The Amyotrophic Lateral Sclerosis Association

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ALS Association

PATHWAYS *to* HOPE

The State of Research into
Amyotrophic Lateral Sclerosis

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PREFACE

The search for answers to the mysteries of Amyotrophic Lateral Sclerosis (ALS), commonly known as Lou Gehrig's disease, is attracting the world's finest scientific minds who are probing the complexities of this deadly disease. More progress in understanding the disease mechanism of ALS and in uncovering new insight into potential treatment has been achieved in the last decade than at any time since ALS was first described by Jean-Martin Charcot more than a century ago.

The ALS Association is at the forefront of many of the bold initiatives described in this report. *Pathways to Hope* chronicles the evolution of scientific investigation into this progressive neurodegenerative disease that holds the name of baseball legend Lou Gehrig, whose courageous struggle symbolizes the fight against ALS.

The ALS Association has raised nearly $100 million in the quest to defeat ALS. The Association's diverse and collaborative research programs have been instrumental in the development of animal models of the disease for scientists to test hypotheses about disease pathogenesis and the viability of therapeutic compounds. The ALS Association is vigorously promoting a collaborative approach to finding a cause or causes and treatments for ALS. This unique research effort brings together leading technology, exploration of gene therapy, stem cell biology, genetic research, environmental factors and patient care in the optimistic belief that there will be effective therapies for the disease.

Pathways To Hope is a comprehensive overview of the current status of ALS research. Importantly, the report provides insight into the growing understanding that many neurodegenerative diseases including ALS, Parkinson's and Alzheimer's have intriguing commonalities—similarities that have led to increasing collaboration and information-sharing among investigators in this field. Common aspects in these diseases have sparked increased interest in the biotechnology and pharmaceutical sectors, which may lead to potential therapies for the disease. It is hoped that through increased collaboration, a breakthrough in one disease may have application to others.

INTRODUCTION

For much of the twentieth century, amyotrophic lateral sclerosis (ALS) occupied a paradoxical place in medical science. On the one hand, the disease gained wide publicity as celebrities such as baseball star Lou Gehrig, British actor David Niven, and astrophysicist Stephen Hawking were diagnosed with ALS. On the other hand, as an "orphan disease" that affects fewer patients than better known neurodegenerative diseases like Alzheimer's and Parkinson's, ALS attracted fewer academic researchers. Sensing a small potential market for ALS drugs, the pharmaceutical industry expressed only minor interest. Until recently, no treatments were available to alter the course of the illness.

In the past decade, this picture has changed dramatically. Today, the research effort in ALS is comparable to Alzheimer's and Parkinson's. Progress in understanding ALS has resulted in FDA approval of Riluzole, the first drug to actually prolong life in ALS patients, and many more drugs are in clinical trials or earlier stages of development. Pharmaceutical and biotech companies are considering expanding their investment in ALS.

This report examines the factors that have led to expansion of ALS research and promise to further accelerate drug development in the near future.

One of the milestones was the discovery, in 1993, of the first ALS gene, which attracted many new researchers to the study of ALS by giving them a tool for studying the disease's biochemical pathways in detail. The growing realization that ALS may share common biological mechanisms with Alzheimer's, Parkinson's and other neurodegenerative diseases is now attracting the interest of the pharmaceutical and biotech industries who feel insights gained from ALS (a small market) can help develop drugs for Alzheimer's and Parkinson's (potentially much larger markets).

Several other genes implicated in the more rare forms of ALS have been identified, and several research groups have narrowed the search for additional genes to small regions of several chromosomes. Identification of these will undoubtedly open up new avenues of research. The availability of a new generation of research tools—robotic drug screening, proteomic, genomic, and metabolomic technology, as well as rapid gene sequencing—ensures that discoveries in the laboratory will be translated more rapidly into therapies that can be tested in the clinic. The ALS Association has launched the TREAT ALS initiative (**T**ranslational **Re**search **A**dvancing **T**herapy for ALS) to be able to rapidly bring new advances from basic research into clinical trials.

As a result of progress over the past decade, ALS experts are hopeful that in another ten years, new treatments will be available that can alter the course of the disease.

DESCRIPTION OF ILLNESS

First described in 1869 by the French physician Jean-Martin Charcot, ALS is a disease of motor neurons, the specialized nerve cells which carry electrical impulses from the brain and spinal cord to the muscles that control movement and respiration. In ALS, motor neurons degenerate and die, leading to atrophy of muscles and, eventually, paralysis. Other regions of the nervous system are relatively unaffected. ALS usually strikes individuals 50 to 60 years of age and progresses quickly, leading to death in one to five years, with the final event being failure of respiratory muscles. In exceptional cases, patients can survive longer, especially when assisted by ventilators.

At any time, ALS affects approximately 30,000 people in the US and is the most common disease of the motor neurons, but its incidence is much smaller than neurodegenerative diseases like Alzheimer's and Parkinson's disease.

Like Alzheimer's and Parkinson's, ALS exists in a familial (inherited) form as well as a "sporadic" form which strikes patients with no prior family history. Familial ALS accounts for 5-10% of all ALS cases, sporadic ALS for about 90-95%. Scientists believe that similar biological mechanisms are at work in both the familial and sporadic forms because the clinical manifestations are similar.

THE FIRST ALS GENE AND ANIMAL MODEL

Until the early 1990s, ALS research was restricted by the inability to study the disease in a living system. Researchers worked primarily with human tissue samples obtained post-mortem, which represented only one "snap-shot" of the disease at its end stage. A new era in ALS research began in 1993 with the identification of the first ALS gene. This discovery, in turn, led to the first ALS animal model, which allowed researchers to study the disease process in much greater detail.

The gene was identified by analyzing the inheritance patterns of ALS families, an approach that narrowed the search to a small region of chromosome 21. DNA sequencing subsequently identified the gene, which encoded a protein known as copper/zinc superoxide dismutase-1 (SOD1). Mutations in SOD1, passed from generation to generation, were responsible for members of these families developing ALS.

Mutations can occur at several locations within the SOD1 gene. Soon after SOD1 was discovered, investigators genetically engineered several lines of mice, each carrying a different SOD1 mutation. These mice exhibited many signs that parallel those seen in human patients: they develop muscle weakness and atrophy which began at 3-8 months (the exact age of onset of muscle atrophy depended on which SOD1 mutation the mice carried.) This time frame (adult onset) is comparable to that of human patients who develop ALS. On a microscopic level, researchers noted changes in tissue and biochemistry which closely resembled those known from human tissue samples.

PROGRESS AND FRUSTRATION

Discovery of the SOD1 gene caused considerable excitement in the scientific community because it was a gene that had already been extensively studied. In healthy individuals, the SOD1 gene produces a protein—superoxide dismutase-1—that detoxifies cells of free oxygen radicals—harmful byproducts of the cell's normal metabolism. ALS researchers initially expected to find that

mutations in the SOD1 gene might contribute to ALS by preventing the SOD1 protein from fulfilling its normal function. If this were the case, researchers would have had an obvious target for drug therapy.

In fact, researchers found that the loss of the normal function of SOD1 is not the cause of motor neuron disease observed in the mouse model, as shown by "knock-out" mice engineered to lack the SOD1 gene altogether—these mice did not develop symptoms of motor neuron disease. Researchers eventually concluded that SOD1 mutations create a misshapen protein that takes on a new, malicious function that contributes to disease by an unknown mechanism. Today, there are several theories attempting to explain this aberrant function, but none has been proven.

Despite the inability of researchers to explain exactly how SOD1 mutations cause disease, the SOD1 animal model has helped clarify many of the biochemical and structural changes involved. (An in-depth discussion can be found in the section Possible Mechanisms of ALS below). Drugs potentially working on these mechanisms have been tested in the animal model and some have proceeded into clinical trials for ALS. Moreover, the availability of the SOD1 animal model has drawn many new investigators into ALS research and helped establish a substantial research infrastructure.

CURRENT CLINICAL TRIALS

Because drug development is a lengthy process (even after a target for drug therapy has been pinpointed), many candidate drugs for ALS resulting from a decade of research using the SOD1 model are just now entering clinical trials. Below is a summary of current theories of ALS and related drug development.

CHALLENGES IN ALS DRUG DEVELOPMENT

While several candidate drugs for ALS have shown promise in mouse models, they have proven less effective in humans, in part because of problems faced by all drug therapies as they move from testing in animals to humans, such as the differences in physiology between the two species. Another is the difference in lifespan—the mouse lifespan is much shorter (and disease progression much faster) than in humans. Many studies in the mouse are carried out at a point in the disease process when there are still many viable neurons, so the effects of drugs in slowing progression of disease may be more dramatic. In contrast, by the time human patients are diagnosed with ALS, they have already lost many of their motor neurons. It may be too late for motor neurons to be rescued by drug therapy, or there may be an insufficient number of neurons to appropriately restore function.

Moreover, researchers believe that some candidate drugs for ALS do not have a therapeutic effect because they never reach the diseased tissues (the motor neurons and surrounding cells). This provides an impetus to find new ways to deliver drugs to the target tissues. Some therapeutic agents may need to be injected into the spinal fluid, rather than administered orally or intravenously. Moreover, because ALS is a "multi-system" disorder (one that involves several different pathways and genes), drug therapies that successfully attack only one of these pathways may not be sufficient to alter the course of the disease.

As mentioned earlier, one of the problems ALS researchers face is that although the discovery of the SOD1 gene has advanced the ability to study the disease, the exact mechanism by which mutant SOD1 protein leads to disease remains

unknown. Despite being unable to map these steps in detail, researchers have nevertheless been able to use the SOD1 mouse model to study a variety of changes that take place on the cellular level and have been able to use this knowledge to develop targets for drug development.

They have also been able to target the SOD1 protein itself. Regardless of how mutant SOD1 protein leads to disease, reducing the amount of SOD1 protein should weaken its effect. Consequently, one approach being pursued is to down regulate SOD1, so that the cell produces less of it. In addition, researchers are attempting to find drugs that can block the aggregation of the protein, a process thought to play a role in the disease. Both these approaches are being tested in animal models.

ALS ATTRACTS INTEREST OF RESEARCHERS IN NEURODEGENERATIVE DISEASES

Commonalities with Alzheimer's and Parkinson's

ALS has traditionally been classified as a motor neuron disease unrelated to neurodegenerative diseases such as Alzheimer's and Parkinson's because these diseases attack different sets of neurons in the central nervous system. Parkinson's attacks dopaminergic neurons in the substantia nigra which control fine motor movement, while Alzheimer's affects neurons in the hippocampus involved in learning and memory. As a result, the clinical manifestation of these diseases differs greatly. In recent years, however, investigators have begun to find similarities among these different diseases:

- Abnormal accumulation of proteins. The type of protein differs with the disease—amyloid in Alzheimer's disease, alpha-synuclein in Parkinson's, and huntingtin protein in Huntington's disease. In the SOD1 mouse model of ALS, two types of protein form aggregations— neurofilament protein accumulates inside motor neurons, while mutant SOD1 protein aggregates inside motor neurons and neighboring glial cells.

- Abnormalities in astrocytes and microglial cells which surround and support nerve cells. These cells normally secrete substances known as neurotrophic factors essential to the functioning of nerve cells; they also detoxify waste products produced by nerve cells. Damage to glial cells may lead to a decrease in the secretion of growth factors and/or a build-up of toxic products that can damage nearby nerve cells. The glial cells seem to be involved in all three neurodegenerative diseases.

- Mitochondrial abnormalities. Motor neurons have a high energy requirement because they need to transport chemicals along the entire length of their axons. As a result, any effect on mitochondria is critical to the functioning of a neuron. In ALS, it is known that the mitochon dria of motor neurons are not functioning to their full capacity, but it is not known if they are directly damaged by mutant SOD1, or whether they are damaged indirectly through another pathway. Recent studies demonstrate that mutant SOD1 protein is abnormally transported into the membrane of mitochondria in spinal cord motor neurons. Mitochondrial damage is also thought to be at work in Alzheimer's and Parkinson's.

Even though the genes and biochemical pathways involved in these diseases differ, they result in the similarities noted above—the accumulations of protein, as well as the abnormalities of glial cells and mitochondria. These in turn are all mechanisms thought to trigger the

process of programmed cell death that results in actual loss of neurons. Because of the similarities in mechanisms, researchers believe that drugs targeting them may be useful in several different neurodegenerative diseases.

ALS Offers an Easier-to-Study Animal Model of Neurodegenerative Disease

While the similarity among neurodegenerative diseases is one reason researchers are increasingly studying ALS, another is the accessibility of the SOD1 mouse model itself. Animal models for Alzheimer's and Parkinson's have been developed, but the SOD1 mouse model for ALS is easier to study, due in part to the differences in the nature of the diseases.

Alzheimer's attacks neurons responsible for learning and memory; effects of memory loss are difficult to measure in mice. Parkinson's affects dopamine cells controlling fine motor movement, another effect difficult to measure in an animal model. ALS, by contrast, affects motor neurons controlling gross movement of the limbs. As a result, the SOD1 mouse strains exhibit overt signs—hind limb dragging—that are easy to observe. The availability of the SOD1 mouse facilitates experimentation and testing of ALS drug therapies.

INCREASING INTEREST OF PHARMACEUTICAL AND BIOTECH INDUSTRY

The realization that ALS may share mechanisms with other neurodegenerative disorders and also offers an easier-to-study animal model has led to interest from the pharmaceutical and biotech industries, which are traditionally interested in more prevalent diseases like Alzheimer's and Parkinson's. One goal of The ALS Association is to actively cultivate the interest of pharmaceutical and biotech industries.

THE NEXT DECADE OF ALS RESEARCH: ACCELERATION AND TRANSLATION

The Search for Additional ALS Genes

Researchers believe that ALS, like other neurodegenerative diseases, is a "multi-system" disorder involving several different biological pathways linked to several genes. While the discovery of the SOD1 gene and the development of the SOD1 animal model greatly accelerated ALS research, frustration with understanding how mutations in SOD1 lead to disease highlights the need to identify more genes linked to the disease.

Several research groups have narrowed the search for additional ALS genes to small regions of specific chromosomes. Because some of this research is unpublished, it is difficult to give an exact figure of the number of genes under investigation. Identification of new ALS genes by research groups can be expected in the next few years.

Discovery of new ALS genes will advance research in several ways. First, as with the discovery of SOD1, it will allow the engineering of new animal models incorporating mutations in these genes. This will further accelerate the study of the mechanisms underlying ALS and help identify new targets for drug development. In addition, it could help understand the mutant SOD1 protein, which is known to play an important role, but whose function is still unknown. Researchers note that several Alzheimer's genes discovered in the past decade have been linked to a common biochemical pathway. Similarly, several Parkinson's genes have been identified and shown be involved in another pathway. As additional ALS genes are identified, researchers will try to determine if they are linked to SOD1. If so, this would greatly increase our understanding of SOD1 in the disease process.

Gene/Environment Interactions in ALS

While genetics plays a central role in ALS, epidemiological studies suggests that environmental factors are also at work. The most well established "ALS cluster" was among the Chamorro people of Guam, where a high percentage of the population developed a disease termed "ALSPDC" (ALS-parkinsonism-dementia complex) in which patients developed symptoms of all three of these diseases; incidence of this disease among the Chamorro has fallen sharply in recent years. Another group that has been found to develop ALS at an abnormally high rate is Gulf War veterans; recently published research shows that this group develops ALS at twice the rate of the wider population.

ALS clusters like these are intriguing, but as with other types of disease, it is very difficult to determine which of many environmental factors may be at work. Further confusing the search is the fact that the actual disease is caused by a complex interplay between environmental factors and genetic factors that are inherited.

In the case of the Gulf War veterans, investigators cannot yet suggest a specific cause. Research among the Chamorros of Guam has been ongoing for decades, yet the cause cannot be given with certainty. One suspect identified, the cycad plant, which forms part of the native diet, is not confirmed by all investigators. The plant contains potential neurotoxins that might harm neurons through excitotoxicity, that is, over stimulation of neurons. (See Glutamate Toxicity). The reason for the decreased incidence of disease in recent years is unknown, although changes in diet may play a role. Scientists are also investigating the role of the genetics of the indigenous peoples in the region.

NEW ALS ANIMAL MODELS

While the SOD1 mouse model has yielded a wealth of information about ALS mechanisms, certain studies are difficult to perform in the mouse because of its small size. Investigators are overcoming some of these problems by developing SOD1 models in a larger species—the rat. The SOD1 rats, with their larger nervous systems, are facilitating studies such as development of procedures for stem cell transplantation and the detection of early ALS biomarkers in the cerebrospinal fluid, which would be used to develop diagnostic tests for the early diagnosis of ALS.

Biomedical research over the past 20 years has revealed a remarkable similarity between the genetic makeup of humans and other species, opening the possibility that useful research on human diseases can be performed in lower species such as flies and worms. The roundworm C elegans is a favorite of researchers because its genome has been completely sequenced, and it breeds faster and has a shorter life cycle than the mouse. For researchers in neurodegenerative diseases, C elegans offers the advantage that it contains a known number of neurons; in addition, the worm itself is thin and translucent, allowing researchers to directly visualize processes such as protein aggregation or the effects of drug therapies in "real time" without destroying the organism. ALS research using C elegans strains that incorporate the SOD1 mutation is currently underway. Similarly, the zebrafish (a small tropical fish) is also being used as a model system to study disease mechanisms and screen for drugs.

Translational Research

Unlike a decade ago, when the discovery of the first ALS gene was greeted by a small ALS research community, a much a larger community is now poised to exploit new advances. This is one of several factors that are accelerating the pace of ALS research. Another is the range of powerful technologies biomedical researchers now have at their disposal, such as genomic arrays that can measure the activity of thousands of genes in a tissue sample and proteomic technology that can detect the presence of thousands of different types of proteins. Gene sequencing technology allows researchers to much more quickly determine the exact DNA sequence of a gene or chromosomal region than before, helping accelerate the search for new ALS genes.

In ALS, as in other areas of biomedical research, there is now greater emphasis on translational research. Scientists studying the basic mechanisms of ALS collaborate closely with clinically oriented researchers to translate knowledge of these mechanisms into drug development. The process is being aided by new technology, particularly robotic technology that allows researchers to screen thousands of potential drugs at a time.

Advances such as these are helping to break down the traditional boundaries between academic research and the pharmaceutical industry. Conventionally, academic researchers uncover the mechanisms of a disease process, and then the pharmaceutical industry steps in to devote its considerable resources to the screening of millions of compounds targeted at that mechanism. Although robotic technology used by academic centers screen smaller numbers of compounds than industry, they allow academic researchers to take the initiative in screening potential ALS drugs. Among those being tested for ALS are drugs already approved by the FDA for other medical conditions. If any of these show potential for ALS, they can enter clinical trials relatively quickly. If the pharmaceutical industry makes a larger investment, this will further accelerate ALS drug development.

Biomarkers

Presently, a diagnosis of ALS is made primarily by ruling out all other possible diseases. For patients, this is often a lengthy process, filled with uncertainty. An accurate, easy-to-perform ALS diagnostic test would allow more rapid diagnosis, which would, in turn, lead to earlier treatment and a greater chance for altering the course of the disease.

In recent years, several initiatives have been undertaken to develop diagnostic tests for ALS. Most of these focus on finding biomarkers—small molecules or proteins associated with the disease—in the patient's blood or cerebrospinal fluid (CSF). Once these are identified, then diagnostic tests that detect their presence can be developed and commercialized.

Biomarkers for ALS have potential applications beyond aiding in the initial diagnosis. If they are present in different amounts early in the disease than later, it might be possible to use biomarkers to chart the rate of disease progression in an individual. This type of test would also be valuable in conducting clinical trials, making it possible to measure the effectiveness of different drug treatments being studied.

CELLULAR THERAPIES

The goal of conventional pharmaceutical research is to find small, synthetic molecules that affect "targets" in biochemical pathways identified by

researchers. Other approaches use biological products such as naturally occurring proteins and even entire cells to achieve a therapeutic goal. Two cutting-edge cellular therapies—stem cell therapy and gene therapy—are currently being developed for ALS.

Stem Cell Therapy

Stem cells are the "primordial" or "parent" cells which, during the course of normal development, specialize and multiply into the many types of cells that make up the human body. Scientists believe that by proper manipulation, stem cells can be transplanted into the body to replace diseased cells. Stem cell therapy could potentially overcome one of the traditional obstacles to treating neurological disorders and disease—unlike cells in other tissues, neurons of the brain and spinal cord do not effectively make repairs. Once they are lost, they cannot be regenerated.

To treat Alzheimer's and Parkinson's, stem cells would be transplanted into the appropriate area of the brain where, once differentiated into the appropriate type of neuron, the cells would successfully grow axons and re-establish the neuronal connections of the diseased neurons. But stem cell therapy for ALS faces an obstacle the other neurodegenerative diseases do not—to replace the diseased motor neurons, they would need to grow much longer nerve fibers, the axons that reach muscles throughout the body. Currently, the science of stem cell transplantation has not reached a stage where stem cells can be manipulated to grow axons up to a meter in length.

Despite this hurdle, stem cell therapy still holds potential for treating ALS, not by replacing motor neurons, but by targeting the astrocytes and microglial cells that surround and nourish the motor neurons. Recent research in SOD1 mice has shown that healthy astrocytes and microglia can maintain the health of neighboring, diseased motor neurons and greatly extend survival. Currently, ALS stem cell research is advancing along two lines: first, trying to understand which chemical messengers cause the stem cells to differentiate into neural cells, and second, working out procedures for stem cell transplantation in SOD1 rat models to lay the groundwork for stem cell transplantation in humans.

Gene Therapy

Researchers have established that healthy microglia and astrocytes secrete proteins (neurotrophic factors) that prolong the survival of diseased motor neurons. One of the avenues of ALS research in recent years has been to identify these neurotrophic factors and find ways to artificially deliver them to motor neurons.

One approach to delivering neurotrophic factors is gene therapy, which artificially introduces not the factor itself, but the gene encoding the factor, to tissues surrounding the motor neuron. Once the gene has been integrated into tissue cells, the cells' genetic machinery takes over, producing copies of the neurotrophic factor from the gene, increasing the concentration of the factor locally.

Like most forms of gene therapy, ALS gene therapy uses a genetically engineered virus (termed the "viral vector") to deliver the therapeutic gene to target tissues. These vectors capitalize on the ability that viruses have evolved to integrate their genetic material into host cells. In ALS gene therapy, the virus is engineered to carry the gene for a neurotrophic factor; the virus introduces this gene into tissue cells.

This approach is being tested in mice using a viral vector engineered to carry a neurotrophic factor, such as IGF-1. In practice, the viral vector is

injected into muscle tissue; from here the gene or its protein product is transported back through the nerve fiber to the motor neuron cell body. Experiments in animal models have shown encouraging results: gene therapy results in production of IGF-1 at the peripheral nerve, and this growth factor is successfully transported from the peripheral nerve to the spinal cord. The mice in these experiments showed an increase in survival; efforts are underway to translate this approach into clinical trials. More recently, gene therapy has been used to deliver the growth factor VEGF (described in more detail below), which has also increased survival of SOD1 mice.

Introducing therapeutic genes in this manner may help overcome one of the traditional obstacles to delivering these factors and other therapeutic agents to the central nervous system: agents injected or taken orally must cross the blood-brain barrier; as a result they may not reach the target neurons in quantities sufficient enough to have a therapeutic effect.

Initially, both stem cell therapy and gene therapy for ALS will be used to increase the exposure of diseased motor neurons to neurotrophic growth factors (or other factors). Although their initial goals are similar, both approaches are worth pursuing because each represents a new technology that will develop at its own pace and bring its own strengths to the treatment of ALS.

RNAi Technology

RNAi technology, also known as "gene silencing," employs a genetically engineered RNA molecule that prevents specific genes from manufacturing proteins involved in a disease. Like neurotrophic growth factors, RNAi will be delivered by gene therapy (described above).

Most cells in the human body have two copies of each gene in the genome. RNAi technology can be used to treat diseases that derive from a dominant, "gain-of-function" mutation in one of the genes. (The SOD1 gene in ALS is an example.) RNAi would be used to selectively lower or eliminate production of the mutant protein. RNAi may be a valid approach even though, as in the case of SOD1 in ALS, the exact pathways by which the mutant protein causes disease may not be known. A rapidly developing technology, RNAi will eventually be applied as a therapy for the subset of ALS patients carrying a mutation in the SOD1 gene. Once other disease producing genes are identified, RNAi may be aimed at these as well.

Other avenues toward silencing genes include antisense compounds that can also target a particular protein by silencing its production. Antisense strategies for SOD1 mutant ALS are making progress toward clinical testing.

Summary

Over the past decade, many researchers have been drawn to the study of ALS by the availability of the SOD1 mouse model. They have been joined more recently by neuroscientists seeking to apply insights from Parkinson's and Alzheimer's to ALS. The result is an ALS research community whose size and funding approach those devoted to these more prevalent diseases. This community has already helped usher many new drugs through laboratory testing and into clinical trials.

A new generation of research technologies has helped further accelerate development of conventional synthetic "small molecule" drugs as well as newer approaches like stem cell therapy and gene therapy. Closer working relationships between bench scientists and clinicians are ensuring that research findings will be translated to the clinic as

quickly as possible. ALS researchers are poised to exploit new discoveries, such as the identification of additional ALS genes, which may have an impact comparable to the discovery of SOD1.

POSSIBLE MECHANISMS OF ALS

Unique Demands on the Motor Neurons

Several mechanisms thought to be involved in ALS research are related to the unique physiological and structural aspects of the motor neuron. Most neurons in the brain and spinal cord are small (under 50 microns in diameter) and have an extension (axon) that carries nerve impulses to neighboring nerve cells. In most types of neurons, the axon is relatively short because the target nerve cell is nearby. But in motor neurons, which carry impulses from the brain or spinal cord to the body and limbs, the axons are much longer—up to a meter in length. This places unique demands on the motor neuron which are thought to make it prone to certain types of dysfunction that could contribute to ALS.

Abnormalities of Structural Proteins

Because of the unusual length of their axons, motor neurons require large quantities of a protein known as neurofilaments, one of the building blocks of structural fibers that help the axon maintain its shape. In patients with ALS, as well as other neurodegenerative diseases, neurofilament proteins fail to assemble properly, creating disorganized fibers or abnormal accumulations (aggregations) of neurofilament within the motor neuron.

Several genetic variations (polymorphisms) in the genes for neurofilaments have been found in patients with sporadic (non-inherited) ALS. This fact, along with the observation that only the largest diameter axons with the highest concentrations of neurofilament are lost in ALS, support the theory that neurofilament polymorphism is a risk factor for ALS. Another line of research focuses on peripherin, a protein of the same family of structural proteins.

Disruption of Axonal Transport

In most cells, critical substances are produced in the cell nucleus and surrounding structures and are then transported to regions of the cell where they are needed. Some substances spread through the cytoplasm by simple diffusion; others are carried by "motor molecules"—proteins that act as the cell's "beasts of burden."

In motor neurons, motor molecules must transport critical substances such as neurotransmitters the entire length of the axon, which can be up to a meter in length. This unusual requirement for transport may be another feature of the motor neuron that makes it vulnerable to disease. Recent research in both mouse models and humans suggest that disruption of axonal transport may play a role in ALS. In SOD1 mice, abnormalities of axonal transport have been found to occur early in life, although how the SOD1 mutations may cause this disruption remains unknown.

Research into axonal transport has been further stimulated by the recent discovery that an inherited form of lower motor neuron disease is caused by mutations in dynactin, a motor molecule. Although the symptoms of this disease are not identical to classical ALS (ALS involves both upper and lower motor neurons), they are similar enough that ALS researchers are now focusing more on dynactin and the breakdown of axonal transport, using mouse models that incorporate the dynactin mutation.

Glutamate Toxicity

The nervous system is constantly transmitting electrical signals to muscles via motor neurons. The electrical impulse travels along the motor neuron's axon; when it reaches the axon's end, the neuron "fires"—the electrical message is converted into a chemical message (a neurotransmitter) that quickly diffuses across the synapse to a second motor neuron (or the target muscle). The need to constantly control the movement of muscles, limbs, and respiration creates a heavy demand on motor neurons, making them, in theory, prone to excitotoxicity— toxicity resulting from an abnormally high rate of "firing."

One form of excitotoxicity is related to glutamate, one of the neurotransmitters used to carry the "message" across the synapse. Glutamate's involvement was suggested by the observation, first made twenty years ago, that the spinal fluid of many ALS patients contains abnormally high concentrations of the neurotransmitter. This suggests that too much glutamate is present in the synapse and surrounding fluid, a situation that would, in theory, cause excessive "firing" of the target motor neuron. The glutamate system remains a main focus of ALS research and drug development. Riluzole, the only FDA-approved ALS drug, is believed to act on the glutamate system.

Most of the glutamate produced at the synapse is removed by glutamate transporters, molecules located on the surface of astrocytes and neurons surrounding the motor neuron synapse. The transporter responsible for clearing 90% of glutamate in humans is EAAT2; located on astrocytes, EAAT2 is known to become depleted in ALS. The analogous transporter in mice is known as GLT1, which studies show also decreases in SOD1 mice. Researchers believe that a decrease in the glutamate transporters may contribute to the build-up of excess glutamate. Several products that target the glutamate system have completed clinical trials or are currently being tested.

Calcium Toxicity

Abnormally high rates of motor neuron firing may also lead to a second form of excitoxicity the build-up of too much calcium within the cell, which may damage neurofilaments or alter internal cell processes. The concentration of intracellular calcium normally fluctuates following the firing of a neuron, but these fluctuations are usually buffered by intracellular proteins. But the high rate of firing of motor neurons may make this type of neuron less efficient at buffering calcium and more vulnerable to this form of excitotoxicity.

Mitochondrial Damage

With their elevated energy demand and metabolic rate, motor neurons have a large number of mitochondria—organelles that form the "power plants" of all cells. Abnormalities in mitochondria have been found in several neurodegenerative diseases, including ALS. Studies in the SOD1 mouse model have helped researchers study these changes in detail, bringing this area of research to the fore in recent years.

Researchers have found that mitochondrial damage develops early in SOD1 mice, and that mutant SOD1 protein accumulates in the inner membrane of the organelle, although whether SOD1 causes damage to the mitochondria by chemical interaction or physically by these protein accumulations is not known. During the course of the illness, the outer membrane of the mitochondria becomes "leaky," releasing substances normally confined to its interior. One of these substances is cytochrome c, which is known to initiate programmed cell death. Several products targeting mitochondria are currently in clinical trials.

Oxidative Damage

The identification of the first ALS gene in 1993 spurred interest in the possible role of oxidation in the disease process. The gene discovered—SOD1—had already been extensively studied and one of its functions was known to be the elimination of free radicals produced by normal cell metabolism. This led to the initial speculation that the SOD1 mutation might cause disease by impairing the protein's antioxidant properties. This in turn resulted in a flurry of interest in antioxidant nutritional supplements such as vitamin E as possible treatments for ALS.

Subsequent research showed that mutant SOD1 does not, in fact, cause disease by altering the protein's normal antioxidant properties, but rather by giving the protein new, aberrant (and still undetermined) properties. Studies of antioxidant supplements in mouse models showed that they do not, in fact, significantly alter the course of the disease. Although oxidative processes may play a role in ALS, they are likely not to be the key contributor to disease.

Vascular Endothelial Growth Factor (VEGF)

One of the surprising discoveries in recent years is that a growth factor previously thought to be unrelated to motor neuron disease is potentially a risk factor for ALS. Vascular endothelial growth factor (VEGF) was previously known to be involved in the growth of blood vessels in response to changes in blood oxygenation. European researchers serendipitously found that when they deactivated the mechanism that switches the VEGF gene "on or off" in mice, the animals unexpectedly developed signs of motor neuron disease and paralysis. Although the mechanism by which VEGF causes disease is unknown, a preliminary European study showed that people who had one of three different variations (polymorphisms) in the VEGF gene were 1.8 times as likely to develop ALS, suggesting that VEGF may be a risk factor for the disease—it needs to work in combination with other genetic changes or influences from the environment and lifestyle to cause actual disease. Current research focuses on defining the mechanism by which VEGF contributes to motor neuron disease. Using gene therapy, investigators have successfully increased the levels of VEGF and improved survival in SOD1 mice.

Programmed Cell Death

Some diseases do not destroy cells outright, but rather trigger the cell's "self-destruct" program, known as programmed cell death (apoptosis), which scientists believe evolved to help rid the organism of unwanted or diseased cells. Although the disease processes differ in Alzheimer's, Parkinson's, and ALS, researchers believe that these neurodegenerative diseases initiate similar triggers of apoptosis. Research currently focuses on finding compounds that inhibit the programmed cell death pathway. Unfortunately, although several agents have been identified and have been shown to lengthen the lives of SOD1 mice, those tested so far are too toxic for use in humans. Identification of less toxic drugs that inhibit apoptosis would be an advance not only for the treatment of ALS, but for Alzheimer's, Parkinson's, and other neurodegenerative diseases as well.

A NEW FOCUS ON NON-NEURAL CELLS

ALS research has traditionally focused on motor neurons themselves, but researchers have recently observed that neighboring non-neuronal cells—astrocytes and microglia—are also affected by the disease. Recently published studies have shown that the relationship between neurons and non-neuronal cells is not only critical, but may, in fact, provide targets for therapy.

In the SOD1 mouse models used over the past decade, all the cells in the nervous system carry the same SOD1 mutation. In recent experiments, researchers genetically engineered a "chimeric" mouse—some of the animal's motor neurons, astrocytes, and glial cells contained the SOD1 mutation, others did not. Examining tissue samples under the microscope, researchers made an important observation—motor neurons carrying the mutation remained healthy when surrounded by cells that did not carry the mutation. In other words, diseased neurons survive in a healthy environment. In addition, the survival of the chimeric mouse was greatly extended compared to SOD1 mice in which all cells carry the SOD1 mutant protein.

These experiments support the therapeutic potential of using stem cell transplantation in ALS patients to introduce healthy cells into the area surrounding dying cells (See Stem Cell Therapy). Further refinement of the ability of researchers to selectively silence the SOD1 mutation in only certain cell types is allowing progress to tease apart each type's contribution to the disease, and how to target therapeutics appropriately.

Restoring Neurotrophic Factors

One of the known ways that astrocytes and microglia exert a protective effect on motor neurons is by secreting neurotrophic factors, critical proteins needed by motor neurons for proper functioning. These include ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), glial cell line derived neurotrophic factor (GDNF), and insulin-like growth factor-1 (IGF1). As ALS progresses, glial cells deteriorate and stop producing these neurotrophic factors. One of the therapeutic strategies currently being pursued is to artificially introduce neurotrophic factors to restore normal levels.

This approach has shown promise in the laboratory but has been less effective in clinical use, probably due to problems in delivering therapeutic amounts of neurotrophic factors to motor neurons. In the laboratory, researchers can add neurotrophic factors directly to cell cultures derived from patient samples or SOD1 mice. Under these conditions, the neurons are exposed to high concentrations of neurotrophic factors and respond positively. In clinical use in patients, therapeutic agents face two obstacles to reaching tissues in adequate quantities. First, when injected or taken orally, they become diluted in the bloodstream; second, like any agent targeting the nervous system, they must first cross the blood-brain barrier. Researchers believe that inadequate amounts of neurotrophic factors actually reach the target motor neurons. Improving delivery of neurotrophic factors and other agents is another motivation for pursuing gene therapy and stem cell therapy for ALS (see Gene Therapy and Stem Cell Therapy).