from the cytotoxic drugs and confer a selective advantage to their growth. The result would be a hematopoietic system better able to withstand the onslaught of high-dose chemotherapy.

In their current clinical study, Cowan and colleagues transduce half the cells with the MDR1 gene and half with a neomycin resistance gene as a control vector. Patients then undergo four cycles of paclitaxel and four of Adriamycin.

All “Marked”

All six patients treated so far “marked” with MDR1, but only three maintained the marking throughout their chemotherapy. The signal from the MDR1 vector surpassed that of the neomycin vector — a good sign that the selection process is working, Cowan said. But the falloff in MDR1 marking in half the patients, he said, reveals a major hurdle that remains: the selection for gene transfer of a pure batch of pluripotent stem cells, which make up a tiny fraction of circulating cells and are capable of giving rise to all types of blood cells.

The most specific selection researchers can now make is for cells expressing the CD34 antigen, resulting in a mixed bag that includes not only true stem cells but committed progenitor cells as well. Unlike stem cells, these latter cells have a limited half-life and are destined to die after several rounds of replication. For the gene transfer technique to work, the gene must get into enough true stem cells to keep the lineage proliferating.

Until this can be done reliably, the technique is hit-or-miss. Stem cells are notoriously slow growing, Cowan added, and the researchers are searching for the perfect cytokine cocktail to encourage their growth.

“One of the caveats is that the results in mice are much better so far than the results in humans, as far as hematopoietic reconstitution of gene-marked cells,” he said.

But if the technique can be perfected, adding the protective effect of the MDR1 gene might be just the beginning of its utility. Other cancer-fighting genes might be able to tag along.

Cowan envisions three potential benefits for MDR1 gene transfer: “You could give the same amount of chemotherapy with much less toxicity. You could give higher doses of chemotherapy for a longer period of time, which might improve response rates.”

“Third, and probably even more significant, you could use MDR1 as a selectable marker in order to transfer yet another gene into hematopoietic cells. For example, say you had a specific T-cell receptor targeted to a tumor antigen, and you wanted to recruit cells that would target that tumor antigen. You could engraft patients with this T-cell receptor, but it wouldn’t necessarily stay around.”

“Passenger” It

“So you could ‘passenger’ it with the MDR1 gene, select the cells in the patient, and make sure these cells stayed around because they would be protected from the anticancer drugs.”

This technique has been used successfully in tissue culture and animal models, Cowan added. The two approaches to using the MDR1 gene — modulating the gene in tumor cells and transfecting it into normal cells — are incompatible with each other if a normal gene is used, Sikic said, because the modulator would abolish the survival advantage for the normal cells.

— Tom Reynolds

R. Michael Blaese: Still Blazing a Path Of His Own

Reports of his imminent departure, it seems, have been greatly exaggerated. Nearly 7 months after being named chief scientific officer and president of the Molecular Pharmaceuticals Division of a small Pennsylvania biotech company, R. Michael Blaese, M.D. — one of the world’s leading gene therapists — is still on the National Institutes of Health campus, still a federal employee, and still ensconced in his scenic 10th floor office at the NIH clinical center.

But Blaese, chief of the Clinical Gene Therapy Branch at the National Human Genome Research Institute, according to his own “working plan,” will move over to Kimeragen, Inc., in Newtown, Pa., in the next few months, while staying involved in clinical protocols at the NIH for at least another year.

Strong Ties

Then who knows? Something in his manner suggests that after 32 years at the NIH, the ties to his many patients, their families, and to the NIH scientific community, are far too strong to sever completely. “I’ve got the best job in the world right here. . . . I really do,” he says, gesturing to “this fabulous place” out a large office picture window in a recent interview. “And I don’t have any particular reason to want to leave except this is something that has me so excited, I really wanted to push it.”

What so excites Blaese is Kimeragen’s technology, a technology based on small molecules known as oligonucleotides, often called “oligos,” that work together with a cell’s own DNA repair mechanisms to correct misspellings of
genes that cause disease. Because these short pieces of synthetic DNA are designed to be complementary to a gene’s messenger RNA — fitting much like a zipper — they can be targeted to specific errors in gene expression including that of a single base pair mutation. It is that specificity, and oligos’ smaller size compared to traditional gene vectors, that Blaese believes gives them “the potential of really making an impact on the lives of thousands of thousands of patients” with genetic diseases.

Totally Seduced

“I am totally seduced by the technology,” he readily admits. After having struggled through the “wars of gene therapy” for the last decade and a half, and understanding “what’s out there, what’s likely to be available, and what the limitations are,” Blaese says, “I go back to my roots as a pediatrician taking care of disease for my entire career and realize that what’s on the horizon is not going to fix them . . . not going to be very useful.”

One reason for his skepticism is that for traditional gene therapy to fix a genetic disease such as sickle cell anemia, whose cause lies in a single nucleotide error, it would be necessary to use a whole gene, “but you can’t put in the whole gene because we don’t have vectors that are big enough.” So a copy of the coding region of the gene (i.e., a DNA sequence complementary to the gene’s messenger RNA) would have to suffice.

But by using a gene copy, according to Blaese, important regulatory regions are lost and “you are delivering tens of thousands of base pairs when all you need to do is fix one nucleotide.” The resulting delivery problems, he says, are like “enormous delivery vans that are trying to get down a narrow street in Italy — It doesn’t work.”

Dr. R. Michael Blaese

Although delivery problems also plague oligos, Blaese admits, he seems confident that this problem, along with the need to bypass or overcome a cell’s defense mechanisms to prevent enzymatic degradation, can be solved. An oligonucleotide, he says, is much more “like a small molecule drug than it is like a biologic” — making delivery issues, at least, far simpler.

ADA Deficiency

One genetic disease, however, that Blaese feels may eventually be fixed by traditional gene therapy is ADA deficiency, an illness which destroys the body’s ability to fight off ordinarily harmless infections, and whose name is inextricably linked to Blaese’s own.

On September 14, 1990, at the NIH, Blaese, along with two former NIH researchers W. French Anderson, M.D., and Kenneth Culver, M.D., ushered in a new era of gene therapy when Ashanthi DeSilva, then 4 years old, received a transfusion of her own white blood cells (T cells), which had been genetically altered to carry a healthy ADA gene. Several months later, a second Ohio girl, 9-year-old Cindy Cutshall, also suffering from ADA, underwent the same procedure.

Blaese’s belief that the ADA defect may yield to these early genetic manipulations is linked in part to how well both girls appear to be doing 8 years after their initial gene treatments, and in part to the nature of their genetic defect, which lends itself to symptomatic improvement even if only small amounts of the missing adenosine deaminase enzyme can be produced.

pegADA

In fact, because both girls — and subsequently three infants who were also genetically treated for ADA (see sidebar) — have remained on a synthetic enzyme, known as pegADA, some critics have contended that the true value of gene therapy may never be known. But Blaese counters that the
theoretical basis for gene therapy — of using peripheral T cells” did not lend itself to stopping [pegADA] “unless you get absolutely huge numbers of ADA, which we didn’t anticipate we could get.

“There was never really a plan to stop it [the pegADA],” he adds, “and that’s why we haven’t.”

Although both girls appear to be leading normal lives, their immunologic responses to the gene treatments have been quite different, says Blaese, and for many years investigators could not figure out why. Ashi, now 12, has not been treated in 6 years — except for the pegADA — and 50% of her circulating T cells are still positive for the ADA gene 8 years out from her initial gene treatment. In comparison, Cindy, a high school senior “looking at colleges,” has circulating T-cell levels that test positive for ADA in the range of only 2% to 4%, says Blaese — “even though she got treated the same number of times, with the same number of cells.”

Puzzled

Researchers puzzled over this discrepancy in immunologic profiles between the two girls for nearly 5 years, and “all sorts of things” were studied, according to Blaese. “But, ultimately, we discovered, and I think convincingly demonstrated, that what happened to Cindy is — because she had a much milder defect [than Ashi] and the ability to make some antibodies before we ever gave her the gene therapy — she actually became immune to fetal calf serum,” the mixture in which retroviral vectors are often cultured and grown prior to using them to ferry new [corrective] genes inside cells.

Specifically, a lipoprotein in the calf serum triggered an immune response. “So when Cindy was getting her own T cells [reinfused],” Blaese notes, “she now had a new antigen on their membranes and she had enough immunity to respond to that and make an antibody to this new component on the membrane of her cells.”

“Then when she had a next treatment and she had an antibody, she just basically cleared the cells as quickly as they were infused.”

Unexpected Snag

The resulting spikes in immune response meant there was initially some effect [of the gene therapy], more effect, and “then nothing,” says Blaese. This unexpected snag, which later derailed attempts to genetically alter her stem cells, arose in spite of efforts to wash the cells extensively — more than 500 volumes of liquid were used — prior to reinfusion. What happened, Blaese says, is the lipoprotein in the calf serum actually became attached to her cells and it wouldn’t wash out — requiring a new way of doing things. “We’ve had to do a lot of dancing to try and get the retrovirus produced so we don’t have to have bovine serum there.”

Now that the problem is understood, he says, it can be avoided.

Meanwhile, for Ashi, there are no more plans for T-cell therapy, although Blaese and his colleagues expect to attempt a blood stem cell correction, using gene therapy, within the next 6 months. Because stem cells, which originate in the bone marrow, give rise to all blood cells, a successful insertion of a healthy ADA gene into these cells in theory would provide a cure.

It is clear that the decision to attempt to genetically alter her stem cells is a preemptive strike against new pathogens...
that may arise unexpectedly. Although she is capable of mounting an immune response, the range of her response is considered limited because of the high level of genetically corrected T cells still detected in her blood even though she has not had a gene treatment since 1992.

“What this tells you,” said Blaese, “is that there are no new cells entering the pool, or you would dilute out these cells. But since the number of cells remains relatively constant, you don’t have the opportunity to introduce a new repertoire of T cells... which is why we haven’t stopped pegADA. She needs to be able to respond if she is exposed to something new.”

For the impending procedure, there are plans to use a second generation vector, which Blaese estimates is 30 to 50 times more active than its predecessor, and which presumably will allow greater efficiency in inserting the ADA gene into Ashi’s stem cells. Additionally, a different cocktail of cytokines will be used to induce better cell growth.

“One of the real difficulties is you don’t get a very good concordance between what you see in animal models and what you see in human beings,” said Blaese. “So in a way it comes down to — almost as it did in the beginning... Eventually, you have to do it [gene therapy] in man to see what’s going to happen.”

— Susan Jenks

### Gene Therapy Infants Faring Well

As the principal investigator of a stem cell study in infants, R. Michael Blaese, M.D., had tried to keep a lid on the public attention surrounding the novel gene therapy.

But upon arriving at Children’s Hospital Los Angeles, where two of the three infants were undergoing the procedure for a rare, inherited immune disorder known as ADA, Blaese had to wade through a sea of cars and TV cameras. There was no more excitement — he later said — than on the day actor Clint Eastwood was elected mayor of Carmel.

Some 5 years after their initial therapy, Blaese has somehow managed to protect the privacy of the infants and their families far better. But the infants’ clinical course is public, and a partial follow-up of their medical status appears in July’s Nature Medicine. All three children, one of whom was treated at the National Institutes of Health, are doing fairly well, he says, but the system — inserting the ADA gene into stem cells harvested from the infants’ cord blood — is far from perfected.

One baby did come off enzyme replacement therapy, pegADA for 2 months, but was quickly put back on the drug when there was a suggestion that his clinical condition might be worsening because of weight loss and possible thrush, according to Blaese. His immunologic profile was mixed as well. While off the enzyme, the baby’s T-cell counts stayed level and remained functional, but an anti-tetanus subset of these cells lost their ability to respond until the child was placed back on the drug, said Blaese.

What was more surprising, however, is what happened to natural killer cells and antibody-producing B cells, which disappeared rapidly in the infant when pegADA was stopped. This suggests, says Blaese, that even though the ADA gene was transduced successfully into the T cells, the granulocytes, and the monocytes, that did not happen for the other immune system cells.

“We still don’t know exactly what’s happening,” he admits. “But we believe and these experiments clearly show that the T cells are the cells that need to get the [ADA] gene for sure and they do have a selective advantage,” which means that genetically corrected T cells survive longer than those without the correction.

By itself, though, that does not appear to be enough to alter the course of ADA, which without any treatment results in death in the first years of life. That finding is “not terribly surprising for a first go-round,” says Blaese, who thinks future attempts to alter stem cells genetically hold greater promise because of a new, more active vector and cytokines that may better preserve the function of B cells.

— Susan Jenks