simply put in a new copy of the RPE65 gene and get restored function

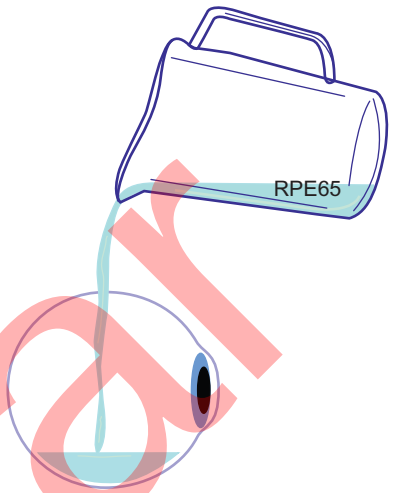

FIGURE 14.2 Gene replacement therapy adds back a functional copy of a gene in cases in which the disease results because defects in both copies of the gene cause loss of the cell's ability to carry on the functions normally handled by the product of that gene. In the case of the Briard dog Lancelot, many good copies of the REP65 AAV gene therapy construct were added into his eye in the vicinity of the retinal pigment epithelium cells that lacked the RPE65 protein activity that normally takes place there. Those copies of the RPE65 gene construct were carried into the cells by an adeno-associated virus (AAV) gene therapy vector.

(Figure 14.2). By using recombinant DNA technologies, the researchers were able to insert the RPE65 gene into a gene therapy vector made from an adeno-associated virus to create the construct.

Why is an understanding of Lancelot's blindness and cure of such great importance to the human population? Defects in RPE65 cause a form of severe, early vision loss in children who have a trait called Leber's congenital amaurosis (LCA). According to the Foundation Fighting Blindness, children with LCA often show substantial visual deficits while they are infants and electrophysiology tests show little or no detectable function of the retina. As one of these children grows up, she might be able to see well enough to count the fingers on a hand held in front of her face. Another child might be limited

to detecting bright lights and the motion of a hand moving in front of his eyes. But the importance of gene therapy for an RPE65 defect reaches far beyond the impact on these individual children. Successful gene therapy for RPE65 provides a strong proof of principle – that gene therapy can work when we get the details right. However, this does not mean that the details that make gene therapy work for RPE65 will work for Huntington disease or cancer.

This study of Lancelot and his relatives is typical of how gene therapy development proceeds. First a gene was identified and shown to be the cause of the disease through studies of animal models and human subjects with the disease. Then gene therapy was tested in an animal model. Finally, once enough was known about the mechanisms of disease pathology, the cell types involved, and the events taking place in gene therapy of the animal model, human gene therapy trials began.

The Human RPE65 Gene Therapy Clinical Trial

Because gene therapy of Lancelot and his relatives was so successful, testing of RPE65 gene therapy in humans has begun. As we write this book, the National Institutes of Health lists seven different clinical trials, six of them in phase I testing of safety and side effects of the treatment, and one of them moving into phase II where testing of larger numbers of subjects will allow further evaluation of vision improvement. To start such a study, very small numbers of subjects receive the gene therapy construct in a test of the safety of the construct. Secondarily, researchers also evaluate visual function. Inclusion criteria for the study call for subjects to have substantial visual impairment but to not be completely blind so that they can carry out visual testing to determine what effect the treatment is having on their visual abilities. So far, not only has the treatment proven to be safe, but the success goes far beyond that. Among the first 12 subjects treated, the gene therapy treatment was found to be safe down to as young an age as eight. *Even though the first phase was just a safety phase, the initial studies now show improved vision in 20 subjects, with the greatest improvements showing up in the youngest subjects!* Additional projects will move the studies into phase II to further evaluate safety and efficacy, and additional phase I trials will test safety in other groups such as even younger subjects. So far the vision research community is very excited at evidence that gene therapy for RPE65-defective LCA appears to work, and the outcome appears to persist.

When we succeed in doing gene therapy for a particular trait, or targeted at a particular tissue or pathway, we learn things that let us improve our ability to treat other traits with similar features such as affected tissue or mode of inheritance. There are many other genes that can cause LCA when defective, including other recessive forms of LCA. In developing RPE65 gene therapy researchers learned to work with a gene therapy vector (something we will discuss in more detail later in this chapter), and once they have the vectors and delivery systems worked out then it will potentially be much faster to develop replacement of the next LCA gene. There are several kinds of recessive retinal degeneration caused by defects in both copies of a single gene that could likely respond to almost exactly the same therapeutic protocol, with almost the only change from Lancelot's treatment being the choice of which gene to put into the eye. We expect that as more details of RPE65 gene therapy are worked out, the information gained will apply not only to treatment for disease caused by RPE65 defects but will begin helping with design of gene therapy for other forms of LCA and for many other single-gene loss-of-function vision defects (Box 14.1). One of the biggest next challenges will be moving beyond LCA to treat other more complex situations such as Usher syndrome – which affects both the eyes and the ears.

BOX 14.1

RESTORING FULL COLOR VISION

Many of the traits we talk about treating are rare single-gene traits, but some of the problems looming on the gene therapy horizon are not so rare. A common form of colorblindness is found in about 8% of men. There are a variety of different mechanisms that can cause such colorblindness but in many cases the man with the color vision defect is simply failing to make either the red opsin (the photoreceptor protein that sees red light) or the green opsin (the photoreceptor protein that sees green light). Researchers at the University of Washington recently did gene therapy in an animal model of colorblindness, male spider monkeys who do not make a red opsin and cannot see a full spectrum of colors. By adding a human red opsin gene, they were able to give the monkeys the ability to detect colors they have never before been able to see. It took about five months after the treatment before the monkeys started showing the ability to detect red, and two years later they were still able to see red. This exciting result suggests that it may become possible to use a similar approach to treat color vision deficits in humans. This is especially happy news for Scott and millions of men world wide who wonder what the world looks like to those who make three different kinds of color opsins.

14.2 REPLACING A LOST GENE – ADA DEFICIENCY

In 1972 David Vetter was born with severe combined immune deficiency (SCID). Up until this point in time, children with SCID routinely succumbed to fatal infections, as had David's older brother, who also had SCID. When David was born he was transferred into a plastic containment bubble, and was raised there in sterile isolation until the age of 12. In an effort to free him from his terrible, isolated existence, a bone marrow transplant was performed. Although at first the transplant seemed to be working, he succumbed to one of the complications that sometimes accompanies bone marrow transplants, cancer caused by a virus that had been undetected in the donated bone marrow. David had grown up with friends who had to interact with him across the divide of his sterile barrier. He grew up with family who loved him, but who never got to touch him until he was dying. David's life, lived just out of reach of the people who loved him, has been the subject of books, documentaries, and movies. Science has now taken steps beyond that primitive use of simple mechanical barriers to save the life of the child often referred to as the "bubble boy." One of the first treatment steps was the administration of the actual ADA enzyme as a medication, and work then progressed to use of gene therapy for SCID due to adenosine deaminase (ADA) deficiency. The story of ADA gene therapy reflects the kinds of advances and setbacks that have kept gene therapy moving forward while keeping it from moving out of the research arena into the offices of all of our local family doctors.

ADA deficiency is a life-threatening trait that results from a defect in a single gene. Bone marrow transplant continues to offer a potentially permanent cure, but it can be difficult to find a donor who is an adequately close match, and a transplant risks outcomes such as the one that took David Vetter's life. Since the time when David Vetter lived in his bubble, there has been progress with ADA deficiency on several fronts. Children with ADA have been treated with

PEG-ADA, a version of the ADA enzyme that helps to clear out some of the toxic metabolic intermediates and improves immune system function but does not restore complete health. The first case of human gene therapy, carried out in 1990, was treatment of ADA deficiency. ADA deficiency was an attractive target for development of one of the first gene therapy projects for several reasons:

- The severity of the trait cried out for a new treatment approach.
- The therapeutic strategy was simple – replace a single missing gene and gene product.
- The gene was known and had been cloned.
- The gene was small enough to be put into a gene therapy vector.
- Treatment was expected to work even if they did not fix 100% of the cells.
- They had good assays for whether or not the treatment was succeeding.
- Treatment could target a very accessible set of white blood cells, not something complex and inaccessible buried in the brain.

The First ADA Gene Therapy Treatment in 1990

The toddler Ashanti DeSilva had ADA deficiency and the treatments with the PEG-ADA version of the enzyme were gradually having less and less effect. Introduction of the ADA gene into circulating blood cells resulted in cells that could produce the enzyme, but circulating cells in the blood are a transient population of cells that has to be renewed from the bone marrow. Over the next few years Ashanti stayed healthy but needed the gene therapy treatment repeated periodically along with supplemental treatment with PEG-ADA.

The next big breakthrough in ADA gene therapy came when treatment was carried out on cells from the bone marrow. This approach seemed to be succeeding, producing a self-renewing population of cells that had been corrected for the genetic defect. Then, two of the patients who had had their ADA deficiency repaired developed leukemia! Investigation showed that the leukemia was the result of something specific to the particular viral vector that had been used. Newer vectors look like they have eliminated this problem and there is optimism that gene therapy for ADA deficiency will be able to keep moving forward.

As each new study of this kind stumbles and picks itself back up to keep going, the goal is to save the lives of those who desperately need help. These studies can only proceed through the participation of the study subjects who help to test whether the treatments are safe and then whether the treatments work. There are now thousands of gene therapy clinical trials ongoing and many more that have been completed. Out of all of these we can tally four deaths, but have you ever thought of how many people die on a regular basis in response to a standard often-used medication? How many have died because they had an allergic reaction to a drug, or because they lost control of body temperature in response to general anesthesia? We lack the data that would let us compare the rate of deaths from gene therapy to the rate of deaths from other kinds of studies, or the rate of death of individuals with similar health histories who are not participating in clinical trials. Each study participant is a hero, someone brave and determined, engaged in a fight for the lives of those who need the treatments that are being developed (Box 14.2).

14.3 TARGETING DOWNSTREAM DISEASE PATHOLOGY

In some cases, we may be trying to compensate for a problem that is too genetically complex to tackle at the point of the disease gene itself; in other cases, the trait may not even be genetic in its origins. In such cases, we may need to simply

BOX 14.2

A HERO AMONG US

In 1999, when Jesse Gelsinger was 17, he had a goal that was amazingly different from that of his high school classmates in Tucson, Arizona. Across North America, seniors in the spring of 1999 were talking about what colleges they would attend, applying for jobs, planning weddings, and deciding whether to enlist in the service. While they planned educations and careers, Jesse was waiting to turn 18 because that was the magical age that would let him become a human subject in a gene therapy research project. How did this young man come to such an extraordinary, selfless view at a time when many his age were focused on themselves and the complex transitions going on in their lives? Some of the answer comes from Jesse's own medical history. Jesse suffered from a mild form of the same recessive disease, ornithine transcarbamylase (OTC) deficiency, that kills the severely affected babies he wanted to help. Jesse could identify with the danger to these infants, even though he had never met them, because Jesse himself could not make enough of the OTC protein, which is part of the urea cycle that is used to remove excess nitrogen that enters our bodies when we consume proteins. If the urea cycle doesn't work, the nitrogen from the proteins accumulates in the form of ammonia that can cause brain damage. Ammonia production can be limited by a low-protein diet and medications, but the one baby in 25,000 who is born with OTC deficiency can usually be expected to go into a coma within days of birth. Even with medical help, many OTC children suffer permanent brain damage; many die before they are one month old, and almost half die before the age of five years. However, in some individuals like Jesse the disease is less severe because only some of their cells carry the genetic defect. Seventeen other people before Jesse had walked safely away from participating in the phase I test of safety of the OTC gene therapy protocol. Jesse responded to the treatment by going into multiple organ failure. There remain questions about why he died, whether or not the particular viral vector choice might have played a role, and whether partial OTC-deficiency played a role in Jesse's death. The result was that the OTC gene therapy trial was discontinued. Jesse's act of heroism ended up improving the whole field of gene therapy by causing re-evaluation and changes throughout the field. But OTC gene therapy in humans has not resumed. We wonder whether Jesse would see the OTC gene therapy program itself as the second casualty in this terrible circumstance. Jesse was a true hero among the many heroes who have made gene therapy possible, and we look forward to the day when his dream finally comes true, when babies with OTC get to live healthy, normal lives.

bypass the whole issue of which gene (or what else) is causing the disease, or even how many genes are involved, and target some other aspect of the disease pathology, things that take place downstream of the initiating events of the disease (see Figure 14.3). Sometimes what is needed is to add a different gene that can supply a function that improves the body's ability to put up with the damage being caused, or that provides a mechanism to assist the body in recovering from damage that has been caused.

An exciting example of this kind of "end-run" gene therapy are some of the approaches being developed to treat cardiovascular disease. Going after the downstream pathology becomes especially important for some phenotypes such as

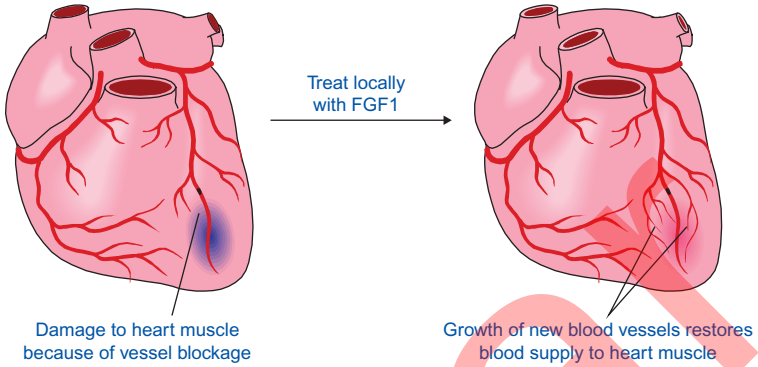

FIGURE 14.3 End-run gene therapy. In some cases, gene therapy can be used to treat a disease without going after the primary causes of the disease. This artist's conception shows how uses of growth factor FGF1 can cause new blood vessel growth in a local area of the heart to restore blood supply to a region previously supplied by a blocked vessel. Successes of this kind have been seen in animal models, and some early human studies in gene therapy of cardiovascular diseases are ongoing.

cardiovascular disease because the patient frequently becomes available for treatment of any kind only after damage has taken place – often a heart attack that has damaged the heart muscle. A complex array of genetic and dietary features lead to a heart attack, and the medical community is already working on that end of the problem. But we will continue needing a way to intervene in those cases where damage has occurred. We can accomplish a lot if we target some of the downstream problems by looking for ways to clear plaque from arteries or improve the health of the damaged heart muscle.

Researchers have shown that a growth factor called FGF1 can be used to stimulate local growth of new blood vessels to supply heart muscle in cases in which blockage is reducing the blood supply to the heart (Figure 14.3). In patients with damaged heart muscle, the combination of genetic and environmental factors that could have caused this is likely complex and different for different individuals. Yet a single treatment approach that goes after the secondary problem of getting a blood supply to the heart could completely ignore the difference in underlying causes among the patients yet still successfully restore oxygenation of heart muscle. Introduction of genes encoding other growth factors such as vascular endothelial growth factor (VEGF-1) are also being developed for this purpose.

Other gene therapy projects target many different aspects of cardiovascular disease. One study used gene therapy to provide an APOE gene that produces an APOE protein that helps reduce "bad" cholesterol, resulting in disappearance of plaque attached to blood vessel walls. Some studies are working on ways to deliver gene therapy into the walls of blood vessels to help clear plaque and improve the health of the vessels. Other studies are looking at how gene expression is changed by the use of medications commonly used to treat heart disease.

14.4 SUPPRESSING THE UNWANTED GENOTYPE – USE OF siRNAs AND miRNAs

In the case of gain-of-function mutations (remember the concept of the monkey wrench) such as Huntington disease, we can't use the

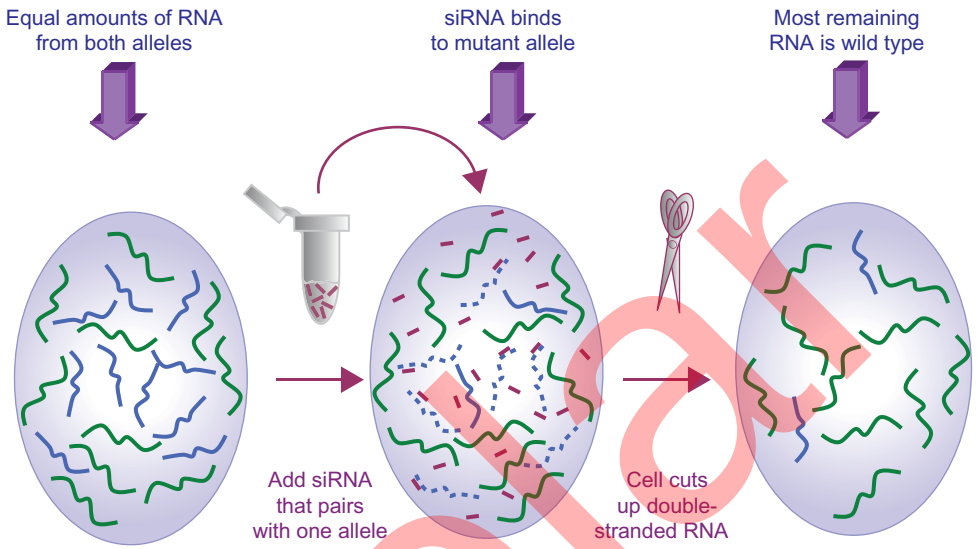

FIGURE 14.4 Gene suppression therapy. If the problem can best be solved by reducing the amount of a gene product (or its activity levels) a variety of technical approaches can be used. RNA interference is one of them. Small interfering RNAs (siRNAs) can trick the cell into digesting and getting rid of RNA to which it binds and thus reduce the amount of the gene product in the cell. If the siRNA is homologous to the causative mutation and binds to it, but not to the normal sequence, then the cell will selectively chew up the RNA to which the siRNA is bound (the RNA carrying the mutation) while leaving the wild type RNA relatively untouched. The outcome will be a cell that still has a normal amount of the wild type RNA for that gene, but that has reduced amounts of the RNA from the gene copy with the mutation. This works well where the cell can tolerate some reduction in total RNA from that gene and where improvement can result from reducing the amount of the RNA with the mutation without completely eliminating it.

approach we used for RPE65. There is already one good copy of the gene present in the cell and putting in more good copies of that gene may not help the situation. However, the situation can be helped by therapeutic approaches aimed at getting rid of the unwanted monkey wrench or the by-products of its misbehavior. So if the problem involves a toxic by-product, the use of gene therapy techniques to reduce the amount of a specific RNA can lead to reducing the amount of gene product being made. As you may recall from Chapter 3, small interfering RNA technology can reduce the amount of transcript coming from the offending gene; the treatment adds many copies of an RNA that is so small that it is readily taken up by the target cells. The sequence of this small RNA is complementary to the sequence of the mRNA produced by the disease gene allele. Because of the sequence complementarity, the *small interfering RNA* (*siRNA*) can bind to the mutant transcript and get the cell to destroy the RNA coming from the disease gene (Figure 14.4). In some cases, it is conceivable that the siRNA can be designed so that the transcript from the disease allele will be destroyed at a higher rate than is the transcript from the normal allele, allowing for the possibility of reducing the amount of a toxic byproduct while still allowing for some normal protein to carry out the normal function. Other strategies work at the level of the gene product, by adding in a gene whose product will chemically activate or inactivate the problem gene product.

In some cases the problem is more complex than the simple presence of the defective allele. For some genes we not only need to get

rid of the monkey wrench, but we also need to keep a normal level of the transcript. Some projects working on this strategy are using two approaches together – siRNA to reduce the level of the defective alleles plus gene therapy to restore the overall level of transcript from the normal allele to its normal level. This is only needed for genes that cannot tolerate a reduction in overall RNA levels.

MicroRNAs (*miRNAs*) have been shown to play a role in a large number of different biological processes. In the case of heart disease, miRNAs have been shown to be associated with the development of cardiovascular problems such as cardiac arrhythmias, heart failure, and atherosclerosis. Among approaches to cardiac disease being explored we find the use of anti-miRNA oligonucleotides (AMOs). These AMOs are short nucleotides homologous to the miRNA shown to cause a problem. When the AMO binds to its complementary miRNA, it effectively reduces the final amount of protein product resulting from the mRNA. One of the problems with the development of AMOs is that they are small enough that their short runs of sequence can often match up to sequences from many different mRNAs in the body, not just the target we are after. Because therapeutics makes use of artificially synthesized oligonucleotides, and because we have the whole human genome sequence available, we can select regions of sequence that are unique to the target mRNA when we design our synthetic AMO; we are not limited to using the miRNA sequence that is available in a human cell.

14.5 GENE SUPPLEMENT THERAPY – MORE OF THE SAME

In some cases, tissues in the body simply need to be making more of something they already make. The item to be supplemented is not missing and the gene is not mutated. One of the situations in which this approach is being used is to get cells to make the proteins necessary for the formation of new bone material (Figure 14.5). In these cases, the patient does not have a defect in bone formation but rather has an injury of some kind that is more than his own body can heal easily. Gene therapy treatment of skin cells with bone morphogenic protein before placement of the cells into a region of bone erosion in periodontal disease can lead to formation of new bone in the region. Another approach places the gene therapy agents and cells into a gel placed at the point of a break in a bone, with gradual release over time resulting in sustained expression of the genes being used in the treatment.

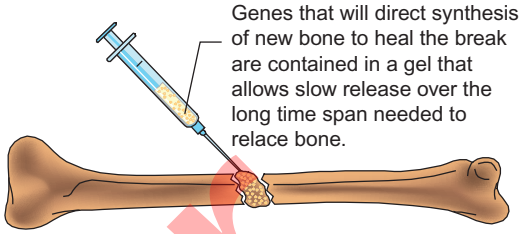

FIGURE 14.5 Gene supplementation therapy. An example of this strategy is the use of gene therapy agents that can induce cells in the bone to manufacture new bone. This is especially important in cases of severe fractures and fractures that do not heal well. By embedding the gene therapy agents in a gel at the site of the break, it is possible to have slow release of the DNA and gradual expression of the relevant genes over the extended time period needed for bone healing. By using a bone morphogenic protein at the site, bone growth is stimulated locally without such activity going on at unwanted locations elsewhere in the body.

There are many other phenotypes that call for this kind of supplementation. Any defect caused by hemizygosity – having only one functional copy of the gene instead of two – could benefit from this kind of strategy. This includes a variety of transcription factors, where diseases such as aniridia or Rieger syndrome result from having only one good copy of the gene. While the idea of restoring copy number seems like a simple fix for this problem, it is important not to overshoot the

amount, not to end up with multiple additional copies. In the case of Rieger syndrome we see that the disease not only results from lacking a copy of the gene but can also result from having an extra copy of the gene. When we give someone a new copy of a gene, it does not simply replace the existing copy, so the new gene goes into the cell in a new context where the regulation of expression may be different. So as we do gene supplementation therapy we face the added problem that for some genes we add back the dosage has to be exactly right, not just in terms of having enough but also in terms of not having too much.

14.6 STRATEGIES FOR CANCER THERAPY

Gene Therapy to Target Tumor Cells

In some cases, especially with cancer, what we really want is to be able to destroy specific cells while leaving the surrounding cells intact. There are a lot of different ways to kill cells, and we can see some of the diversity of possibilities when we look at available cancer therapies. Similarly, gene therapy approaches to cancer use many different strategies. While some focus on killing cancer cells, other strategies may simply aim to give the cell back the ability to control the cell cycle and regulate its growth.

One of the approaches that has made it as far as phase III clinical trials in the United States, and is in clinical use in China, is the delivery of p53 into tumor cells. As you will recall from Chapter 10, loss of p53 is a primary cause of some cancers, and p53 is also lost in secondary steps of some cancers as tumor stages progress. Adding p53 back through gene therapy has been reported to be beneficial in treatment of cancers of the head, neck, and lungs. There remain a variety of problems with such delivery since we normally cannot get effective introduction of the construct into every cell in a tumor.

An especially ingenious idea was developed by researchers who want to use a "suicide vector" approach to destroy malignant brain tumor cells while leaving the surrounding brain cells untouched. Brain cells are not usually thought of as growing or dividing. Some viruses will infect either dividing or non-dividing cells, but there are types of viruses that infect only actively dividing cells. By selecting the kind of virus that infects only actively dividing cells, we can target the gene therapy into any actively dividing cells in the treated region while not treating the non-dividing cells that surround them. Use of such a virus lets us target an aggressively growing tumor while leaving the brain cells surrounding it untouched. Administration of an antiviral drug called gancyclovir will expose many of the brain cells to gancylcovir, but the drug will be harmless to most of the cells in the brain. It will specifically kill only those cells that have taken up the virus, so the tumor cells will die but surrounding tissues will remain intact (Figure 14.6).

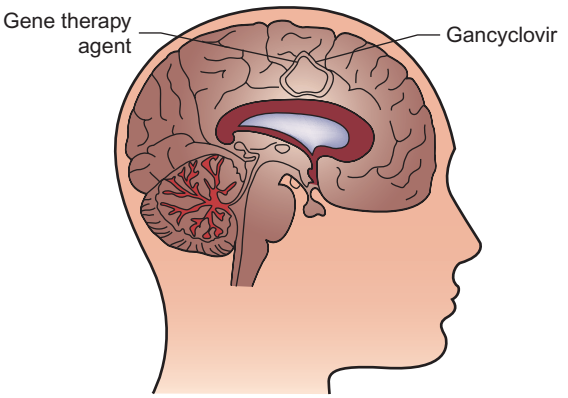

FIGURE 14.6 Magic bullet therapy. Many different strategies are being developed for being able to target therapy in such a way that only the tumor cells die while the normal cells remain healthy. One strategy is to use two different therapeutic agents that are each benign alone and kill cells only where both agents are present. Use of a gene therapy virus that can only infect dividing cells will tag tumor cells while sparing surrounding nondividing cells. A secondary treatment kills only tagged cells. This strategy would not work in many tissues of the body.

This concept, that cell death will occur only where two separate events coincide, resembles a process in current use in cancer treatment. In this process, low-level radiation administered from multiple different directions spares the surrounding tissues while killing only those cells present at the point where multiple radiation beams come together at the same place to result in a dose high enough to kill the cells. Other research groups are trying a variety such approaches that call for cell death to occur only where two different events come together, thus sparing any cells that are exposed to only one or the other of the two items. And one of the ways in which gene therapy approaches can best help limit delivery is through use of viruses that in some way selectively infect the tumor cells as compared to cells of the surrounding tissues.

Gene Therapy to Improve Effectiveness of Traditional Cancer Therapies

An intriguing concept in cancer therapy is to increase the effectiveness of chemotherapy by doing gene therapy that lets the patient tolerate a higher level of chemotherapy. If we put a gene encoding a protein that pumps specific chemicals out of the cell (a pump protein) into bone marrow cells to increase their resistance to the effects of anticancer drugs, while not putting that same gene into the tumor cells, we can increase the therapeutic dose without increasing the damage to the bone marrow that is usually one of the worst complications of treatment (Figure 14.7). Clearly this approach will not work for any blood-based cancers such as leukemia, but could be a real boon to anyone with a solid-tissue tumor who has to undergo chemotherapy.

When we look at what gene could be added to provide such protection we are struck by the idea that we might even be able to get this effect without having to add in a gene! How could that be? One of the genes that we would most like to be able to add to the bone marrow in someone who has to undergo chemotherapy for cancer elsewhere in the body is a gene encoding a pump protein. But we know that the body has natural mechanisms for up-regulating expression of such pump proteins. In fact, this up-regulation in tumor cells can sometimes be a problem that can interfere with treatment. So if we can learn how to regulate expression of this gene, then the optimal approach would be to down-regulate the pump protein in the tumor cells and up-regulate it in the bone marrow and lining of the gut. This then becomes a gene-based therapy that is not actually gene therapy in a classical sense.

A similar strategy, whether through gene therapy or through regulation of expression of genes such as those encoding pump proteins, might reduce the hazard of living or working in a contaminated environment, including environments

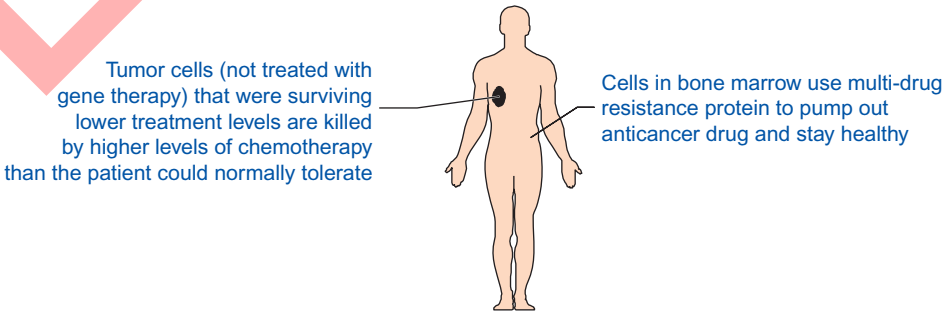

FIGURE 14.7 Supplemental gene therapy. Another use of supplemental gene therapy is to boost the ability of the patient to survive higher levels of chemotherapeutic agents being used to attack the tumor cells.

that increase our risk of cancer. During our lives, we suffer a variety of exposures that can be directly harmful or can increase our risk of things such as cancer. As we learn more about the normal mechanisms used by the body to eliminate toxic substances, more about biochemical pathways that can convert toxic substances into safe (or safer) substances, and more about ways to get compounds pumped out of cells or excreted from the body, we gain the potential to use gene therapy to protect us from exposure or to clean up our internal environments once we are exposed. The same pump proteins that can affect whether therapeutic levels of chemotherapy are getting into target cells or other cells in the body can also serve to help pump out some toxic compounds coming in from the environment.

We have presented only a couple of examples of things being tried, but we want you to understand that for cancer, as for many of the other traits we discuss in this chapter, a wide variety of approaches are being tried. We are struck by the intelligence and creativity that seems to go into the design of many of the new strategies being developed.

14.7 GENE-BASED THERAPY INSTEAD OF GENE THERAPY

Use of Recombinant Proteins as Therapeutic Agents

Sometimes the answer is gene therapy, and sometimes the answer is gene-based therapy where we are using genes to produce the therapeutic agent without actually putting the gene itself into the patient. This concept of gene-based therapy rather than gene therapy is not even terribly new. One form of gene-based therapy is the use of a recombinant protein, that is to say a protein that has been produced through use of an expression vector that contains a copy of the gene encoding that protein. For many years, recombinant tissue plasminogen activator (TPA) has been produced outside of the body and then injected so that it can help reduce clotting in individuals such as those having heart attacks or strokes. Recombinant human insulin, made from a cloned version of the human insulin gene, allows diabetics to use human insulin instead of pig or cow insulin. A variety of other growth factors, blood clotting agents, and other products that our own bodies normally make can now be synthesized outside of the body and then added back in. In forms of hemophilia, as different individuals have been found to be lacking a specific blood clotting factor it has become possible to purify or produce a recombinant form of that blood clotting factor to be used therapeutically. Knowing that insulin is what is missing in children with type I diabetes lets doctors supply them with insulin, the protein product of the insulin gene. In each case, what we are talking about is not gene therapy, because we are not putting any genes into any of these patients, and yet it is gene-based therapy because the therapy is designed directly from the understanding gained when it was determined what gene or gene product was missing.

Use of Drugs That Can Affect Transcription or Translation

One of the approaches being used to alter gene expression for therapeutic purposes involves inducing the cell to turn on an alternative gene to compensate for the gene that is functioning properly. As you may recall from Chapter 5, sickle cell anemia results when both hemoglobin copies are the HbS allele, resulting in red blood cells that take on a sickled shape and get stuck in capillaries. Studies in a mouse model of sickle cell anemia showed that gene therapy could add back a normal globin allele and convert sickle cell anemia to sickle cell trait. But for many years it has been known that sickle cell anemia could be helped through the use of a gene-based therapy that is not itself gene therapy; by using a chemical called hydroxyurea doctors are able to turn on

expression of a fetal form of hemoglobin to help compensate for the defective form of adult hemoglobin that is the hallmark of the disease. Thus at this point the development of the pharmacologic approach using hydroxyurea is being used even as gene therapy for sickle cell anemia is being worked on.

In the case of Duchenne muscular dystrophy some treatment approaches being tried may generalize to use for other traits. Clearly these drugs are not yet optimal but they are both promising enough that work on them is continuing. The demonstration of effective treatment with these drugs would lay the groundwork for similar approaches to other traits. One drug being tried is an anti-sense oligonucleotide that binds to the transcript and leads to efficient skipping of exon 51, an exon in which many Duchenne's mutations are found. Boys with exon 51 causative mutations constitute 13% of the Duchenne muscular dystrophy population, and skipping of this exon restores an open reading frame to the protein. Targeting of exon 51 is expected to be especially effective since studies of large deletions spanning this region show that onset of symptoms is delayed until very late in life. Studies in animal models suggest that this type of treatment can be tolerated (and work) for a long period of time. A lot of work will be needed to develop a similar exon-skipping drug and protocol for each of the 76 exons in the gene that have mutations that cause Duchenne muscular dystrophy. In phase I clinical trials muscle biopsies show the presence of dystrophin protein, something these boys were not previously making and studies have moved onto a stage I/II clinical trial to continue testing safety while looking at efficacy!

Other researchers are looking for ways to get the muscle cells to read through nonsense mutations that cause truncation of the dystrophin protein. The first drug found, an antibiotic called gentamicin, results in production of dystrophin but it is too toxic to take long term. Screening of large numbers of compounds has led to the identification of other possible drugs that seem to be able to get this read-through effect for dystrophin without interfering with use of the normal stop codons present in other genes. It remains to be seen whether they can be tolerated for continuous treatment lifelong. Variations on this approach are also being tried for cystic fibrosis.

Drugs That Target the Biochemical Defect

Recently researchers set what may well be a new record for the elapsed time from the gene hunt to the treatment. In 2003 Francis Collins and his colleagues reported that mutations in lamin A (LMNA) cause progeria, a severely premature aging syndrome. Progeria is so rare that there are usually fewer than twenty individuals in the whole United States who have this trait at any one time. The average life span of these children is 13 years, but some of these children may occasionally live as long as 20 years. Those affected are very short and often under-weight for their height. They have large, bald heads, prominent eyes, an unusual gait, and many other distinctive features. Although many of these features of appearance are how we so readily recognize these children, the key issue in progeria is the cardiovascular disease that ends up taking their lives. By 2005 researchers knew that a key to the disease process was a modification of the truncated lamin A protein produced in the bodies of these children; if a farnesyl chemical group is present on the protein, the protein remains anchored in the membrane. The result is messed up nuclear membrane architecture that has a typical appearance called blebbing. Researchers found that if they used a drug called a farnesyl transferase inhibitor (FTI) to keep the protein free of this chemical modification, they could help restore the nuclear architecture. As a sign of how successful this was, the nuclear blebbing disappeared. Researchers went on to show that treatment with FTIs could help prevent the cardiovascular disease in a mouse model of progeria,

and could even undo some of the damage in progeria animals who had already developed cardiovascular disease. In 2007, just four years after the cause of the disease was first reported, the first clinical trial began that uses this gene-based therapeutic approach – treatment with FTIs. There are now three different clinical trials under way to test a combination of three drugs together, a statin to take a traditional approach to cardiovascular problems such as cholesterol, a farnesyl transferase inhibitor to directly address the underlying cause of the disease, and a bisphosphonate to deal with other problems of aging such as osteoporosis. Although there are some aspects of the phenotype that may not yet be solved by this treatment approach, the mouse model suggests that this may help give these children more years of life, and years with higher quality of life. For three of the children in this study who are older than 13, this treatment breakthrough has come just in time for kids feeling like they were living on borrowed time.

14.8 DELIVERING GENE THERAPY

One of the tricky parts of gene therapy turns out to be getting the gene into the cell. We end up balancing a trade-off between the efficiency with which we get the DNA into the cell and the side effects that take place as a result of the delivery method. Some of the methods with the lowest side effects are so much less efficient than viruses that they cannot achieve a therapeutic level of gene transfer. Does this mean that we give up on them? No, it means that we keep working on the technical development of these approaches in search of breakthroughs that could offer new options.

Use of Viral Vectors

One of the earliest gene delivery strategies involves the use of *viral vectors*. Such vectors are engineered to remove their disease-causing properties and to give them the ability to carry human genes along with their own DNA. There are several advantages to the use of such viruses. They greatly enhance the ability to get DNA into the cells, and such efficiency turns out to be critical to the success of such projects. Another advantage is that viruses can sometimes help restrict which cells get targeted for treatment. This lets us put the treatment into some cell types while keeping it away from others.

Viral vectors also allow for mass production. No, we are not seeking mass production for some industrial economic reasons (although the economic issues are worth considering, too!). Rather, being able to do mass production allows for kinds of quality control that we cannot do if we manufacture one dose of something each time we do a treatment. If you can make one gene therapy construct, prepare huge amounts of it, and do extensive testing on it, you can still have enough at the end to use in treatments. This lets you know that the batch you are using to treat people with is the same batch that passed all of the safety testing.

The biggest disadvantage to viral vectors is the tendency of the body to mount an immune reaction. In some cases, if what is desired is the destruction of a particular cell type, the use of vectors that invite an immune reaction may actually enhance the therapy, but it would not be useful in situations that call for repeated treatments because the virus would not be able to get to the target cell once the body develops an immune response to the virus.

There are differences between the different viruses used in vector construction. Adeno-associated virus integrates into the genome to give stable, long-term expression, and it can infect both dividing and non-dividing cell types. It can only be used for some gene therapy projects since it cannot accommodate really large genes, and the processes for producing it are less efficient than for some of the other vectors. Adenoviruses can take larger inserts but tend to produce a much stronger immune reaction than the

adeno-associated viruses. Other viruses offer differences in sizes of genes that can be accommodated, how immunogenic they are, whether they can infect particular cells types like nerves, and how stable or transient the resulting gene therapy will be. This gives gene therapy designers a lot of choices, but it is not possible to do a simple mix-and-match selection of an exact profile of features the researcher wants. There are a limited set of viruses currently under development and in most cases the advantages end up having to be weighed against disadvantages.

Non-viral Delivery Systems

There are other ways to get genes into cells that don't use viral vectors. In some cases, DNA copies of the gene can be packaged into *liposomes*, lipid packets that surround the DNA and help carry it into the cell. In other cases, direct injection of DNA can be carried out but would only get the DNA into a very limited set of cells. There are some promising approaches being used that call for removing cells from the body, carrying out the delivery of DNA in a cell culture dish, and returning the cells to the body.

One very important alternative delivery system uses a plasmid instead of a virus as the delivery system. By including a nuclear localization signal normally present on a virus, the researchers are able to get the DNA to go on into the nucleus. To get around the lack of an efficient viral system for getting the DNA into the cell and then into the nucleus, DNA bound to a protein called phi31C is delivered into the cell through use of hydraulic pressure to push the DNA into the cell efficiently. The phi31C bacterial recombinase is a protein that specifically inserts DNA into one of a very small number of locations in the human genome. Once inside the cell, the gene being introduced is stable and can't come back out or move to a new location. The small number of ph31C sites in the genome are not located in any of the oncogenes or other coding sequences of concern, so this system helps to overcome one of the problems we face with gene therapy: that up until now we have had little control over where the gene ended up once it got into the human genome. Thus each new gene therapy event that involves stable insertion of the gene into a chromosome risks having that insertion event interrupt a crucial gene such as a cancer gene. Through the use of phi31C Michelle Calos and her collaborators are putting genes into a variety of cell types including skin, muscles, and white blood cells. The phi31C recombinase system is especially important because it lets researchers target genes to locations that are known to be safe, and to keep the gene there once it is inserted. This strategy is offering a new approach to gene therapy for hemophilia A.

One of the most intriguing new areas of gene therapy design is coming out of the field of nanotechnology. Dendrimers are large, branching, tree-like macromolecules that are water-soluble and have the ability to surround and encapsulate other molecules. Some kinds of dendrimers have been developed for use in drug delivery, and can serve a similar purpose for getting a copy of a gene into a cell. When biodegradable polymers containing DNA dendrimer complexes are used, it is possible to apply these polymers to the target tissue and limit the transfer of DNA on a very tightly localized basis.

14.9 DO WE HAVE TO TREAT THE WHOLE BODY?

Controlling Delivery

In some cases, if something is missing that is used by every cell in the body, we would like to be able to carry out a treatment that will restore the gene throughout the body. But in some cases, even if most or all cells make a gene product, we may be able to get away with putting the gene back selectively into some place like circulating blood cells or the liver and find ourselves solving the problem without having to treat every cell.

In many cases, we would actually prefer to avoid treating the whole body if we can, partly to help limit the immune reactions going on, partly because treating fewer cells means less risk of rare side effects, and partly because in some cases there will be cell types in the body that actually need to not be expressing the gene we are trying to get into one specific organ or cell type. Even for genes that are expressed throughout the body, disease resulting from a defect is often specific to a few organs or even one specific cell type. So we would prefer to limit the gene therapy agent very specifically to just the cells we want to treat.

One way to limit which cells end up with the gene therapy agent involves the selection of the type of gene therapy vector. Some vectors will treat only actively growing cells, whereas others will treat cells in any state of growth. Some vectors are derived from viruses that already have some specificity in terms of which cells in the human body they prefer, such as viruses that preferentially infect cells of the central nervous system. If we were wanting to treat the eye, we would want to ask whether we could build a vector from a virus known to infect the eye. If we wanted to treat cystic fibrosis, we would want to build our vector from a virus that infects lung cells. Now, in most cases, we do not have the luxury of starting with viruses that show absolute specificity for just the cell we want to target, but we can again do a least a bit of limiting where our treatment goes, depending on the vector we select.

Controlling Expression

If we include a promoter region in our construct, we can further limit the localization of expression beyond what was accomplished by the gene delivery process. So far, in studies of transgenic animals, all too often a promoter region placed artificially into a cell does not grant a pattern of gene expression identical to the natural pattern usually directed by that promoter. The promoter will give very specific expression in just one cell type when present in its natural location on the chromosome but the transgenic version of the promoter will not give expression in all cells of that type, and it may also give some expression in other cells when present as part of an external construct added to the cells. This may be happening in part because the *endogenous promoter* (the one that was there in the first place) is affected by other regional things, such as the structure of the chromosome in the local region, existing methylation pattern, or other sequences present at some distance from the promoter such as enhancer sequences. Thus, although use of a promoter specific to a rod cell may allow us to get something expressed in some of the rod cells, we do not yet have a way to exactly mimic the natural pattern of expression for that gene.

One strategy for treating only the cells you want to treat is to remove the target cells from the body, treat them in culture, and then return them to the body once they are fixed. This can be done with blood cells if you just need to end up with some treated cells and do not need to fix every single cell. However, if you need to treat every cell in the liver, this approach will not work.

Controlling Immune Reactions

Another strategy for limiting delivery is to deliver into a localized region. In treating the liver, some efforts to limit delivery involve injecting into vessels that feed directly into the liver, but this still results in some of the gene therapy agent ending up in other parts of the body. In treating bone, the clever use of a gel to hold the gene therapy agent in a localized position seems to help.

The eye and brain are expected to be good targets for gene therapy because there are some ways in which they are isolated from the rest of the body. The normal immune surveillance experienced by most of the body does not extend to the eye and brain. Thus some kinds of immune reactions that eliminate the gene therapy agent

or kill the treated cells elsewhere in the body can potentially be avoided for eye and brain. On the other hand, it is a well-known phenomenon that the eye can end up being attacked by the immune system if it attracts too much attention from the immune system, so testing of gene therapy approaches to the eye and the brain have to be explored very carefully.

14.10 WHAT ARE THE BIGGEST PROBLEMS WITH GENE THERAPY?

The RPE65 gene therapy story so far looks like a big success, and the things that have helped it to succeed make it look like similar strategies for treatment of other forms of retinal degeneration might also expect such happy outcomes.

Immune Surveillance

One of the distressing early findings in many gene therapy efforts was that, in many cases, positive results from treatment ended up being transient. In cases in which a repeat effort at treatment was tried, often the result the next time was much reduced or even nonexistent. This turns out to be the result of the action of the immune system that normally protects us from infection by bacteria and viruses. The immune system is very good at rapidly mounting a defense against such an infection. In many cases, the mechanism for getting the gene into the cell is an altered virus that can carry the gene into the cell. The use of cloning technology has allowed researchers to create gene therapy vectors that are derived from viruses that normally infect human cells but that have had the genes removed that make the virus able to cause disease. In place of the removed genes, the researchers place the gene that is due to be introduced into the cells. However, the protein coat that protects the viral DNA as it moves through the bloodstream and into the cell is the same protein coat that normally stimulates your *immune system* to attack the virus and keep you from becoming ill. And our bodies have evolved to be very efficient at recognizing and attacking infectious agents such as viruses.

Researchers have worked to change those viral coat proteins to make them less visible to the immune system, but the ability of the body to eliminate viruses is rather amazing. The first time the gene therapy agent is administered, the viral particles avoid being eliminated but stimulate the beginnings of an immune response. If expression of the introduced gene drops off over the course of six months, the body is effectively well immunized against that virus by the time another attempt at treatment is made. The next time the same gene therapy construct is injected, the ability of the immune system to remove the virus may be so effective that none of the constructs will ever reach the cells that need to be treated.

The immune system may also recognize treated cells as foreign, which would result in the body trying to destroy the treated cells. This has turned out to be a problem in the treatment of Duchenne muscular dystrophy. During the 1990s, as frantic parents were asking how they could get their children into gene therapy trials, saying that they would be willing to do even very risky things rather than just sit and watch their children die, it was not possible to move ahead with trying gene therapy on the children because of concerns that the treatment for this particular trait not only would not cure them but actually might make them worse if the immune system were to attack the treated cells. Researchers have been working at changing the gene therapy vectors to make them less likely to induce an immune response. There are now vectors that have fewer problems with creating an immune reaction, and progress has resumed now that researchers have developed a mini-version of the DMD gene that could fit into the vectors that have serious size limitations.

A very different issue is the problem of what happens to the human genome when a new gene is put in from the outside in such a way that the transgene integrates into chromosomal DNA. If the gene integrates into a region of *junk DNA* between genes, it might have little effect other than curing the cell's metabolic defect. However, if the transgene integrates into a gene in the chromosome and disrupts it, the consequences will depend on which gene gets disrupted. If it disrupts one copy of a gene encoding an enzyme involved in metabolism, it may have little effect or perhaps at the worst it will kill that one single cell.

However, if the gene therapy agent is delivered *in vivo* into a large number of existing cells in a human organ, each cell becomes a separate integration event. So even if a large number of cells receive transgenes that integrate safely between genes, it would take only one transgene integration into certain kinds of cancer genes to cause a problem. In the long run, optimal design of gene therapy will need to gain the ability to control where the transgene integrates, or at least to prevent certain kinds of integration events. After the initial tragic events when a seemingly successful ADA gene therapy trial turned up with several cases of leukemia, researchers have figured out why this event happened and have developed an alternative vector that does not cause this problem. Thus in spite of some early set-backs, the field is continuing to advance in overcoming the worst barriers to progress.

Amidst the problems with immune responses and transient expression, the RPE65 treatment that used a viral vector has produced effective results that required only one round of treatment. There were no problems with an immune response. No problems with transient expression. Why did the RPE65 trial not fail like so many of the others? Why did it not elicit an immune reaction against the delivery system?

Many gene therapy programs are succeeding in pushing past these issues. Some groups are working to improve the vectors. Some groups are switching to different vectors. And some groups are developing and using delivery systems that completely bypass the problem of using a virus at all. Use of liposomes and nanotechnology dendrimers offers promising alternatives that may some day render the use of viruses unnecessary.

And yet the RPE65 trials have been succeeding while using viral vectors. So we have to ask, is there something different about gene therapy in the eye? The key to the success of the RPE65 gene therapy efforts may lie in the unique properties of the eye and the central nervous system. The eye, like the brain, is not subject to immune surveillance, the process by which the body monitors and protects most of the other organ systems of the body. Each is protected against some of the problems that have plagued other gene therapy projects because the immune system does not handle the brain and eye in the same way it handles the rest of the body.

The fact that the RPE65 trials seem to be working to provide functional correction of the problem and persistent expression of the genes actually suggests to us that a lot of different ocular traits might be amenable to gene therapy that has not yet paid off systemically.

14.11 SO, WHOM DO WE TREAT?

Which Traits Do We Treat?

Many may well wonder why their particular trait does not have a gene therapy clinical trial taking place. They may well wonder who is getting treated if they are not. There are a lot of very serious problems out there that all need to be solved, and many of them do not yet have gene therapy efforts going on. So if we are not developing therapies for some of those problems, then whom do we treat? The answer is a rather pragmatic one. You would think it would be simple – solve the most severe things first, solve the

things that affect the most people first. But the real answer is not that simple.

The real answer is that a variety of factors go into determining which of the problems are tackled first, factors that can be determined by figuring out how many of the following questions can be answered with the word "yes":

- Has the gene been found?
- Is the trait genetically simple?
- Is the trait free of environmental complications?
- Is the trait severe?
- Is the underlying pathology understood?
- Do we have an available animal model for preliminary studies?
- Do we have a cell culture model and biochemical assays?
- Do we know which cell types we want to treat?
- Can we limit treatment to avoid tissues that need to be left untouched by therapy?
- Is the gene small enough to fit into existing delivery systems?
- Do we have some leeway on dosage if expression levels vary?
- Do we have a clever new idea that seems like it would apply to this particular situation?
- Have we learned things from other past studies that would make this one easier?
- Are the cells we need to treat still alive in the target population?
- Do we expect the treatment to be free of unsafe complications?

Clearly there is no simple formula for putting together all of these factors. One of the important factors is that other intangible thing – where do new ideas arise? In some cases a trait may be highly meritorious as a target for gene therapy according to the above list, but if the people working on it have not been able to come up with a good set of strategies for how to go about it, then the research is not feasible, no matter how important the problem. In other cases, something may not be the most severe trait, but it may be the ideal trait for trying out some brave new idea in how to get gene therapy to work, to develop new methods that would apply to a lot of other traits in need of treatment.

So what it amounts to is this: is the available combination of genes and strategies one that can make gene therapy development feasible? Some of the most desperate cases may not have gene therapy development going on because something about the needed therapy is not yet feasible. In some cases, traits that seem less terrible in their consequences may have ongoing gene therapy development because they seem as if they would be much easier situations to treat or would teach us something important that would move the overall field of gene therapy forward. By working on these more feasible cases, advances in gene therapy take place, teaching us important things that we need to know to be able to tackle some of the more difficult problems. And no matter how terrible a trait, and no matter how desperately everyone would like to see it cured, if it is too complex in its origins, if we do not know enough about the causes, we may not yet be able to develop gene therapy for it.

Who Are the Study Subjects?

One of the other key issues deals with the selection of specific individuals to participate in gene therapy trials. In some cases, the inclusion and exclusion criteria for a study may include only some stages of a disease. This can result in people who are excluded and don't understand why they can't join the study. In some cases, they may be excluded because the therapy that is currently feasible is not expected to work on their stage of the disease. In other cases, their stage of the disease may be considered to be at much higher risk of potential hazards of the study. Once again, the determining factors are often quite pragmatic. In the treatment of Huntington disease, one might imagine that the

most advanced cases might offer the most compelling arguments for treatment, as well as the greatest opportunity to demonstrate gains from the therapy. However, when we look at the disease pathology we see that cells in the brain are dying, and quite frankly if the basis of the treatment is to put a neuroprotective gene into brain cells to help keep them alive, it is simply not going to work in cases where those cells are no longer there to be protected. Other strategies aimed at getting cells to grow and regenerate might work well in that same case, but that is irrelevant if the gene therapy trial you are wanting to join requires that you still have cells that you no longer have. So often simple issues of what can and cannot be made to work will over-ride the seemingly dominant issue of who most needs the treatment.

In the first round of a clinical trial, when a small number of individuals are tested to determine whether the treatment is safe and perhaps to pin down the appropriate dosage, there are questions about who is most appropriate to treat. To many of us, it seems obvious that those with the most to lose without treatment and the most to gain from treatment would logically be the ones to take the risks in these early tests of safety. In a gene therapy study aimed at treating OTC deficiency, a bioethicist ruled that the most appropriate participants would not be infants at high risk of dying of OTC deficiency. Some might think it appropriate that those with the most to gain (or lose) would be the ones to take the largest risks. Instead it was decided that the pressures that the child's desperate health status place on the parents to put the child into the study, combined with the inability of the child to decide for himself if he is willing to be a study subject, seemed to make it ethically unacceptable to include these children in the first gene therapy tests. Why? Because the consent to participate in the study would be considered to have been given under undue pressure. To some on the outside of the study, this seems surprising. Anyone participating in such studies is under great pressure to participate because of their health status, and anyone watching from the outside would wonder at how this supposed ethical dilemma is balanced against the ethical dilemma of expending a potentially lifesaving treatment on some unaffected individual who cannot benefit instead of offering it to an incredibly ill child who could potentially be saved if the therapy turned out to work.

Clearly, the complex situation in which a patient dying of cancer agrees to a treatment becomes incredibly more complex when the decision is being made by parents if the child cannot decide for herself. However, on some levels the issue is the same and the reasons in favor of participating are the same. A whole field of bioethics has grown to include very active consideration of very complicated situations such as these, and each new trait and treatment protocol seems to raise new questions about how to walk the fine line between treatment risk and disease risk, between informed consent and undue pressure to participate, or between death from non-intervention and the risk of death if there are unforeseen consequences of the intervention.

Where To From Here?

There are those who sometimes discuss the idea of treating the germline, going beyond the individual treatments that are now being tried; they propose making changes that could pass along to the next generation so that we do not have to keep re-treating each new family member. However, treating the germline is not currently on the horizon. Any manipulation of the germline has the potential to reach far beyond the health of any one individual to impact the human population, and the technical problems of messing with the germline substantially exceed any problems we have presented here. Any delving into artificially directed "evolution" of the human genome calls for vast wisdom and ethical insights that are still being developed. So for now, the field is focused on the most

immediate issue, finding somatic cures for individuals without touching the germline in hopes of moving beyond research to use in real medical settings.

Research is the first key to solving the problem. For gene therapy to arrive in your local doctors' offices, much work remains on the part of people with many different kinds of expertise. Some of the smartest people in the world are working on the development of these technologies. Geneticists go after the right genes to use. Biochemists characterize the gene products and sort out the pathways. Molecular biologists design constructs that bring together human and viral DNA. Nanotechnology researchers are developing coated delivery systems. Stem cell researchers work to develop the ideal cells for use in bioengineering. Cell culture workers and animal model researchers test out preliminary ideas to pioneer new approaches and identify where improvements are needed. Virologists work to develop the vector systems for delivery of the genes. Immunologists study immune responses against the vectors. Biostatisticians evaluate the outcomes to help us tell whether something has actually worked, and help tell us how many subjects are needed in a study to be able to get a meaningful answer. Doctors work to improve systems for delivery of treatments and for monitoring the health status of treated individuals. And gradually, like a building being erected, the many pieces of the treatment puzzle are coming together towards a finished product.

But the other key to the whole process of developing gene therapy is the patients themselves, an often-unmentioned group who seem to us to be the real heroes in this story. The gene therapy story is about them, and the answers we seek are for the benefit of the many who cannot currently be helped by traditional medicine. Through a partnership of the patients who need the cures and the researchers developing the cures, eventually we will arrive at that seemingly magical moment when babies born with a terminal illness can be treated and sent home to grow up along with the other children who were born healthy, just as Jesse Gelsinger wished.

> Will you make me some magic with your own two hands?
> Could you build an emerald city with these grains of sand? ***—Jim Steinman***

Study Questions

1. What is gene therapy?
2. What is adenosine deaminase (ADA) deficiency?
3. What are the pros and cons of AAV versus AV gene therapy vectors?
4. What is nanotechnology and how may this be useful in gene therapy?
5. How can miRNA or siRNAs be useful for alternative gene therapy approaches?
6. What major problem with gene therapy does not happen when gene therapy is delivered to the eye, and why is gene therapy in the eye different?
7. Why is integration in gene therapy a concern?
8. Who should be involved in a gene therapy trial?
9. What are two different strategies for using gene therapy to treat cancer?
10. What are two gene therapy strategies other than simple replacement of a missing gene?
11. Why was RPE65 a good candidate as a target for gene therapy?
12. Why was the treatment of the Briard dog Lancelot of importance to humans?
13. Which trait was the first human trait on which gene therapy was tried, and why was it the trait selected?
14. What went wrong with the ADA gene therapy trial and why have efforts to treat ADA continued?
15. What causes damage in OTC deficiency?
16. What kind of "end-run" approach to gene therapy can be used to assist healing following a heart attack?

17. What good does it do for gene therapy to add in more of something that is already present in the body?
18. How can gene therapy help heal a bad break in a bone?
19. Why are the strategies for gene therapy of cancer so different from the strategies for treatment of metabolic disorders?
20. How else can a cloned gene help us treat a disorder if we do not use it for gene therapy?

Short Essays

1. In humans, some aspects of visual function in the brain develop through use of the visual system. As scientists consider how to treat color blindness there has been concern that putting the missing gene back into the cells of color blind men would not be enough because their brains would not have the capability to use the information. How has our understanding of this situation been informed by the recent gene therapy treatment of color blind monkeys? As you consider this question please read "Monkey see monkey juice" by Evan Lerner in *Seed Magazine*, September 18, 2009.
2. Researchers carrying out gene therapy for severe combined immunodeficiency (SCID) found that the gene therapy construct that they used caused an acute T cell leukemia in some of the study subjects. They thought the leukemia was the result of a very specific combination of genes involved in the vector and the chromosomal integration event. However, another effort at gene therapy for chronic granulomatous disease (CGD) using a different, but related, vector led researchers to think that they had accidentally generated a model for human T cell leukemia. What did the researchers learn and what did it tell them about gene therapy vector design? As you consider this question please read "Gene therapy activates EVI1, destabilizes chromosomes" by Cynthia E. Dunbar and Andre Larochelle in *Nature Medicine*, 2010;16:163–5.
3. Hematopoietic stem cells are the precursors to the various cell types found in human blood. The HIV virus infects one of the differentiated blood cell types, the CD4+ T cells, but CD4+ T cells that carry a mutant form of the CCR5 receptor protein are resistant to HIV infection. How can gene therapy be used to fight HIV in infected individuals, and why is it not enough to treat the CD4+ T cells? Why will it be difficult to turn this approach into something that can be easily applied to large populations of infected individuals? As you consider these questions please read "Can HIV be cured with stem cell therapy?" by Steven G. Deeks and Joseph M. McCune in *Nature Biotechnology*, 2010;28:807–10.
4. Since RNA interference was first discovered in the worm *Caenorhabditis elegans* researchers have tried applying it to many different problems in human biology. Why does RNA interference turn out to be especially appropriate for some of the dominant neurodegenerative diseases that result from simple sequence repeat expansions? As you consider this question please read "Allele-specific RNA interference for neurological disease" by Edgardo Rodriguez-Lebron and Henry L. Paulson in *Gene Therapy*, 2006;13:576–81.

Resource Project

There are a variety of resources that let us check on what is happening with clinical trials in the US and elsewhere in the world. Go to the Gene Therapy Net website and look at the Clinical Trials Databases section. Check on what is happening with gene therapy for cystic fibrosis in the US and two other countries and write a brief essay comparing what is happening with gene therapy for this trait in these three countries.

Suggested Reading

Articles

"Making a play at regrowing hearts" by Kenneth Chien in *The Scientist*, 2006;20(8):34.

"Whither gene therapy? Success has been mingled with failure; a few technical modifications could make the method safer" by Alain Fischer and Marian Cavazzana-Calvo in *The Scientist*, 2007;20:36.

"Kenyon's ageless quest: A San Francisco scientist's genetic research renews the ancient hope for a way to slow aging" by Stephen S. Hall in *Smithsonian Magazine*, March 2004 (Available at www.smithsonianmag.com/science-nature/quest.html).

"Egg sharing in return for subsidized fertility treatment – ethical challenges and pitfalls" by Boon Chin Heng in *Journal of Assisted Reproductive Genetics*, 2008;25:159–61.

"Gene therapy may switch off Huntington's" by Bob Holmes at NewScientist.com, March 13, 2003 (www.newscientist.com/article/dn3493-gene-therapy-may-switch-off-huntingtons.html).

"To build a killing machine: David Kirn can't turn his back on a century-old quest to pit oncolytic viruses against tumors" by Andrew Holtz in *The Scientist*, 2007;21(5):48.

"Gene therapy in a new light: A husband-and-wife team's experimental genetic treatment for blindness is renewing hopes for a controversial field of medicine" by Jocelyn Kaiser in *Smithsonian Magazine*, January 2009 (www.smithsonianmag.com/science-nature/Gene-Therapy-in-a-New-Light.html).

"Latest developments in viral vectors for gene therapy" by Kenneth Lundstrom in *Trends in Biotechnology*, 2003;21:117–22.

"Interspecies SCNT-derived human embryos – a new way forward for genetic medicine" by Stephen Minger in *Regenerative Medicine*, 2007;2:103–6.

"Gene therapy to improve the body's ability to eliminate cancer" by R. A. Morgan, M. E. Dudley, J. R. Wunderlich *et al.* in *Science* 2008;314(5796):126–9.

"Re-engineering humans" by S. Jay Olshansky, Robert N. Butler, and Bruce A. Carnes in *The Scientist*, 2007; 21(3):28.

"Subtle gene therapy tackles blood disorder" by Danny Penman at NewScientist.com, October 11, 2002 (www.newscientist.com/article/dn2915-subtle-gene-therapy-tackles-blood-disorder.html).

"The return of the phage: As deadly bacteria increasingly resist antibiotics, researchers try to improve a World War I era weapon" by Julie Wakefield in *Smithsonian Magazine*, October 2000 (available at www.smithsonianmag.com/science-nature/phenom_oct00.html).

"DNA nanoballs boost gene therapy" by Sylvia Pagán Westphal at NewScientist.com, May 12, 2002 (www.newscientist.com/article/dn2257-dna-nanoballs-boost-gene-therapy.html).