

## STORIES OF DISCOVERY

### **Statins: They're not Just for High Cholesterol Anymore**

In early 2001, approximately 13 million Americans were taking statin drugs to lower their cholesterol levels. That number is expected to more than double as physicians learn of recommendations in the recently released “Third Report of the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults” (also known as “Adult Treatment Panel [ATP] III”) from the NIH National Cholesterol Education Program (NCEP). The recommendations convey a new sense of urgency about lowering cholesterol levels with an increased reliance on drug therapy, and that means an increased reliance on statins. Statins now seem to have potential for preventing or treating a much wider variety of conditions, even ones apparently unrelated to heart disease.

Statins were used initially to treat a rare genetic disease called familial hypercholesterolemia (FH). FH results in extremely high circulating cholesterol levels and its victims typically suffer heart attacks in childhood or early adulthood. NIH-supported researchers Michael Brown and Joseph Goldstein discovered that the cells from patients with severe forms of FH could not remove low density lipoproteins (LDL, or “bad,” cholesterol) from the blood because they lacked a protein, the LDL receptor. This discovery, which earned the researchers the Nobel Prize in Medicine, clarified how cholesterol is metabolized, prompting Brown and Goldstein to hypothesize that the same statin drugs with which they were treating FH patients could be used to lower cholesterol in persons who did not have the FH genetic defect. Once their lifesaving potential was recognized, physicians began prescribing statins for prevention of coronary heart disease in millions of Americans with high cholesterol levels.

Subsequent evidence has established additional heart-related benefits of statins. They reduce the risk for first heart attacks in people with *average* cholesterol levels, prevent recurrence of heart attacks in patients regardless of their cholesterol levels, and reduce the likelihood that heart attack patients will need bypass surgery or angioplasty.

Recent results indicate that statins also may help to prevent diabetes. Although the results are barely statistically meaningful, there is a plausible mechanism to explain this protective effect: statins reduce circulating levels of proteins associated with insulin resistance. The same proteins are associated with inflammation, and the evidence that statins have anti-inflammatory properties is quite strong. Their ability to reduce inflammation contributes not only to protection against heart attacks, but also against stroke. Moreover, by altering inflammatory responses, statins significantly improve survival of heart transplant patients.

Recently, statins have been implicated in reducing risk of dementia associated with Alzheimer's disease, which also is thought to be exacerbated by inflammation. However, the protection may be mediated by additional, previously undiscovered, effects of statins – reduction of levels of B-amyloid peptides A-beta 42 and A-beta 40 in the brains of patients with Alzheimer's disease.

Statins may be useful in treating or preventing other diseases. Researchers investigating bone formation noted that statins alter the same metabolic pathway that is affected by a class of drugs commonly used to treat/prevent osteoporosis. Statins stimulate bone formation in rodents, and researchers have observed that statin use among older persons is associated with a diminished risk of fractures.

And so, research continues. Statins have emerged from their humble beginnings as a treatment for young patients with a rare disease to a prophylactic therapy for one of the leading causes of morbidity and mortality. Even if the tantalizing potential for prevention of diabetes, Alzheimer's disease, or osteoporosis fails to materialize, the millions of Americans for whom statins prevent heart disease and strokes, recurrence of heart attacks, and the need for bypass surgery would not be wrong if they considered statins to be "miracle drugs."

## **Enhancing Treatment Adherence in AIDS and Schizophrenia**

AIDS and schizophrenia are extremely destructive disorders that until recently elicited fear and fatalism in equal measure. Both, however, have been transformed by increasingly effective treatments over the past decade. Cures remain elusive, but properly administered treatment can vastly improve the quality and duration of life. To fully realize the public health benefits of treatment, however, patients need to be highly engaged in their care and motivated to keep taking medications that significantly interfere with their perceived quality of life. They also must participate in psychosocial therapies over long periods, especially when symptoms lessen. Moreover, treatment regimens must be designed to reach disenfranchised populations, including the urban poor. Communities lacking resources and access to high-quality care have suffered the greatest toll from mismanagement of severe mental illness, and continue to be disproportionately affected in the AIDS pandemic.

The efficacy of antiretroviral drug therapy (ART) has dramatically altered the landscape of HIV treatment. Treatment can inhibit virus replication and reduce virus load to undetectable levels – with much improved clinical outcomes. But not all patients are willing or able to maintain complex medication regimes, and partial or poor adherence can lead to the resumption of rapid viral replication, poorer survival rates, and mutations to form treatment-resistant strains of HIV. The widespread transmission of resistant HIV strains is becoming an increasingly serious public health concern in the U.S. and throughout the world.

Through the combined efforts of NIH staff and extramural researchers, innovative interventions to strengthen HIV treatment adherence have rapidly evolved to meet the needs of the changing pandemic. Moreover, advances in the HIV/AIDS arena have paralleled increased attention to enhanced models for treatment adherence for severe mental illnesses, such as schizophrenia. Like their counterparts living with HIV, many people with schizophrenia face difficult life circumstances that impede their capacity to benefit from effective medical and psychosocial treatments. As a result, many individuals reject therapy outright, discontinue treatment prematurely, or selectively ignore components of prescribed treatment programs. Non-adherence heightens the likelihood of symptom relapse, collateral behavioral disability, and a downward spiral of chronic impairment.

Effective psychosocial interventions for HIV adherence have capitalized on factors related to the individual (motivation, self-efficacy, substance use, neuropsychiatric symptoms, treatment of co-occurring depression), characteristics of the treatment environment (access to care, therapists' attitudes and behavior, social reinforcement of change efforts), and features of the natural environment (family support, social networks, stigma related to mental illness and/or HIV/AIDS). For example, in the mid-1990s, NIH-funded researchers found that with a theoretically grounded behavioral intervention, adequate HIV treatment adherence levels could be reached even among the most difficult of populations: homeless men and women with severe mental illness and substance abuse. There had been debate at that time about whether protease inhibitors (PIs) should be given to people living in streets, shelters, and residential hotels because their residential instability may compromise adherence to treatment. Using three

biological and behavioral measures, investigators evaluated adherence in HIV-infected homeless and marginally housed people on PI therapy. Contrary to expectations, adherence was good, with 38 percent of the population having over 90 percent adherence. Moreover, adherence was strongly related to viral load; a 90 percent adherence rate was required to produce 60 percent undetectability of virus. These findings provided evidence to providers across the country that treatment decisions should not be based solely on age, ethnicity, or socioeconomic status. If the degree of behavioral, social, and systemic intervention matches the patients' needs for support, substantial barriers can be overcome.

Other NIH-supported researchers have examined how health literacy skill among inner-city medical patients affects HIV treatment adherence, and how interventions can bridge this gap to reduce disparities in health outcomes. They found that HIV-infected patients who miss taking at least one antiretroviral medication in a 2-day period have greater difficulty comprehending simple medical instructions than do people who are treatment adherent. Further, men and women with higher health literacy had significantly lower viral loads than their less literate counterparts. As AIDS continues to afflict those living in poverty, and as antiretroviral medications become increasingly available in the developing world, treatment and adherence interventions for people with limited literacy skills are a public health necessity. Behavioral intervention strategies that rely on pictographs and other non-verbal modalities are being developed and evaluated. These kinds of strategies also have enormous potential to compensate for the cognitive deficits found in schizophrenia.

Several research teams have collaborated on cross-cutting interventions grounded in principles of motivational interviewing – a directive, client-centered approach to counseling that can increase patients' motivation to adhere to treatment recommendations. Instead of simply telling patients to follow a prescribed treatment, this intervention attempts to elicit and reinforce their own reasons for adherence (such as avoiding rehospitalization). These techniques help both psychiatric and HIV-positive patients to recognize discrepancies between their stated goals and current problematic behaviors (e.g., non-adherence with aftercare). They also increase patients' confidence in their ability to remove barriers to adherence (e.g., arranging for medical transportation). A randomized trial was recently conducted to test how well motivational interviewing can increase attendance at aftercare appointments by patients with both psychiatric and substance use disorders. Among patients given standard discharge instructions plus a brief motivational intervention just prior to hospital discharge, the rate of attendance at initial appointments was more than twice that of patients given only standard discharge planning.

The entire clinical and research community has greatly benefitted from the redoubling of efforts in adherence that was fueled by urgency as ART medications proved highly successful. The conceptual and methodological advances were quickly adopted in cross-cutting efforts among persons with severe mental illness. Subsequent NIH-funded projects will continue to reach special populations with behaviors and environments that call for a broader understanding of 'adherence,' which captures a fuller range of desired outcomes. These projects will target integrated service delivery of both mental health and medical services, staff training, economic

and sociocultural barriers, and other structural factors that affect access and adherence to effective treatment of AIDS and schizophrenia.

## **A Brighter Future for People with Lupus as a Result of Medical Research**

When patients hear a diagnosis, the reaction is typically a mix of relief and anxiety. The relief comes as the symptoms they have been experiencing finally have a name. The anxiety comes as they explore the implications of the diagnosis and look to the future. Twenty-five years ago, women – and it usually is women – who were diagnosed with systemic lupus erythematosus (SLE), or lupus, faced a future that was uncertain, but clouded. Today, a patient with lupus faces an altogether different prognosis for a whole variety of reasons. The underpinning for all of those reasons is advances through medical research. The story of discovery in lupus is one of the most exciting stories from a patient perspective, as the following highlights illustrate.

Lupus is an autoimmune disease that can affect many parts of the body, including the joints, skin, kidneys, heart, lungs, blood vessels, and brain; it is a serious public health problem that mainly affects young women. The disease often starts between the ages of 15 and 44. Nine times more women than men have the disease. It is also three times more common in African-American women than in Caucasian women, and is more common in women of Hispanic, Asian, and Native American descent. African-American women tend to develop the disease at a younger age than Caucasian women and to develop more serious complications.

The primary reason for the optimism for the future of lupus patients comes from seminal studies from the NIH Intramural Research Program that determined that treatment with immunosuppressive drugs (cyclophosphamide and prednisone) can prevent or delay kidney failure due to nephritis, one of the most serious common complication of this disease. NIH clinical investigators continue to study and refine treatment regimens for lupus nephritis.

Although the cause of lupus is unknown, applied genetics have dramatically improved the pace of research by discovering the genes that contribute to lupus susceptibility, severity, and mortality. Using experimental animal models of lupus, investigators are uncovering the genetic factors involved. NIH-supported researchers have identified, in mouse models of lupus, 7 to 10 gene regions that are linked to the disease. Some features of human lupus are readily apparent in these animal models. Recently, researchers have found an association between lupus and a region on chromosome 1. Fine mapping of this region has identified another candidate gene involved in immune function, specifically in the processes of DNA repair and cell death, both of which have been reported to be abnormal in lupus. The results to date suggest that lupus susceptibility genes are very similar in mice and humans, and that these same genes may be important in all racial groups. In addition, the identification of genetic risk factors in lupus could indicate which patients may potentially develop severe disease and therefore merit early, aggressive treatment. Recent studies yielded two such risk factors – absence of the C4a gene and changes in an Fc receptor gene. C4a and Fc receptors are involved in the removal of proteins known as immune complexes, which, if not removed, can cause tissue injury.

In other approaches to lupus, the NIH is funding the first clinical trial on the safety of estrogens for women with lupus. At the present time, women with lupus are usually advised not to take any medications that contain estrogen in the belief that it will worsen their disease or cause

problems with blood clotting. This leaves women limited options for contraception during child-bearing years and for hormone replacement therapy during postmenopausal years. These studies will focus on the effects of oral contraceptives on disease activity in women with lupus and on the effects of hormone replacement therapy with estrogen and cyclic low-dose progestins in postmenopausal women with lupus. Many of the patients recruited for this trial are minority women. The outcomes of this trial are expected to have a major impact on the treatment options, health, and quality of life for patients with lupus.

With regard to modifiable risk factors for health outcomes in patients with lupus, we know that patients with chronic diseases have poorer outcomes when they have low socioeconomic status. Socioeconomic status includes such factors as education, employment, occupation, income, insurance, and access to medical care. Several studies have found an association between lower socioeconomic status and higher morbidity or mortality in black patients with lupus. In a large multicenter study, lupus disease activity and health status were most strongly associated with potentially modifiable psychosocial factors such as self-efficacy for disease management. Cumulative organ damage was most highly associated with clinical factors such as age and duration of disease. None of the outcomes measured was associated with race. These results indicate that education and counseling, coordinated with medical care, might improve outcomes in patients with lupus. Psychosocial interventions were also suggested whereby patients could increase their role as self-advocates in the management of their lupus.

Information dissemination is a vital dimension to the work of the NIH. Joined by voluntary partners, the NIH recently published, "Lupus: A Patient Care Guide for Nurses and Other Health Professionals," which will be a valuable tool and resource for nurses and all health professionals who work with lupus patients. We have also targeted our information to particular areas of need and to diverse populations (including printed information and our toll-free information line in Spanish and in English). We will continue to strive to make our information accessible to the vast and diverse populations affected by lupus and other chronic diseases.

In summary, the NIH has undertaken research on multiple fronts. Our long-term investment in research has meant that a young person who has lupus today faces a much brighter future than even 25 years ago. Significant progress has been realized over even the last decade from the investment in research on lupus. Through research, we have learned much more about the causes of lupus, we have improved diagnostic abilities, the treatments are significantly better than in the past, and newer treatments are on the horizon, taking advantage of emerging areas of science.

## **Bringing a New Medication to Market: Shifting Treatment From Clinics to Doctors' Offices**

Although it may seem like new medications for treating diseases come on the market every day, this is clearly not the case for drug addiction, where new medications appear on the market or are approved for use much less frequently. For example, the last anti-heroin addiction medication to become available was LAAM which was approved for the management of opiate dependence in 1993. Unfortunately, like the only other agonist medication approved for treating opiate addiction, methadone, both medications are only available in a limited number of clinics across the country. There are strict state and federal regulations that control the use of these medications, frequently making it difficult for the estimated 900,000 individuals in need of opiate treatment to receive it. The good news is, there are two new medications that once approved by FDA, will be available to qualified physicians across the country to prescribe in their own office settings. Having safe and effective alternative pharmacotherapies for treating opiate addiction that can be dispensed in doctors' offices will dramatically increase access to drug addiction treatment.

The two new medications that NIH has just brought to fruition are the sublingual tablets, buprenorphine (*Subutex*) and buprenorphine/naloxone (*Suboxone*). Both products contain the active ingredient buprenorphine, a partial agonist that functions on the same brain receptors as heroin, but does not produce the same high, dependence, or withdrawal syndrome. Buprenorphine actually prevents heroin from binding to opiate receptors, thus blocking its pleasurable effects. Buprenorphine also blocks withdrawal discomfort by keeping the receptors occupied. It is long-lasting, less likely to cause respiratory depression, well-tolerated by addicts and when combined with naloxone, has very limited diversion potential.

Bringing these buprenorphine products to their current state of development was not an easy task. In fact, the search for a medication like buprenorphine has been underway since at least 1929. It was in the 1920s when the U.S. began to seriously recognize that Americans were abusing and becoming addicted to opiates, especially as a way to treat pain. A number of formal projects were initiated to replace opiates with medications that could treat pain, but did not have addictive properties. Thousands of synthetic opioids were produced and tested. As these formal efforts to identify opioids of low abuse liability waned during the 1970s, the last major compound to be tested as a result of this effort was buprenorphine.

Buprenorphine was first synthesized in 1969 in England by Dr. John Lewis of Reckitt and Colman Products and subsequently developed as an analgesic. It was initially being looked at purely for its abuse liability potential, but was discovered to have properties that could be used therapeutically. It was first marketed in the United Kingdom in 1978 for injection and in 1981 and 1982 as an oral tablet. It is being marketed in over 40 countries today as an analgesic. It has been marketed in the U.S. since 1985, but only in its injectable form. In the mid 1970s researchers at the Addiction Research Center in Lexington, Kentucky (at the time, the intramural program of NIH's National Institute on Drug Abuse) began to take an interest in buprenorphine as a medication that might work for treating opiate addiction. In 1978, Dr. Donald Jasinski and

colleagues in a landmark clinical study showed that buprenorphine can in fact block the euphoria produced by opiates (Archives of General Psychiatry 35:501-516). Other researchers were also reporting that daily administration of buprenorphine decreased heroin self-administration in opiate abusers.

Studies on buprenorphine continued throughout the 1980s and 1990s. Congress, recognizing the need to stimulate the availability of addiction medications, passed several pieces of precursor legislation during the 1970s and 1980s indicating its intention that NIH initiate and promote research into the creation, development, and testing of pharmacological substances for treatment of addiction. NIH administratively created a Medications Development Division to focus on this effort in 1990. By 1992, Congress passed the “ADAMHA Reorganization Act” (P.L. 102-321) which statutorily established the Medications Development Program at NIH. By this time, the new medications program included a number of major studies to document buprenorphine’s safety and efficacy in the opiate-abusing population. Researchers had accumulated data from more than a half-dozen controlled clinical trials involving over 1,000 patients, using outcome measures of illicit opiate use and retention in treatment to substantiate buprenorphine’s clinical safety and efficacy. With the bulk of the research completed, NIH established a Cooperative Research and Development Agreement in 1994 with the original developers of the medication, Reckitt and Colman Pharmaceuticals, Inc. This was a team effort to bring this drug to a marketable status for treatment of opiate addiction in the U.S. In 1999, Reckitt submitted all of the study data to the FDA in support of a new drug application (NDA) for buprenorphine in the treatment of opiate dependence. Further information was requested by FDA in 2000 and 2001 as part of the approval process. Once final approval comes from the FDA, these medications will become the first of their kind to be dispensed more like other commonly prescribed medications, such as those used to treat diabetes or hypertension.

On October 17, 2000, an historic day for addiction treatment, the President signed a bill that allows qualified physicians to prescribe certain anti-addiction medications in an office setting, including buprenorphine and buprenorphine/naloxone (after approval by the FDA). Although the Drug Addiction Treatment Act is a small provision in the Children’s Health Act (HR 4365), it is a gigantic leap for science and for those in need of addiction treatment. This treatment provision amends the Controlled Substances Act, which placed strict requirements on practitioners dispensing narcotics for treating addiction. For the first time, the disease of addiction will be put on an equal footing with other chronic diseases. Buprenorphine and buprenorphine/naloxone products are expected to increase the amount of treatment capacity available and expand the range of treatment options that can be used by physicians.

## NIH Scientists Develop the First Typhoid Vaccine that Protects Children Under Age Five

In collaboration with the government of Vietnam, NIH scientists have finished testing the first vaccine ever that protects children under age 5 against the age-old scourge, typhoid fever. The vaccine confers greater protection against the disease, and causes fewer side effects, than any of the other vaccines currently available. The researchers succeeded where others had failed because of an ingenious approach that they pioneered in the early 1980s.

The testing was completed in Dong Thap province, in the Mekong Delta. The seasonal floods in the area are a boon to agriculture, but a threat to human health. Each year, during the heavy rains of the late fall, the Delta's rivers overflow their banks. Water covers the countryside, as far as the eye can see. The local people wade through the floodwaters and string hammocks above the 12 to 24 inches of water that floods their homes every year. When the waters finally retreat, they leave behind a fertile residue of silt and debris that nourishes the next season's crops. But the flat terrain and high waters also make it impossible to build an effective sewage system in the area.

As a result, typhoid fever and other diseases that are spread through raw sewage run rampant. Typhoid, a potentially debilitating and life-threatening illness, is caused by the bacterium, *Salmonella typhi*. Untreated typhoid causes persistent high fever, stomach pains, weight loss, loss of appetite, delirium, severe diarrhea (in children), and constipation (in adults). The disease spreads by fecal contamination of drinking water or food, or by person-to-person contact. According to the U.S. Centers for Disease Control and Prevention, about 16 million people worldwide develop typhoid each year, and 600,000 die from it. Treatment consists of antibiotics. However, in Dong Thap province – like many other parts of the world lacking effective sewage systems – the typhoid bacteria have grown resistant to the antibiotics used to eliminate them.

Since no animal models exist for typhoid, developing a typhoid vaccine has been a long and difficult challenge. *S. typhi* inhabits and causes illness only in human beings. Typhoid vaccines currently on the market are ineffective for children under 5 years of age. The new typhoid vaccine was based upon an idea developed by Drs. John Robbins and Rachel Schneerson at the NIH. In 1983, when these scientists opened the NIH's Laboratory of Developmental and Molecular Immunity, most vaccines against bacterial diseases consisted of whole bacteria that had been killed or weakened. Although usually effective, such vaccines often caused unpleasant side effects, and, in rare instances, the disease itself. The NIH scientists' idea was to develop a vaccine based on a single target substance on the bacteria's surface.

The NIH scientists' approach involved developing a vaccine with a sugar molecule, called a polysaccharide, on the outer capsule encasing the bacteria. Previously, many researchers believed that the immune system was incapable of recognizing a polysaccharide. In their early research, the NIH scientists tested a vaccine they developed against *Hemophilus influenzae* type b (Hib). At the time, Hib infection was the leading cause of acquired mental retardation in the U.S. Even with effective antibiotic treatment, 5 percent of those who contracted the disease

died, and about 30 percent had damage to the central nervous system, including mental retardation, deafness, or seizures.

Robbins and Schneerson soon tested a Hib purified polysaccharide vaccine, and found that it was safe. In older children and adults, it resulted in protective levels of antibody – the immune system molecules that target invading microbes for later destruction. This approach, however, failed in infants, the age group with the highest incidence of serious Hib infections. The infants' immature immune systems failed to identify the polysaccharide, and could not make enough antibodies to protect them against it.

The NIH scientists then developed a new conjugate, or linking, technology to create a vaccine. This involved chemically linking the poorly-recognized polysaccharide to a protein the immune system could recognize easily. The conjugate vaccine was soon found to be effective in infants as well as in older children. Since 1987, Hib conjugate vaccines have been licensed and marketed, and have become part of the routine pediatric immunization series given to infants. An added benefit of the conjugate technology is that it results in fewer side effects than whole-cell vaccines. Because the immune system attacks many molecules in a whole-cell vaccine, the immune response is strong. Byproducts of this response include fever as well as swelling and soreness around the injection site. With a conjugate vaccine, however, the immune system recognizes only one molecule and the response tends to be much less pronounced.

Similarly, the NIH scientists also began experimenting with a conjugate version of a typhoid vaccine. The polysaccharide on the bacteria's outer coat proved difficult to isolate, but the NIH scientists discovered how to do so and soon made their first non-conjugate vaccine against the disease. In 1987, the scientists reported completing the initial testing of the typhoid vaccine among villagers in Nepal. The success of this early effort led to larger studies, among them a successful test on 7000 Nepalese adults. These tests led to licensing of the non-conjugate vaccine in the U.S. and many other countries.

Because this vaccine was not sufficiently protective of young children, the NIH scientists applied the technology they developed for the Hib conjugate vaccine to make a typhoid conjugate vaccine. After the conjugate vaccine proved to be safe and effective for adults, the researchers began the final phase of testing, in children. Joined by NIH scientists Kimi Lin and Shousun Szu, they undertook the study in Dong Thap Province, where there is a high rate of typhoid fever – roughly 413 cases for every 100,000 people. More than 90 percent of the typhoid strains present in the area are resistant to the antibiotics used to treat the disease.

In all, 11,091 Vietnamese children ranging from age 2 to age 5 took part in the study. The children received two injections, 6 weeks apart. Half received the vaccine, and the other half, a placebo. In the following two years, both groups were observed by their physicians throughout the study. Those who developed typhoid fever received the standard treatment of antibiotic therapy for the disease. *S. typhi* was isolated from only 4 children who had received both injections of the vaccine while the placebo group had 47 cases, for an effectiveness rate of 91.5 percent. By comparison, typhoid vaccines currently on the market have a 70 percent

effectiveness rate even in adults or older children and fail to protect children under age 5 against the disease. Fewer than 2 percent of children experienced any side effects, and these were mild and limited to swelling at the injection site, or to mild fever that went away within 48 hours. The effectiveness of the vaccine – 91.5 percent – is the highest reported for any typhoid vaccine.

When the NIH scientists published their results in the *New England Journal of Medicine*, the study authors wrote that they next plan to test the vaccine in children under two, to see if it can be administered at the same time as the routine vaccination for Diphtheria, Tetanus, and Pertussis. Because of the high levels of protective antibodies the vaccine brought about in young children, the study authors suggested that the vaccine would probably be at least 90 percent effective in individuals above 5 years of age, “including the military and travelers to areas with high rates of typhoid fever.”

## **Making Drugs Safe for Children**

Until recently, over-the-counter and prescription drugs were considered safe for everyone, including children, after the drugs were tested only in adults. Children, however, in addition to their smaller size, also differ from adults metabolically. These differences may mean that a drug that is safe for adults could be very dangerous for children.

The most well known consequence of failing to see if a new drug is safe and effective for children resulted from the now infamous drug, thalidomide, which was marketed in Europe to combat morning sickness in women. Thousands of children whose mothers took the drug during pregnancy were born without arms or legs. Other drugs that are also presumed safe after being tested in adults may cause problems for children. For example, newborns given tolazoline to treat a form of high blood pressure in the lungs developed bleeding in the gastrointestinal tract and kidney failure. Another example occurred when newborns given the drug chloramphenicol developed “grey baby syndrome,” resulting in shock and, sometimes, death.

In fact, three-quarters of all medications marketed in the U.S. are not approved as safe and effective for use in children. In response to this situation, the NIH founded the Pediatric Pharmacology Research Unit (PPRU) network. Created in 1994, the network demonstrates that studies of drugs can be ethically and efficiently conducted in children.

The PPRU network serves as a resource for researchers who conduct studies on the safety and effectiveness of drugs for infants, children, and adolescents. The network consists of a partnership among the NIH, members of the pharmaceutical industry, and scientists at research and academic institutions throughout the country. The research institutions supply the laboratory space and the scientists who lead the study, the industry supplies funding for the technical and patient costs of the study, and the NIH provides funding for additional personnel and equipment. In addition, the PPRU network trains pediatricians and other researchers interested in developing safe and effective drug treatments for children. The PPRU network also helps to identify pediatric diseases that need new drug treatments, new uses for drugs that are already on the market, and better ways to analyze information to determine if existing drugs are suitable for use in children.

The studies in the PPRU network are designed to minimize discomfort for patients and do not disturb family life, striving to develop child-friendly protocols with minimal risk for all pediatric patients, regardless of their condition. Patients who participate in the studies include those with common disorders such as allergies, asthma, and upper respiratory infections, as well as those with less common disorders such as cystic fibrosis, severe infections, AIDS, sickle cell anemia, and childhood depression.

The PPRU studies typically involve children from early childhood through adolescence, to provide information relevant for children of all ages. Among the many PPRU projects, the activities of numerous enzymes involved in metabolizing drugs are being studied, as well as how these processes may change as children mature. In addition, the researchers are investigating

how the drugs are absorbed, distributed throughout the body, and, eventually, eliminated. Researchers are also studying how the drugs affect the targeted tissues at the level of the cell surface.

The pediatric provisions of the Food and Drug Administration Modernization Act of 1997 (FDAMA) have greatly influenced the growth of the PPRU network. Along with its other provisions, FDAMA called for more information on drugs used to treat children. Before FDAMA was enacted – from 1994 through 1997 – the PPRU network conducted 17 studies. During 1998, the first year after FDAMA was enacted, the PPRU network undertook 21 studies. By 1999, there were 54 studies in the network – almost a 200 percent increase. Then in 2000, there were 73 active studies, with a total of more than 1,000 patients participating. In addition, within the last two years, the number of pharmaceutical companies sponsoring studies in the network has increased by 41 percent.

By the end of the year 2000, the PPRU network tested the effectiveness in children of 7 drugs already on the market for adults. However, the majority of the network studies – 66 percent – do not involve the study of previously-approved drugs. Among the drugs studied are:

- \_ a drug to treat viral meningitis in infants
- \_ a new drug to treat asthma in children
- \_ a drug treatment for Kawasaki disease (a disorder involving inflammation of the blood vessels)
- \_ a new drug to treat conduct disorders
- \_ a treatment for systemic fungal infections
- \_ inhaled insulin for the treatment of Type 1 diabetes

Because the network has increased in size, capacity, and potential public health impact, researchers within the network have created working groups to deal with a growing number of issues in pediatric pharmacology. Among these, the diabetic group has reported considerable progress. The group is concerned not only with testing new anti-diabetic drugs, but also with treating other disorders that can occur in diabetic children, such as depression. Diabetes can influence how the drug is distributed within the body, and thus affect treatment outcomes. The PPRU network researchers are also conducting seminal work in refining techniques for monitoring blood sugar. The technology made it possible to detect periods of unusually high or low blood sugar in children with diabetes. Moreover, the development of such monitoring technology is crucial before efforts to develop an artificial pancreas can succeed.

In addition, the neonatal (newborn) medicine group recently developed a set of guidelines for conducting drug treatment studies in low birth weight infants. The working group targets conditions that are common in low birth weight infants such as bronchopulmonary dysplasia (BPD), an irritation of the lungs that sometimes occurs in about 30 to 40 percent of premature infants. Currently, treating BPD involves steroids, which the infant inhales through a mask. Unfortunately, these drugs may only reach as far as the mouth and throat. To address this problem, the working group is testing a new steroid formulation. The size of the individual

particles of the new preparation is much smaller than those in current formulations, and so the new preparation should be able to penetrate the lungs more deeply.

## The Story of Gleevec

May 10, 2001, marked an important milestone in the fight against cancer. News outlets all over the country announced that a promising drug called Gleevec™ had been approved to treat a serious blood cancer known as chronic myelogenous leukemia (CML). This drug is one of the first of its kind to be approved – a **targeted** agent that hones in on specific molecules in cancer cells, leaving healthy cells unharmed.

What the news reports did not emphasize was that Gleevec™ is far from an overnight breakthrough. The road to its discovery was paved by knowledge culled from more than 40 years of studies probing the molecular events associated with cancer development, emerging new technologies that enabled these studies, and quite often, unanticipated opportunity.

***The Philadelphia Chromosome: Uncovering the Fundamental Nature of CML.*** The story really began in 1960 when Drs. Peter Nowell and David Hungerford – two Philadelphia-based physicians – made a curious discovery. They noticed that cells from CML patients were missing a short segment on one member of the 22<sup>nd</sup> pair of chromosomes. This shortened chromosome became known as the “Philadelphia chromosome.” It was the first chromosome abnormality ever found to be associated with a specific cancer. Its presence also was the first indication of the startling possibility that tumors might indeed arise from a genetic mutation in a single cell.

Although the link between the Philadelphia chromosome and CML led scientists to suspect a causal relationship, the location of the missing DNA from chromosome 22 and how it might lead to CML remained a mystery over the next three decades. Then, in the early 1970s, new staining techniques offered a way to more precisely visualize band patterns – characteristic markings that can be used to identify individual chromosomes. With this technique, Dr. Janet Rowley determined that chromosome 9 in CML patients was lengthened by the same amount that chromosome 22 was shortened. From this observation, Rowley proposed that the genetic material from the two chromosomes was reciprocally exchanged, or translocated.

Using newly developed approaches for molecular analysis, scientists in the early 1980s determined that the genetic rearrangement that leads to the Philadelphia chromosome occurs when genetic mistakes cause breaks in the middle of two vital genes located on chromosomes 9 and 22. They found that the break on chromosome 22 occurs in the middle of the *bcr* gene and that the break on chromosome 9 occurs in the *abl* gene. On the shortened end of chromosome 22, the genetic rearrangement produces the abnormal *bcr-abl* gene, the source of CML development.

In 1986, Dr. David Baltimore and his research group determined that, like the normal *abl* gene, the defective *bcr-abl* gene carries the code for tyrosine kinase, a class of proteins that plays an important role in regulating cell growth and division. The normal *abl* gene will turn on or off, producing tyrosine kinase to promote cell growth as needed. The aberrant *bcr-abl* gene, however, is always turned on and lacks the critical piece that enables the gene to turn itself off. As a consequence, *bcr-abl* floods the cell with the instruction to divide constantly and also

prevents the leukemia cells from undergoing normal programmed cell death or apoptosis, a process that helps to regulate white blood cell numbers. Several laboratories confirmed the link between the defective gene and CML through studies showing that the *bcr-abl* gene was all that was needed to induce leukemia in mice.

***Developing a Targeted Treatment.*** During this same time period, advances in molecular biology were revolutionizing the field of drug discovery. In the laboratories of Ciba-Geigy, scientists were able to apply unfolding knowledge about the workings of cellular pathways and communications systems in a number of drug development efforts. In one research program, scientists were looking for agents to inhibit protein kinases – a group of cell signaling proteins that includes the Abl protein. A number of such agents were found, including one that they labeled STI571.

Meanwhile, American oncologist Dr. Brian Druker was interested in determining how the Bcr-Abl protein, the product of the *bcr-abl* gene, fits into the complicated circuitry of cell signaling. His research led him to believe that the Bcr-Abl protein could be a powerful target for a drug that could impede the activity of the protein and be an effective treatment for CML. When he learned about Ciba-Geigy's complementary research, Druker asked scientists there for candidate protein kinase inhibitors that he could test against leukemia cells. At the end of 1993, the pharmaceutical company sent him several candidates, including STI571. Druker screened the chemicals and found that STI571 halted the growth of the leukemia cells but had little effect on healthy ones.

While this was an exciting outcome, there were still many obstacles to overcome. The process of developing a new drug and getting it approved for use is lengthy and expensive. The steps include discovery, efficacy testing, lead agent development, pharmacology and toxicology studies, filing with the FDA, and finally evaluation through clinical trials. STI571 posed an additional risk for drug developers because the incidence of CML is quite low, and two moderately effective treatments already were available for CML, although both sometimes caused serious side effects.

Despite reservations, Novartis agreed to produce enough STI571 for an initial clinical trial. Dr. Druker began the Phase I trial, conducted to identify a safe dose level, in June of 1998. By December of 1999, he and his colleagues reported that white blood cell counts for all of the 31 patients receiving a high dose of STI571 had returned to normal, an effect that was sustained for the eight months that the patients stayed on the drug. In 9 of the 20 patients who were treated for five months or longer, no leukemia cells could be found, confirming that the drug was eliminating the source of the cancer. In addition to these remarkable results, the drug had minimal side effects. Rarely are such dramatic results seen in a Phase I trial. As the news spread, more and more CML patients began to request the treatment.

In response to these exciting findings, Druker and his colleagues conducted a larger study and reported in April 2001 that STI571 restored normal blood counts in 53 of 54 CML patients, all of whom had resisted previous chemotherapy. Of these patients, 51 were still doing well after a

year on the medicine, with most reporting few side effects. Following “fast-track” review on these findings, the Food and Drug Administration approved STI571, now known as Gleevec™, as a treatment for CML in May 2001.

***The Story Continues.*** But the story of Gleevec™ as a treatment for CML is not complete. Patients receiving the drug need to be followed for longer periods to determine whether the positive effects will last and whether long-term treatment can cause side effects. Unfortunately, most patients with advanced disease relapse within a year. The cause of resistance is now known, so scientists are trying to overcome it. Like most successful treatments, Gleevec™ will undoubtedly spawn a host of refinements.

The story does not end with CML. In addition to the Bcr-Abl signaling protein, the drug appears to target two other protein kinases, the c-kit receptor and the PDGF receptor. The c-kit receptor is active in gastrointestinal stromal tumor (GIST), a cancer that affects connective tissue in the digestive system. The PDGF receptor is associated with many types of cancer, one of which is a form of brain cancer called glioblastoma. Cancers of the breast, ovary, and lung may also be effectively treated with Gleevec™. Both NIH-supported and private-sector scientists are currently conducting a number of different clinical trials to determine the effectiveness of Gleevec™ against these other cancers.

The success of Gleevec™ offers substantial hope that molecular targeting is a highly effective strategy in the fight against cancer, provided that the target is carefully chosen and validated. As scientists identify additional cellular mechanisms that drive tumor growth, it will be possible to design tailor-made agents that selectively take aim at these targets to thwart the growth of specific cancers. It is likely that Gleevec™ is the first of many potent, but safer, targeted preventive and treatment drugs to be developed as a result of advances in our understanding of cancer at the molecular level.

## Salivary Glands: Potential Target Site for Gene Therapies

For millions of people who suffer from diseases such as diabetes, growth hormone deficiency, or hemophilias – diseases caused by a deficiency in a single protein – gene therapy may some day offer an attractive alternative to available treatments. Current therapies for these disorders are invasive. Primarily administered by injection, the treatments are costly and often dependent upon patient self-monitoring and compliance. Furthermore, they are not cures. Gene therapy offers the potential to correct the underlying disorder in these patients by actually replacing the missing protein.

NIH researchers are studying the possibility of using gene transfer in salivary glands to produce missing hormonal proteins. Gene transfer to salivary glands is an exciting prospect because it is noninvasive. The ducts of major salivary glands exit into the mouth and can be readily accessed without any surgical procedure. Furthermore, salivary glands are a natural protein-producing site, making them an appealing choice for the delivery of gene products. Although salivary glands are exocrine glands – they secrete outwardly through ducts – scientists have speculated for years that they could also act as endocrine glands, which secrete internally into the bloodstream.

NIH scientists began gene transfer studies about a decade ago. Their first important step was to demonstrate that using gene transfer technology, salivary glands could indeed be coaxed into producing “foreign” proteins and secreting them into the bloodstream. Delivery of a therapeutic gene requires the use of a vector, or vehicle, to carry it. Using an adenovirus (cold virus) as the vehicle, the researchers inserted a gene that encodes for alpha-1-antitrypsin – a serum protein normally made and secreted by the liver – into the adenovirus. They then administered the adenovirus into the salivary glands of rats with the hope that the glands would begin to produce the protein. Results of the study clearly showed that salivary glands could be made to produce and secrete something other than their own natural product. The research provided the first direct evidence that salivary glands could secrete a transgenic protein, alpha-1-antitrypsin, into the bloodstream, as confirmed by measurement of the protein levels in blood. In every rat examined in the study, levels of alpha-1-antitrypsin were higher in the veins coming out of the salivary gland than in those going in, clearly showing that the protein was being secreted into the bloodstream.

Next, the investigators needed to determine if transgenic proteins secreted into the bloodstream from salivary glands were biologically active. That is, did the transgenic proteins have the ability to perform their intended function? NIH researchers used an adenoviral vector encoding human growth hormone (hGH) to find out. After delivering the hGH gene to rat submandibular salivary glands, they analyzed serum from the treated animals. They found several chemical changes indicating that the hGH protein was functioning as intended. Furthermore, the animals' hGH blood levels were three times those required physiologically, proving that the salivary gland can produce the transgenic protein in abundance.

But how exactly were the transgenic proteins produced by the salivary glands getting into the bloodstream? For salivary glands to be useful in gene therapy, transgenic proteins must be efficiently directed into the bloodstream for systemic use. Proteins are normally secreted from cells via two general pathways – so-called constitutive and regulated paths. While many cells have constitutive pathways, a regulated pathway requires a sophisticated cell. NIH investigators knew that salivary gland cells secrete salivary proteins through a regulated pathway. They suspected that the cells might also be using a constitutive pathway to secrete the transgenic proteins into the bloodstream. This hypothesis was tested using three proteins, each inserted into rat salivary glands using an adenoviral vector. The investigators then measured the amount of each transgene product in saliva and blood. While two of the proteins were secreted primarily into saliva, the third was secreted into the bloodstream. The study revealed that salivary glands are able to sort the transgenic proteins into the two distinct pathways by recognizing sorting signals presumably encoded in the proteins. Understanding these signals and how to manipulate them would help in the efficient delivery of these therapeutic proteins to specific sites.

However, the studies exposed a major concern – that some of the proteins, including hGH, were predominantly secreted into saliva where they are not biologically useful. NIH researchers tested their theory that a common manipulation used in cell biology sorting experiments might be able to re-direct protein secretion from salivary glands into the bloodstream. The researchers injected the rats with the alkalinizing agent hydroxychloroquine (HCQ), an FDA-approved drug, prior to delivering the adenovirus into the rats' salivary glands. HCQ changes the pH level of intracellular vesicles, which are the “cargo ships” carrying secretory proteins along their route within the cell; when this happens, their internal compasses malfunction. With their pH level off balance, the vesicles become lost and confused, and are more likely to exit through the back of the cell into the bloodstream (the endocrine path) rather than through the front (the exocrine path). Results of the study indicated that HCQ markedly enhanced, by more than 10-fold, the proportion of transgenic proteins secreted from salivary glands into the bloodstream. As an added benefit, investigators learned that the addition of HCQ allows them to use significantly less virus to transfer the gene into the glands. This may someday yield a safer procedure for clinical use in humans.

NIH's decade-long pursuit of producing biologically active substances in human salivary glands now appears to be within reach. Animal studies have revealed how to deliver hormonal proteins from salivary glands into the bloodstream at useful levels, without using high doses of a viral vector. Researchers now need to determine if this gene transfer technology – including the use of HCQ – will enhance secretion of transgenic proteins in humans. Further studies also are needed to find a way to regulate secretion of the hormones such that they are made only as needed and in the amounts required. Clinical testing is the next important step in this journey.