Amyotrophic Lateral Sclerosis

A report on the state of research into the cause, cure, and prevention of ALS

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Presented to the Department of Public Health, State of Massachusetts
By the ALS Therapy Development Foundation

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THE EPIDEMIOLOGY AND ETIOLOGY OF ALS

An Executive Summary[1]

Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurological disorder that causes weakness, atrophy, paralysis, and eventually respiratory failure due to the selective degeneration of neurons responsible for voluntary movement. Since its discovery in 1874, ALS has remained a medical mystery - researchers remain unable to identify any clear cause, cure, or effective treatment for the disease.[2] Throughout the history of research on the disease, epidemiological and public health research approaches have played a key role in informing policy and advancing knowledge on the disease. Epidemiological studies have helped change early perceptions of ALS as an extremely rare disease, and as a result the disease has become an increasing focus of policy interventions and research funding. Epidemiological and public health research perspectives have also provided important clues to the etiology of ALS, and to the biological, genetic, environmental, and social factors that impact individuals' risk of developing ALS.

This review provides a concise overview of Colin Pritchard & Sanjay Sunak's extensive technical report on epidemiological and public health research on ALS.[3] This review is primarily intended for a policy and advocacy audience and addresses the major findings and key arguments contained in that report.

In brief, Pritchard & Sunak provide compelling evidence that the incidence of ALS is increasing in the United States and throughout most of the Western world. Based on both the published epidemiological literature on ALS and original research, Pritchard & Sunak demonstrate that these increases follow distinct patterns – with ALS incidence increasing the most rapidly in women and

in younger age brackets than those in which have historically had the highest incidence of ALS. Some researchers have attributed apparent increases in the incidence of ALS to increased longevity among the general population, but Pritchard & Sunak argue that the observed patterns of increased incidence are at odds with this hypothesis. Convinced that increases in ALS incidence cannot be ascribed to changing age demographics alone, Pritchard & Sunak conduct an extensive review of the literature on biological, genetic, environmental and social factors that may have played a role in ALS' changing patterns of incidence over the past several decades. Pritchard & Sunak argue that no single research discipline can convincingly explain the changing patterns of ALS incidence. Instead, ALS appears to be influenced by a complex array of bio-socio-environmental dynamics; Pritchard & Sunak implicate a wide array of social and environmental changes over the past several decades in the increased incidence (and, hence, the underlying etiology) of ALS. If anything, Pritchard & Sunak imply that the continued focus on seeking a single, simple explanation for both the etiology and changing epidemiology prevents a more complex understanding of the disease. Pritchard & Sunak conclude their review by highlighting the importance of holistic public health and epidemiological approaches to future research on the disease.

1. Background on ALS

Although ALS was first identified in 1874, it is unclear whether the disease is a relatively modern affliction or one that has affected people throughout history. A number of historical documents describe diseases involving paralysis and atrophy, but given the wide range of conditions that could be described by these criteria it is difficult to determine whether or not these references are evidence of ALS.[4]

American researchers frequently use the term ALS to refer to not only classic ALS (degeneration of the lower and upper motor neurons), but also to several variant motor neuron syndromes, including progressive muscular atrophy (PMA, disease affecting primarily the lower motor neurons), primarily lateral sclerosis (PLS, disease affecting primarily the upper motor neurons), and progressive bulbar palsy (PBP, disease affecting the brainstem lower motor neurons controlling the facial, tongue, and pharyngeal muscles.) In the

United Kingdom and elsewhere, the term "motor neuron disease" is more frequently used to describe these four conditions.[5] Because ALS is the most common motor neuron disease, and because PMA, PLS, and PBP more often than not are re-diagnosed as ALS as the disease progresses and other groups of motor neurons become affected, both this review and research in general focuses primarily on classic ALS.

Roughly 90% of all ALS cases appear to occur at random (termed 'sporadic ALS'), while 10% are familial in nature. Generally, ALS is perceived as affecting men more frequently than women (a ratio of roughly 1.4:1.0), although in the early years after the disease's discovery women were perceived to be at higher risk for the disease.[6] Epidemiological studies set the incidence of ALS at between 10 and 30 cases per million population and the prevalence at between 30 and 50 cases per million population. Clinical and laboratory research descriptions of ALS have tended to favor the higher end of these estimates; ALS is commonly described as affecting between 5,000 and 8,000 new people per year in the U.S., with a prevalence of 30,000 cases nationwide.[7]

Although a number of hypotheses have been proposed over the years, there is still little conclusive data on the causes, contributing factors, or possible cure of ALS. Key findings of the past six decades have included the discovery of unusually high incidence of ALS on Guam and the Kii peninsula in Japan, the discovery of a series of mutations in Cu/Zn superoxide dismutase-1 (SOD1) responsible for familial ALS, and the identification of glutamate excitotoxicity as a possible target for therapeutic intervention (via the marginally success of the anti-glutamate agent Riluzole in slowing disease progression.) These and a wide range of more recent research findings have provided clues, but so far no major breakthroughs, in unlocking the mystery of ALS.[8] While current laboratory research on ALS will be discussed in greater detail in a later report, this summary focuses primarily on the role of epidemiological and public health approaches in understanding both the etiology of ALS and the implications of laboratory and clinical research on the disease.

2. Epidemiology of ALS

Because ALS is perceived as a primarily sporadic disease, changes in the incidence of ALS have traditionally been dismissed by researchers as an artifact of increased longevity. This hypothesis, known as the Gompertzian hypothesis after the early 19th century mathematician on whose work it is based, holds that as acute, early-onset diseases have declined and human longevity has increased, the incidence of a range of late-onset sporadic and genetic diseases has increased as a result of a greater number of people reaching the age at which such diseases typically develop. In order to evaluate the effectiveness of such a hypothesis at explaining changes in ALS incidence, Pritchard & Sunak examine the changing patterns of ALS incidence, prevalence, and mortality from both a public health/policy and epidemiological/statistical perspective.

2.1. Public Health and ALS

A number of phenomena over the years have raised the possibility of ALS as target of public health action. The most common reason for possible public health involvement in ALS has been the identification of disease clusters – particular geographic locations or social relations in which the incidence of ALS is markedly higher than in the general population. The earliest such clusters were those found on the island of Guam and the Kii peninsula of Japan. In both cases, rates of ALS incidence were between 50 and 100 times greater than average, although it was not clear that the Guamanian and Kii manifestations of the disease were necessarily classic ALS – Guamanians and members of the Kii peninsula appeared to suffer from a range of neurological disorders and symptoms (often referred to as the ALS/Parkinsonism-dementia complex or ALS/PDC) in near-epidemic patterns. A number of key research studies have focused on Guam and the Kii peninsula in an attempt to understand both the nature of the clinical syndrome (whether it is the same 'ALS' as observed in the rest of the world) and the reason for the increased incidence, with mixed results.[9] Since the 1960's, the incidence of neurological disorders on Guam and the Kii peninsula have been steadily declining with increasing Westernization, lending credence to theories that indigenous dietary practices or environmental factors may have caused the increased incidence of the disease.[10]

Dramatic variation in ALS incidence has

also been identified in less exotic geographic locations (including Massachusetts, Italy, and Finland.)[11] There has also been evidence of conjugal clustering of ALS (in which marriage partners are both affected by ALS – an occurrence that is highly statistically improbable.)[12]

2.2. Epidemiological Findings

In their comprehensive review, Pritchard & Sunak interrogated the major epidemiological studies on ALS in both the United States and elsewhere in order to develop a detailed picture of the changes in ALS epidemiology over the past three decades.[13] The key study which served as the baseline for their analysis was that of Noonan et al., who found that both the absolute numbers and the rate-per-million of ALS deaths has been increasing steadily since the late 1960s.[14] Noonan and colleagues also found that ALS deaths were increasing among women at a faster rate than among men. The evidence of the Noonan et al. study provides the first contradiction to a simple Gompertzian explanation of increased ALS incidence rates. If the increased numbers of ALS deaths were due simply to more people living to reach the typical age of onset of ALS, then the absolute *numbers* of ALS deaths would increase while the *rate* of ALS deaths would remain constant.

The increase in the rate of ALS mortality in the 'younger' age brackets (i.e. those below the average age of ALS mortality: 60) is particularly troubling. To understand the possible implications of this trend, Pritchard & Sunak turned to reported increases in ALS incidence among Gulf War Veterans.[15] A number of studies on the phenomenon of increased rates of ALS among Gulf War Veterans revealed that both military service in general AND deployment to the Persian Gulf were associated with a higher risk of developing ALS later in life.[16] Deployed troops had an ALS incidence of 57.5 per million while non-deployed troops had an incidence of 37.5 per million – both above the national average of between 10 and 30 cases per million. Pritchard & Sunak interpret these studies as demonstrating the clear involvement of multiple environmental factors in the etiology of ALS among relative young and early middle-aged patients.[17]

Continuing this line of inquiry, Pritchard et al. turn to international data in order to ascertain whether the changes in ALS

incidence (namely, increased rates of incidence across all age brackets, with the most pronounced increase among younger age brackets and women) are unique to the United States or can be observed in other Western nations.[18] In an original investigation, Pritchard et al. investigated mortality from neurological diseases in a variety of countries, including Australia, Canada, England & Wales, France, Germany, Italy, Japan, Netherlands, and Spain.[19] In England & Wales, Pritchard et al. were able to obtain specific data on ALS mortality, while in the other countries they relied on a broad category of neurological disease deaths due to the fact that WHO reporting does not separate out ALS as a diagnostic category.[20] However, Pritchard and colleagues examined multiple sclerosis deaths in the United States and found almost identical patterns to those observed with ALS, suggesting that similar patterns of increased incidence are occurring across the range of neurological disorders which fall under the WHO category employed in their analysis.[21]

In general, Pritchard et al. found similar patterns to those observed in the United States in many of the countries studied. For example, in England & Wales, ALS mortality increased across the board, but most dramatically among women and in 'younger' age brackets than those most frequently affected by ALS. Apart from France, the authors observed substantial increases in both male and female deaths in the 'OND' (other neurological diseases) category between 1979 and 1997. Rates of increase varied from country to country, but were most dramatic in Canada and the United States.[22]

Pritchard et al. observed similar patterns of increase in Mental Disorder Death (MDD) numbers and rates. (The MDD category includes Alzheimer's Disease, another neurodegenerative condition.) As with OND, U.S. rates of MDD deaths rose across the board, with the most dramatic increases among women and in the younger age brackets. These trends were true in a number of the other countries studied, with the notable exception of Japan, in which both OND and MDD neurological deaths in both genders fell for all age brackets under 74 over the period studied.[23]

In an add-on to this study, Pritchard continued his analysis up to and including the year 2000 (the previous study statistics had ended in 1997), and found that the

trend toward increased neurological deaths in Canada, England & Wales, and the U.S. continued between 1997 and 2000; in general, changes in neurological disorder deaths between 1979 and 2000 reached statistical significance in both Canada and the U.S. [24]

Overall, Pritchard and colleagues estimated that the increases in both OND and MDD death rates accounted for a total of more than 11,972 'extra' deaths per year (4,998 among 25-64 year olds and 6,974 among 65-74 year olds). [25]

Comparing deaths from all causes in the U.S. by gender, Pritchard et al. also found that the pattern of higher rates of increase among women was not unique to the neurodegenerative diseases. Rates of death from mental disorders, lung cancer, circulatory disorders, infarction, and other respiratory diseases all increased among women at a greater rate than among men (all statistically significant at the $p<0.00001$ level.)[26]

Based on this data, Pritchard & Sunak conclude that a simple Gompertzian approach is insufficient to explain the changing patterns of ALS and neurological disease incidence over the past thirty years. Not only have numbers *and* rates of ALS and neurological disease deaths increased since the late 1960s, but these increases have followed distinct patterns that are at odds with what might be expected under a strictly Gompertzian model. Pritchard & Sunak interpret these changing patterns of incidence to be the result of broad social, biological, and environmental changes rather than simple demographic shifts. The remainder of their extensive review is devoted to reviewing and evaluating possible influences on ALS incidence that have been proposed in the research literature on ALS. [27]

3. Bio-genetic factors

Although the exact etiopathogenesis of ALS remains unknown, the literature on possible biological and genetic factors in the disease is extensive. While later reports in this series focus specifically on contemporary research topics in ALS, a number of these research topics are directly relevant to the epidemiology of ALS because of their ties to the possible impact of genetic factors or exogenous biological pathogens on the risk of developing ALS.[28] For example, glutamate excitotoxicity (the death of neurons due to an excess of the amino acid

and neurotransmitter glutamate) is a key mechanism hypothesized to be involved in ALS etiopathogenesis. As Pritchard & Sunak demonstrate in their review, there is evidence in the literature that the glutamate excitotoxicity observed in ALS can be linked to both exogenous factors (i.e. diet) *and* genetic defects (not via a heritable DNA mutation, but through problems with the post-transcriptional RNA editing of a specific gene.)[29] The other major topic within the literature – the role of mutations in the Cu/Zn superoxide dismutase-1 gene in causing ALS – is also obviously genetic in origin. Mutant SOD1 is now believed to exert its influence primarily through a gain-of-function mutation.[30] While gain-of-function mutations are traditionally thought of as following a dominant pattern of inheritance (often with complete penetrance), mSOD1 shows a wide range of inheritance patterns in addition to dominant/complete penetrance, including dominant/incomplete penetrance, recessive, recessive with compound heterozygosity, and de novo mutations.[31] The wide variation in inheritance patterns of this single gene point to the possibility that certain cases of 'sporadic ALS' may have rare or as-yet undiscovered patterns of inheritance and gene interaction at their source. The wide range of other proteins and genes implicated in certain forms of ALS certainly lends some credence to this possibility.[32]

Latent viral infection or inherent genetic susceptibility activated by a 'trigger' later in life has also been proposed as a possible bio-genetic factor in the etiology of ALS.[33] The risk of death from ALS appears to be correlated slightly with poliomyelitis infection in childhood.[34] Despite this evidence in favor of a possible viral etiology, studies searching for specific evidence of a virus in ALS have been contradictory – a small number of studies have detected evidence of viruses in the spinal cord of some but not all ALS patients.[35] There is also evidence of a possible correlation between allergic disorders (e.g. asthma) and the later development of motor neuron disease. A study on the development of progressive muscular atrophy following radiation therapy hypothesized that the radiation therapy served as a trigger for the ill effects of a previous viral infection to become symptomatic.[36]

In the absence of a clear understanding of the genetic or viral origin of sporadic ALS

(or the possible interaction of genetic susceptibility, viral infection, and exogenous factors in bringing about the disease), twin studies may provide important data on the overall heritability of sporadic ALS.[37] Unfortunately, only one such study has been conducted, and found only 2 cases in which identical twins were concordant for ALS out of a total of 21 pairs in which one twin had been diagnosed with ALS (and no cases of concordance among fraternal twins), yielding a heritability of between 0.38 and 0.85.[38]

4. Socioenvironmental Factors

While bio-genetic factors appear to play a role in ALS – especially in familial ALS – it appears that bio-genetic factors alone may not be sufficient in explaining the rise in ALS incidence over the past thirty years. To further expand the range of possibly etiological factors, Pritchard & Sunak complete their review with an inquiry into research on socio-environmental determinants of ALS risk.[39]

4.1. Occupation

Studies on the relation of occupation and risk of developing ALS have revealed surprising and at times puzzling correlations between certain occupations and increased risk of ALS.[40] For example, an increased risk of ALS has been observed among pilots and navigators.[41] A study by Schulte and colleagues found an increased likelihood of developing ALS among electrical workers, construction workers, lawyers, judges, and archivists.[42] Interestingly, such studies typically consider only *increased* risk and the conditions of a particular occupation which might lead to that increased risk, while the possible neuroprotective conditions of occupations with lower correlations to ALS risk are rarely considered. Studies of occupational risk and ALS are ultimately inconclusive in that they cannot reveal whether the source of the increased or decreased risk is environmental, social, biological, or even psychological in origin.[43]

4.2. Electromagnetic field (EMF) exposure

Interest in the possible connection of electromagnetic field exposure to ALS risk has persisted for two decades, and has been bolstered by Schulte and colleagues' finding of an increased risk of ALS among electrical workers.[44] Electro-magnetic field exposure and electric shock has also been anecdotally linked with ALS-like symptoms.[45] More rigorous studies have yield mixed results. Most do find a slight correlation between

electromagnetic field exposure and risk of death from ALS, but not all such correlations reach statistical significance.[46] The correlation between EMF exposure and ALS was much more consistent than that between EMF exposure and Parkinson's Disease, and most studies found little correlation between EMF exposure and Alzheimer's Disease.[47] A number of methodological problems in EMF studies (including the need to estimate EMF exposure and account for the possibility of non-reported electrical shock incidents in certain occupations) need to be resolved as research moves forward. At the very least, researchers can convincingly argue that electric shocks are associated with a slightly increased risk of developing ALS later in life. [48]

4.3. Heavy metals and ALS risk

The toxicity of heavy metals is well known, and both lead and mercury poisoning are able to cause symptoms mimicking those of ALS. Pritchard & Sunak explore the literature on less dramatic concentrations of heavy metals, e.g. through occupational exposure, dental amalgams, and drinking water contamination.)[49] There appears to be little evidence that trace amounts of heavy metal contribute to the risk of developing ALS, although accidental exposure to very high (7x) levels of selenium was associated with a four-fold increase in ALS incidence in the area affected. [50]

4.4. Exposure to toxic chemicals

A number of studies have considered the possible impact on ALS risk of occupational or environmental exposure to toxic chemicals including solvents and pesticides.[51] In a study based on the Scottish MND register, for example, occupational exposure to solvents was associated with a 3.3:1 odds ratio.[52] Several studies found an overrepresentation of ALS among farmers and agricultural workers, suggesting a possible link between pesticide exposure and ALS.[53] Another study found that environmental risk factors like solvent exposure were especially pronounced among 'younger' cases of ALS (<59.)[54] This data fits with observed changes in incidence patterns over time – if environmental exposure has the greatest impact on risk among younger age brackets, then increases in exposure to a variety of environmental risk factors would be expected to cause the greatest increases in incidence among the lower age brackets.

5. Synthesis

Pritchard & Sunak conclude their review by reviewing the evidence in favor of a bio-environmental view of ALS etiology.[55] Based on the unusual patterns of increased incidence, the inability of biogenetic explanations to account for or explain the initiating cause of pathology in most cases of ALS, and the association of specific socio-environmental factors with increased risk of ALS, Pritchard & Sunak conclude that increases in ALS incidence are unlikely to have come about as a result of increased longevity.[56] This conclusion has implications both for etiological research on ALS and for public policy. The Gompertzian approach to explaining increased ALS incidence implies, in a sense, that increases in ALS incidence are due to the *success* of public health efforts. That is, by reducing mortality from (and in some cases eradicating) infectious and chronic diseases that affect young and middle-aged adults, public health efforts have ensured that far greater numbers of people reach the age at which ALS symptoms typically develop. Pritchard & Sunak argue, on the other hand, that increases in ALS incidence may well be a *target* for public health intervention, and at the very least a target for future research on the exact nature of the multiple biological, social, and environmental factors which appear to be contributing to alarming increases in ALS mortality among women and in younger age brackets. It is their belief that public health perspectives are needed now, more than ever, in the search for the cause, cure, and prevention of ALS.

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[1] This report is largely an executive summary of: C. Pritchard & S. Sunak, *The epidemiology and etiology of ALS: an interactive and interdisciplinary perspective* (ALSTDF, 2005). It is intended to summarize for an advance general audience the points made and argument advanced in that report. Additional comments and data which do not appear in Pritchard & Sunak's original report are indicated clearly in the references.

[2] Scholars vary on the date they ascribe to the discovery of ALS. The first use of the term "amyotrophic lateral sclerosis" in the published scientific literature occurred in 1874 in a paper by French neurologist Jean-Martin Charcot. However, the cases cited in that paper had been previously described in 1869 as cases of progressive muscular atrophy (a broad diagnostic category of the 19th century out of which Charcot later identified ALS as a particularly aggressive variant disease.) As early as 1864, Charcot had begun his work separating out unique cases of progressive muscular atrophy affecting both the lower and upper motor neurons. See: J.M. Charcot, "Deuz cas d'atrophie musculaire progressive avec lésions de la substance grise et des faisceaux antéro-latéraux de la moëlle épinière," *Archives de physiologie normale et pathologique,* 1869, 2:744-60; J.M. Charcot, "Des amyotrophies spinales chroniques," *Progrés Médicale,* 1874, 2:573-4.

[3] C. Pritchard & S. Sunak, *The epidemiology and etiology of ALS: an interactive and interdisciplinary perspective* (ALSTDF, 2005).

[4] Pritchard & Sunak cite examples by Keats and J.C. Harris as well as references to palsy in the Bible. (See Pritchard & Sunak, pg. 5.) The historical record on palsy, wasting palsy, and creeping paralysis – the names by which ALS would likely have been described before it emerged as a distinct diagnostic category – is extensive and beyond the scope of either this or Pritchard & Sunak's review. However, the diaries, letters, and other papers of selected patients suggest that ALS-like symptomatology can be observed at least several centuries before Charcot's clinical discovery.

[5] Pritchard & Sunak, p. 5.

[6] This latter fact was likely an artefact of the very small numbers of ALS patients on which such studies were based. See J.M. Charcot, "Des amyotrophies spinales chroniques," *Progrés Médicale,* 1874, 2:573-4.

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[15] Pritchard & Sunak, pp. 24-29.

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[20] Pritchard & Sunak, p. 31.

[21] *Ibid*.

[22] *Ibid*., pp. 32-34

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[24] *Ibid*., pp. 37-38.

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[26] *Ibid*., pp. 41-44.

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The Epidemiology and Etiology of Amyotrophic Lateral Sclerosis

An Integrated and Inter-Disciplinary Perspective

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A Working Report to the Department of Public Health, State of Massachusetts on behalf of the ALS Therapy Development Foundation, Massachusetts.

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THE EPIDEMIOLOGY AND ETIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS

An Interactive and Interdisciplinary Perspective.

Amyotrophic Lateral Sclerosis (ALS), first described by Charcot in 1864, is the "most common form of progressive motor neuron disease and arguably the most devastating."[1] More than 140 years after its discovery, ALS still has no known cause, cure or effective treatment, and is "relentlessly progressive, leading to death from respiratory paralysis…[within] 3-5 years."[2] The impact of such a prognosis on patients and their families is not difficult to imagine, and such a reality should spur our efforts to find an effective treatment, cure or prevention. Fortunately ALS is relatively rare, with an incidence of 1 to 3 per 100,000 people, although there is evidence this incidence may be increasing.[3]

The adage `no cause no cure', which has been one of the most effective medical and public health dictums of the past century, is particularly relevant to the search for an effective treatment for ALS. Over the years, an assortment of possible causal associations have emerged from different disciplines, including virology (the possibility of an early infection with a long period of latency), immunology (the possibility of certain allergic reactions leading to ALS), genetics (the possibility of a genetic predisposition to ALS developing from various molecular biological processes involving possible mutations, oxidative stress, neurotoxic metabolic processes), and environmental epidemiology (the possibility that the symptoms of ALS are caused by interaction with external influences such as exposure to heavy metals, solvents, pesticides, and electromagnetic fields, social factors such as occupation or residence, neurotoxin-rich diets, and even contact with animals or domestic pets.) With such wide variation in the research fields from which etiological hypotheses on ALS arise, it is perhaps understandable that relatively few studies, if any, encompass all these topics.

Interestingly, apparent increases in the number of deaths attributed to ALS have typically not been considered relevant to the search for the cause of ALS, and instead have somewhat controversially been dismissed as an artifact of increases in the longevity of the general population.[4] However, there is evidence that over the past 25 years the incidence of *all* neurodegenerative diseases has increased significantly throughout most of the Western world.[5] As with arguments on the etiology of ALS, here too there are unanswered questions: is the incidence of ALS, a disease arguably at the most severe end of the spectrum of neurodegenerative diseases, also increasing? If so, are external factors influencing these

changes?

The range of research relevant to understanding the epidemiology and etiology of ALS is considerable, and while this reviewer has virtually made a career out of crossing disciplinary boundaries in such problematic areas as sudden-infant-death-syndrome, suicide, child abuse, as well as studies in neurosurgery, cancer and neurology, this has proved to be the broadest challenge to date.[6] The extent of the material explored, and the time taken to assemble it, has meant that to meet the deadline, this study is in the nature of a working report. Nonetheless, this paper offers a comprehensive review of recent and current research literature on ALS and related topics, and is specifically designed to highlight the possible involvement of both bio-genetic and socio-environmental influences in the etiology of ALS.

Most importantly, this study provides new evidence that death rates from ALS and a range of other neurodegenerative diseases are rising, especially in the United States. These changes in mortality cannot be explained by a simple genetic predisposition/longevity hypothesis. It is hoped that this report will in some small way add to the understanding of this most dreadful disease, and aid in the search for improved care and treatment until the prevention of ALS can be achieved.

Declaration of Interests

Over the past twenty or more years there has been a need in medical research to declare any direct or inadvertent possible clash of vested interests inherent in the results of any research. We can state unequivocally that we are totally independent of any organization either in the USA or in the European Union, which might have an `interest' in this report. Our only concern is a disinterested search for greater understanding. Consequently, any conclusions drawn in this report are those of the authors and are not the policy or views of either the Department of Public Health, of the State of Massachusetts, nor of the ALS Therapy Development Foundation of Massachusetts.

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Finally, this report is dedicated to Andrew Child 1930-1997, whose gallantry in the face of motor neuron disease' overwhelming odds was inspirational, demonstrated what the human spirit can achieve, and how when the battle is lost it but means a renewal and re-definition of the struggle others must continue.

Setting the Scene: Disease of the Motor Neurons

Although disease of the upper and lower motor neurons was first described by Charcot in 1864, it is difficult to ascertain whether ALS is a recent human affliction or a historical disease. Progressive muscular atrophy (disease of the lower motor neurons) was known throughout most of the 19th century and was the original diagnosis of Charcot's first two ALS patients. The word `palsy' date back to at least 1582.[7] Although palsy is most often associated with `shaking palsy', the medical term for Parkinson's Disease prior to 1817 and a popular folk term for the disease throughout the 19th century, Keats used the word palsy to intimate degeneration and loss, and the Black American diarist JC Harris infers link between palsy and age when he said "wen de folks get ole an stricken wid de palsy they muss speck to be laffed at." Yet palsy is also mentioned in the bible and not specifically about the elderly, but seemingly about wasting and disability.[8] So ALS might well have presented in earlier times, and perhaps has long afflicted humankind. However, these broad descriptions of palsy can hardly be used to extrapolate an accurate diagnosis of ALS in bygone centuries; it is entirely possible that ALS may truly be a `modern' disease known only in relatively recent times, which could account for increases in ALS death rates in Japan coincident with the increasing westernization of Japanese lifestyles.[9]

Amyotrophic Lateral Sclerosis (ALS) and related syndromes are often referred to as Motor Neuron Disease (MND), and the two terms are used interchangeably in many textbooks.[10] Symptoms are due chiefly to the degeneration of motor neurons in the cerebral cortex, cerebellum, brain stem, and spinal cord.[11] Variations in the exact population of affected motor neurons leads to a range of 'types' of motor neuron disease, although Amyotrophic Lateral Sclerosis is by far the predominant type, accounting for 85% of all MND cases. The progressive bulbar palsy and progressive muscular atrophy forms of the condition often eventually progress to include both upper and lower neurons and must be recategorized as ALS.[12] In the United States, the condition is predominately known as Amyotrophic Lateral Sclerosis (ALS) and this term will be used throughout this report, even though MND is sometimes used by American researchers.[13]

Since 1990, the World Federation of Neurology `El Escorial' diagnostic criteria have been used for research purposes.[14] According to Harvard University Professor of Neurology Robert Brown, Jr., "simultaneous upper and lower motor neuron involvement with progressive weakness and the exclusion of alternative diagnoses" are essential to the diagnosis of ALS, but he and others accede a degree of diagnostic

`plasticity' - if not all of the El Escorial criteria are present, neurologists may use the term probable or possible ALS.[15] While the El Escorial criteria are acceptable for research, they have been criticized as not taking into account the vagaries of clinical practice.[16] The final disease process, death, erases any previous doubts on the diagnosis.[17]

Amyotrophic lateral sclerosis is still a relatively rare condition, and until recently was thought to affect about one person in 24,000 around the world, or between 20 to 66 people per million.[18] In the United States in 1975, the rate was estimated at about 64 per million.[19] Most cases of ALS appear to emerge in the later part of the fifth decade, and ordinarily the disease is quite rare in people under the age of 45. Between 1969 and 1998, the United States National Center for Health Statistics (NHCS) reported the prevalence of ALS to be only 0.9 per million for people under the age of 45. The risk of death from ALS increases with age – between 1969 and 1973, the NCHS estimated the prevalence of ALS at 34.5 per million for people aged 55-64, 58.3 per million for those aged 65-74 years, and a slightly lower prevalence for those over 75 years old.[20]

Because of this age-associated increase in prevalence, the condition has always been thought to be associated with ageing. Some textbooks even seem to imply that ALS occurs as part of the *natural* process of ageing - a view previously held about Alzheimer's Disease. Though once considered a natural consequence of aging, Alzheimer's Disease is now increasingly recognized as a distinct disease in which prevention of possible etiological factors should be taken seriously.

There are two patterns of ALS incidence: `sporadic ALS' (SALS), in which incidence appears to occur randomly and which constitutes about 90% of all ALS cases, and 'familial ALS' (FALS), in which the patient has blood relatives who have been diagnosed with ALS. Up to 10% of all ALS cases are familial in origin. With the exception of people living on the island of Guam and the Japanese peninsula of Kii, the incidence of ALS is thought to be similar throughout the world.[21] The disease typically affects men more frequently than women (a ratio of roughly 1.4:1.0) but as will be seen in the course of this report, this pattern is changing.[22] Because of the short survival time after diagnosis, the incidence to prevalence ratio is relatively low - an incidence of about 10 to 30 per million compared to a prevalence of 30 to 50 per million.[23]

One of the key problems of studying a rare condition is the limited patient population available for participation in clinical investigations: published papers on ALS from the mid 1960s to the mid 1980s have regularly reported extremely small sample sizes – from 168 participants down to only 2![24] In the late 1960's, the NCHS was reporting an average of 1100 deaths per annum from ALS, which may appear insubstantial in the face of the 1.9 million deaths in the United States at the time. However "every man's death doth diminish me" and it needs to be remembered that for every death there are probably at least 10 other people deeply affected by that one loss.[25]

The limitations of clinic-based samples are self-evident, hence the importance of the larger epidemiological / public health orientated studies. For example, there have been a number of studies surrounding apparent `clusters' of ALS which occur in a particular geographic location or as a result of particular social relations (e.g. marriage and cohabitation).[26] However, as Proctor et al found while

investigating a possible ALS cluster in Massachusetts, because of the rarity of the condition and the small numbers of patients involved in these 'clusters', it is often difficult to establish a statistically significant difference between ALS rates in the location of the cluster and in the broader population.[27] Occasionally these clusters have been attributed to higher rates familial ALS (FALS) genes in a particular population, or to unusual distributions of ethnicity in a particular geographic location – e.g. people of Swedish origins concentrating in Rochester, Minnesota.[28]

Initially there was considerable excitement surrounding the discovery of significantly higher rates of ALS among residents of the island of Guam and the Japanese peninsula of Kii. These increased rates of ALS appeared to be either related to a concentration of genetically affected families or linked to diet, but as will be seen, the implications of the increased incidence in Guam and the Kii peninsula are less clear cut than they at first appear.[29]

Later investigations emphasized the genetic sources of FALS and the association of mutations in the enzyme super oxide dismutase as chromosomal rearrangements had been identified.[30] In a concise explanation, Robert Brown Jr. brings together human and transgenic data to offer an hypothesis as to how excitotoxic neurotransmitters are involved in the degeneration of neurons in ALS, citing problems in cellular glutamate metabolism and mutations in the enzyme SOD1, which "detoxifies the free radical superoxide." How these SOD1 mutations exert a neurotoxic effect is uncertain.[31] Recent and contemporary research on this issue will be explored in Part Two in greater detail. ALS has also been linked to broad environmental causes, including heavy metals, pesticides, various industrial and agricultural chemicals, occupation, background electro-magnetic field exposure and diet.[32]

Further complicating the issue, there is no single or simple diagnostic test for ALS. Although the presence of a mutated SOD1 gene comes closest to a diagnostic test, mutations in SOD1 responsible for FALS are present in fewer than 3% of all cases of ALS.[33] Despite the El Escorial criteria, researchers await a break-through in diagnostic technology, which will lead to a better understanding of `cause then cure' paradigm. Dr. David Chad of the University of Massachusetts suggests that further development of contemporary neuro-imaging techniques (which measure the degeneration of motor neurons through motoneuron-specific metabolite levels) may prove an aid in diagnosis, but as yet, autopsy is still the definitive means of demonstrating motor neuron degeneration characteristic of ALS.[34]

For ease of presentation this report is schematically divided into three parts: the Epidemiological, the Biological and the Socio-environmental. This distinction, of course, is an artificial one - the ALS patient is an integrated whole and all aspects are relevant to his or her diagnosis, treatment, and experience. The end of this report attempts a synthesis of the epidemiological, biological, and socio-environmental data - an approach which more accurately reflects the reality of ALS.

PART ONE: EPIDEMIOLOGY AND PUBLIC HEALTH

The great insight of Durkheim was that changes in mortality rates reflect for better or worse, changes in society. For this reason, we begin by addressing the epidemiology of ALS in order to provide a context for etiological factors. Because ALS is associated with a late age of onset (diagnosis at an age younger than 55 is very unusual), early indications that the incidence of ALS was increasing were dismissed as an artifact of increased longevity.[35] This is related to the Gompertzian hypothesis that due to relatively recent increases in lifespan, people susceptible to ALS who would have previously died from other diseases, e.g. infections, are now living longer and are able to develop ALS or other age-related neurological disorders such as Parkinson's Disease (PD) and Alzheimer's disease (AD).[36]

At first glance, this concept appears to have the merit of `common sense' – the basis of the Gompertzian idea is that `competition' between various disorders leading to fatality has shifted over time. As people have become less vulnerable to diseases of youth and early adulthood, they have lived longer and have therefore been able to develop those late-onset diseases to which they are genetically susceptible. However, this explanation assumes a highly simplistic hereditary or environmental etiology of disease, infers that neurological disease is an inevitable consequence of ageing, and ignores the question of why neurological deterioration appears specifically in older people. It tacitly accepts as inevitable the development of ALS- like disorders.

The very foundation of medicine, however, is to question and challenge and not passively accept that which in earlier centuries has been seen as `natural and inevitable'. The Gompertzian hypothesis will be discussed in greater depth later in this report; though there is likely some truth to the hypothesis, it fails to explain why a range of disorders such as cancer and brain disease are increasing disproportionately and, crucially, starting in earlier age bands and differentially between the genders.[37] More importantly, the Gompertzian position infers that we need not worry about any possible environmental triggers that may exacerbate genetic `susceptibility.' Furthermore, it implies that the ultimately genetic nature of ALS risk exculpates society from responding to the increased rates of disease. The Gompertzian position, in a sense, is in conflict with the common cultural view that many illnesses are essentially random, i.e., there but "for the grace of God go I."

1. Public Health

1.1. Clusters of ALS

Amyotrophic Lateral Sclerosis has always been considered to be a relatively rare disease; for this reason, the discovery of apparent clusters of ALS cases has traditionally been met with great interest as, theoretically, these clusters provide researchers with an opportunity to test theories about genetic and environmental factors, and on possible interaction between the two. The discovery that certain parts of the Western Pacific – namely the island of Guam and the Kii peninsula in Japan - had significantly higher rates of ALS than elsewhere in the world spurred an intensive study of the manifestations of ALS in those regions. Guam and the Kii peninsula had an incidence of ALS that was between 50 and 100 times the Western World rate of 10-30 per million.[38] However, the type of ALS found on Guam does not conform strictly to the El Escorial criteria for the diagnosis of ALS; Guamanians affected by ALS-like symptoms suffered from an apparent combination of degenerative conditions which has since been termed the ALS - Parkinsonism-Dementia Complex (ALS/PDC.) From the start, the Western Pacific epidemiological discoveries were recognized as highly complex, not least because several studies identified a possible association between ALS/PDC risk certain dietary practices, namely the inadequate cooking and detoxification of cycad flour and a possible excess of manganese in the drinking water.[39] Figlewicz et al identified a possible link between mutations in cu/zn superoxide dismutase (SOD1) and cases of ALS/PDC.[40] (Mutations in SOD1 had initially been thought to be associated only with ALS) The 'natural history' of the ALS/PDC observed on Guam and the Kii peninsula differed significantly from that of simple ALS; ALS/PDC affected relatively young people, some as young as 30 years old, and having a less speedy fatal end point than the average 3 years post-diagnosis survival associated with ALS.[41] Matsumoto's earlier work reported that spinal cord neurofibrillary tangles were present in both ALS and PDCC, and later authors treated these conditions in Guam as inter-changeable.[42]

1.1.1. Neuroradiological study of patients with ALS and PDC on the Kii Peninsula of Japan

A fascinating study from Japan undertook a comparative, neuro-radiological study of patients with ALS and PDC on the Kii peninsula.[43] Kokubo and Kuzuhara's study started from the premise that the ALS and PDC observed on Guam and the Kii peninsula were different clinical manifestations of a single disease entity. Using Computed Topography (CT), Magnetic Resonance Imaging (MRI) and a SPECT scanner they studied 4 patients with ALS and 10 with PDC, utilizing the El Escorial diagnostic criteria for ALS. In both sets of ethnically Kii Japanese patients, Kokubo and Kuzuhara observed a decrease in cerebral blood flow, but only Parkinsonism-dementia patients demonstrated atrophy of temporal and frontal lobes on CT and MRI. Interestingly, 2 of the 4 ALS patients and 8 of the 10 PDC patients had family histories of their conditions, which as will be seen later, is a far higher rate of familial ALS than that reported in the West. This high rate of familial ALS among residents of the Kii peninsula may help highlight why such clusters exist – namely, residence in tight geographical area, probable limited social and geographic mobility, and restricted opportunities for genetic dilution. There are, however, methodological

problems with Kokubo and Kuzuhara's study. Despite the study appearing in a major research journal, the sample size is quite low and it is unclear exactly how the patients were selected for the study. There were also clear differences in the duration of the two conditions; apart from gender, little attempt was made to match prognostic and other factors between patients in the ALS and PDC groups. Most troubling is the fact that the authors were unclear on whether they were analyzing the ALS-PDC complex per se or whether the two sets of patients were different, so the reader was left uncertain as to their specific research focus. Nonetheless, there were some interesting results, namely that Parkinsonism-dementia complex patients appeared to have mild to severe frontal and temporal lobe atrophy, which increased over the duration of the disease, but that this did not occur in the ALS people. And of course the PDC patients were eventually associated at the later stages with growing dementia, whereas the reduced CBF in both sets of patients did not appear to be related to any obvious atrophy or clinical dementia.

Patients with ALS and PDCC on Kii showed a degree of hypometabolism of the frontal temporal lobes, resembling frontotemporal dementia syndrome but different from Alzheimer's Disease.[44] Kokubo and Kuzuhara concluded that in regard to "neuro-pathological and neuro-imaging findings," Kii people with ALS and PDC can be considered to suffer from a single disease entity (as in Guamanian ALS/PDC) and that Kii ALS/PDC "is a manifestation of a single taupathy of frontotemporal degeneration."[45] Despite their marked confidence, methodological problems remained, even in such as well established area of `clusters' of ALS type degenerative disease.

1.1.2. A perceived cluster of ALS cases in a Massachusetts community

Some authorities deal with ALS and PDC in Guam interchangeably, suggesting that clusters like the one observed on Guam are in effect `variants' of what might be described as classic `Western' ALS.[46] This was true of a very important study from Massachusetts in which Proctor et al explored an apparent cluster of ALS patients in the State.[47] Proctor's study measured person–years for an observed incidence of ALS deaths, and found the rate of deaths from ALS to be higher than the national average over the period of 1969-1985 (25.1 per million compared to an average of 16.4 per million.) However, the results were not statistically significant, in part because the number of cases in the cluster was quite small. Proctor's study nevertheless highlighted the problems of teasing out apparent clusters and possible environmental exposure to a range of potentially neurotoxic factors. The only way forward is to adequately explore potential links in larger populations of ALS type disease than are presently available for study.

1.1.3. ALS among the migrant population to Piemonte, Italy

At the same time, certain clusters can be explained by an unusually high geographic concentration of people from a specific ethnic or family background that is associated with increased genetic susceptibility to ALS. This was demonstrated in an Italian study which explored ALS in migrant and indigenous populations.[48] Chio et al based their study on the discovery of a North to South geographical gradient in mortality and incidence of ALS in Italy, studying ALS in Piemonte, in North West Italy, over the 35 year period between 1940 and 1975. They analyzed the effects of birthplace and migration on the risk of developing ALS between 1971 and 1990. Standardized Incidence

Ratios (SIR) were calculated for patients born outside Piemonte and using Piemonte-born people as a reference point. A total of 962 cases of ALS were identified, equivalent to an annual rate of 13.7 per million. The authors found that people from the three Southern-most regions of Italy and foreign-born people were significantly over-represented in the ALS cases than persons born in Piemonte. The authors' conclusion was that this finding may help explain the impact of migration on genetic and environmental factors.

1.1.4. Conjugal ALS in Southeastern France

An interesting cluster study came from France: Corcia et al discovered a cluster of what they termed 'conjugal ALS' – cases in which a man and wife both developed ALS.[49] The authors identified 9 couples who developed ALS between 1975 and 1992. Mean age of onset was 65 years and the patients had been married for an average of 25+ years. Twelve of patients had spinal onset ALS and the other six had bulbar onset. All the couples lived mainly in the southwest region of France, with 3 couples coming from the same `Department' covering roughly 6.5 million people. The employment history of 16 of the 18 patients was known; two patients were strongly exposed to solvents and pesticides and four had engaged in work involving intense muscular activity. Only one patient had a familial ALS antecedent. This study raises the question of whether a shared environmental exposure to exogenous factors or even a shared `infection' might contribute to the development of ALS.

Unfortunately, while Corcia et al mention that 80% of ALS patients have evidence of enteroviral nucleic acids in their neurons, it is not clear whether they are speaking of their couples or simply making a general point related to other work.[50] Conversely, as Corcia points out, despite the rarity of ALS the collection of 9 conjugal ALS couples could be nothing more than a chance association. Without statistics, modern medicine would virtually be in the pre-scientific era but it needs to be remembered that statistical probability is just that: `chance' results do happen. This was dramatically experienced by this author in a prospective regional study of subarachnoid hemorrhage in a general population of some 4.5 million people. In the first 10 months of the 24 month study, 52% of our SAH patients were male, totally at odds with all the textbooks. By the end of the two years, the consecutive cohort had settled down to textbook numbers of 35% male and 65% female SAH patients.[51] Chance results do happen - especially when relatively few people are affected by a particular condition.

1.1.5. Spatial clustering of ALS in Finland

Sabel et al from Scotland, with Finnish colleagues, resolved the numbers problem by examining 1,000 ALS patients who died in Finland between 1985 and 1995, a rate of 19.1 deaths per million (at the higher end of the range compared with most West countries.)[52] Sabel et al examined both place of birth as well as place of death. The populations ranged between 350 to 870 thousand people from a general population, which was fairly static over the time the majority of ALS cases were born. The authors were able to identify two significant population clusters which contained over a third of their ALS cases but only 24% of Finland's population, and were able to demonstrate that these clusters were related to place of birth and not just to place of death. The identification of clusters at both birth and death is etiologically important and clearly at sample sizes this large appears to be reliable. Of course, if one considers that ALS might have a long latency then establishing that such clusters exists gives weight to such etiological considerations.[53]

Elswhere, Peltonen et al. have argued that Finland's history and population concentrations led to "major genetic bottlenecks and the possible enrichment of rare genes," since a number of dominant and recessive disorders exist in higher frequency in Finland than elsewhere in the world.[54] Thus there are grounds to suspect that "genetic factors may be at play in the present study."[55] Conversely says Sabel "it remains entirely possible that environmental factors also explain the clusters."

The authors accept the weaknesses inherent in the study, namely the loss of population during the war years and the impact of changing boundaries, and acknowledge that perhaps the study would have been better if it had examined smaller geographical populations, which might have helped identify smaller potential clusters. Sabel et al. were nevertheless able to establish that there is a statistical link between the risk of death from ALS and place of birth and death, which may be relevant in understanding the etiology of this serious disease.

1.1.6. ALS & PDC of Guam: 40-year follow-up

Perhaps the most important modern study of ALS clusters emerged from Plato et al.'s population cohort studies of patient-control registries.[56] Plato's work in Guam goes back more than two decades, and the two studies reported upon were designed to be prospective. Each patient in the Guamanian registry was matched with controls and with first-degree relatives in both ALS & Parkinson's Disease cases. They were able to obtain data on all ALS (or lytico as it is known by on Guam) and PDC (Guamanian name: bodig) cases, initially from the former NINDS Research Center and then from the extant Guam Clinical Core team who re-commenced ALS research in Guam after the closing of the NINDS center. This yielded five-year data from the case register by age and gender from 1940 to 1999. In addition they traced and interviewed relatives and family members by asking about either Lytico or Bodig (ALS or PDC) and, crucially, counted and measured ALS and PDC separately, as well as identifying a ALS/PDC group, but these were always in the minority, and the ALS/PDC classification has now been abandoned.[57]

Between 1940 and 1999 there were 436 people diagnosed with ALS, 278 males and 158 females, and 493 PDC patients, 312 males and 181 females. Between 1940 and 1944 males aged 50-54 had an incidence of 2220 cases of ALS per million but by 1995-99 this had fallen to 250 per million; in females aged 55-59 the rate was 2130 per million in the early 1940's but by 1995-99 had fallen to 210 per million, illustrating the dramatic but gradual decline in ALS incidence over the period studied. Nonetheless, these rates are still considerably higher than those seen in the rest of the world. Unlike the gradual decline in ALS rates, PDC rates fell in the early years covered by the study, peaked in the 1960's, fell back and then increased in the 1980's, and finally declined again by the end of the study period.

A major strength of Plato et al.'s work is that they took a broad socio-cultural historical view, charting the changes in population, and identified the changing pattern of ethnicity in the general Guam population, which for example from 1960 contained 52% of people of `Chamorro' background and only 13% Filipino, but by 2000 had shifted to 37 % and 26% respectively. During the same time period, the numbers of 'whites' declined and other groups increased from 4% to 30%. This dramatic change in population ethnicity, and the marked increases in population over the period, appears to have influenced the ALS and PDC outcomes.

A very important element of the study, which readers have to dig for, is that the data concentrated entirely upon the predominately Chamorro people; however, there would likely have been some inter-marriage among the island's different ethnic groups. To complicate matters further, beginning in the 1960's the Chamorro people experienced marked socio-cultural and dietary changes, as the majority of the population moved from a rural, fresh vegetable, high fish diet to a Western lifestyle and a diet high in fat, `Westernized' foods – completely changing any underlying environmental factors which may have been influencing the ALS and PDC rates. The authors are confident that despite the rapid ethnic changes in Guam "much more generational time would be needed to bring about the observed changes in incidence," if the changes in ALS incidence were due to genetics alone.

However, a familial component to changing ALS rates in Guam cannot completely be ruled out. When parents, siblings and off-spring of patients were considered, there were significantly greater observed than expected frequency of ALS and PDC amongst them. Moreover, between 1958 and 1998 there were 90 new cases amongst patients' first-degree relatives but only 25 new cases among controls' first-degree relatives. However, there was also a significant excess of observed over expected frequency of ALS amongst spouses, suggesting non-hereditary factors may be implicated in familial diagnoses on Guam. Plato and his colleagues cannot fully account for these marked changes but conclude that the "registry results strongly suggest that ALS and PDC are familial disorders, [but] offer no support for the involvement of simple Mendelian dominant or recessive genes," while the higher "risks of developing the disease in the spouses of patients tend to support the involvement of extraneous factors, with or without the hereditary involvement in the etiology." Looking at the differences between the Guam ALS and PDC results, Plato et al concluded that "It is possible that PDC represents a phenotype that can be triggered by a lower degree of exposure to exogenous factors than ALS, accounting for the slight divergence of incidence of these conditions in recent years."[58]

It is extremely difficult to critique a body of knowledge that dates back more than 30 years and self-evidently has made a major contribution to efforts to untangle the complexities surrounding ALS as well as other neurological degenerative diseases, but the perfect research paper has yet to be written. One problem of taking the climax of this work was that the authors assumed previous knowledge and this left some questions about the methodology a little unclear. Simple omissions might be clearer if readers had access to all previous papers, but no mention was made of the type of questionnaire used in the field study - e.g. whether it was open-ended, structured or standardized in any way. Also, some ALS and PDC patients had a `range of neurological' tests' while others did not and we are not told why and what they found or whether the data from these tests was relevant. It is also unclear, except to the most fastidious reader, that the study is only focused on the indigenous Chamorro people, and of course it would be virtually impossible, not least in terms of cost, to trace details of inter-marriage and what effect, if any, this may have had on genetic weighting. The study also fails to address whether there were ALS or PDC cases recorded in people from the other ethnic groups residing on Guam. Perhaps the biggest weakness of the study was the failure to specifically examine or discuss in these recent papers the possible contribution of excessive consumption of cycad, a nut

containing an N-methyl-D-asparate agonist.[59] Plato et al did, however, stress the marked change from a fish and fruit and vegetables to more `Westernized' fast food and fat diet in the last 20 or more years. Interestingly, the "rapid Westernization of Guam", which many in the `environmentalist' field might read as a pejorative term, is here associated with a reduction in ALS incidence.[60] Nonetheless, the Plato team's research is world class, has made a major contribution to our understanding of the epidemiology of ALS and, like all good research which deepens our knowledge in one area, leads to further questions. It is the epitome of the value of `cluster' research. Plato's work demonstrates that while there are clearly genetic factors in the development of ALS and PDC, the changes in incidence over the past thirty years are the result not simply of genetic shifts, but of "radical socio-economic, ethnographic and ecologic changes."

1.2. Season of Birth and ALS

Another issue emerging from a public health perspective is that of season of birth, which might well be a complicating, confounding variable for place-of-birth studies. At the extreme end of the place-of-birth spectrum lies the concept of `fetal origin of disease.'[61] In brief, this hypothesis is based on research which showed that variation in babies' pre- and post-natal care up to 12 months was associated with a range of disorders as adults, with low birth weight being associated with negative outcomes later in life. Some of these disorders did not manifest themselves until mid-life and onwards. In part this is linked to pre-natal life-style and social conditions of the mother but may also be linked to season of birth. Earlier studies of neurological disorders reported a link between season of birth and with the risk of developing Alzheimer's disease later in life.[62] Seasonal factors have also been associated with other `mental diseases' such as autism, multiple sclerosis and schizophrenia.[63] Kondo & Fujiki, from Japan, looked at seasonality and ALS and found seasonal trends – those born in the spring had a higher risk of developing ALS while those born in the autumnal months had a lower risk of the same.[64] The study, however, was based on relatively small numbers and was case-based.

1.2.1. Season of birth and ALS in Switzerland

Ajdacic-Gross et al. undertook a whole-population cohort study of ALS in Switzerland between 1969 and 1993; during this time period the diagnosis of ALS was quite stable as it was based upon WHO ICD 8 categories.[65] They identified 2178 deaths on the ALS code of 348.0 [ICD 8] and had as high as 92% unequivocal ALS diagnosis, which while considered very high, is on reflection perhaps to be expected as Chio et al found similar levels of accuracy on death certificates.[66] Ajdacic-Gross and colleagues were able to establish the monthly frequencies of all births in Switzerland from 1901-1940, which would cover all people aged up to and below 92 years. Thus apart from missing any ALS cases in people over 93, their cohort was inclusive and comprehensive.

Over the period the expected number of `ALS' births, in comparison to all other births, ranged from 164 to 191 per month from 1901-1940, establishing a statistical `expected' and `observed' actual frequency. They found that people were more likely to develop ALS in later life if they were born in the spring/early summer months of March-June and less likely if they were born in the autumnal and early winter

months of September –December.

Ajdacic-Gross et al proposed a range of possible implications for their findings. They suggested that for some, ALS can be acquired very early, which infers that in some cases there may be a very long latency period, as with schizophrenia, multiple sclerosis and possibly Alzheimer's disease.[67] This `fits' the `Fetal Origin of Disease' theory, demonstrated in a range of conditions such as cardiovascular disease, diabetes, acute myocardial infarction and depression.[68] Moreover, Ajdacic-Gross et al felt that their study confirmed the Japanese findings of Shimura et al and Kondo & Fujiki.[69] However they recognized that introducing the notion of a `latency period' for ALS, added to the "enigma of ALS".

Nonetheless, they emphasized that seasonalities are essentially a singular event per se, rather than what may occur later as an accumulative effect. The fact that they found a relatively narrow peak period perhaps indicates where we should be looking for the key risks, whether pre or post-natal vulnerability. Ajdacic-Gross et al. are very open about the limits of their findings and the problems of interpretation of pre, peri or post the season of risk. Among their concerns was the possibility of a correlation with high periods of infections, especially viral infections , citing the important British study of which found co-terminosity between poliomyelitis and ALS and other such studies.[70] Furthermore, the authors raised issue of possible seasonal `pollutants' such as agricultural chemicals, light dependent processes, allergens etc. This is the problem with seasonal studies, they do not take us very far, but simply add to the clues of etiology.

The Ajdacic-Gross et al study is an important one but has a number of weaknesses. Crucially, the socio-economic, technical and common health care situation of people born before 1940 is enormously different from that of people born post the Second World War. People living prior to World War II had no antibiotics, for example, ate a different type of diet, and engaged in very different forms of work and exposure as the majority of Swiss population at that time would be rural or semi-rural. Moreover, Switzerland's population would be barely five million pre 1940, which while as populous as some U.S. states, is not very large. Because of the relatively limited social and geographic mobility of those times, any concentration of `genetically' vulnerable residents would skew the findings. Another issue is climate change. Pre 1940 Swiss temperatures, relative to today's, would be far colder for longer periods of time than temperatures post 1970's.

The seasonality issue does appear to be a factor, but how important may well depend upon not only season of birth, but place of birth, whether this is linked with genetic clustering, viral infections and/or social background. Thus the clue may lay in seasonality being an early risk factor which potentates later risks. Some authors are convinced of the primary importance of seasonality in the development of such conditions as schizophrenia, epilepsy and the neuro-degenerative diseases, such as ALS, Parkinson's and Multiple Sclerosis.[71] With MS, ALS and Parkinson's appearing to have an excess of spring births, and epilepsy and cerebral palsy being more linked to summer births. Sabel et al sought to explore the issue of apparent long latency between emergence of ALS symptoms and the exposure to potential environmental risks, but concluded that while seasonality and place of birth are factors, more research is needed to understand the problem.[72] Researcher have yet to identify what the key interactive features are between possible predispositions, timing and exposure to risks.

1.3. Smoking and ALS

One of the greatest recent achievement of Public Health was to alert and change the Western world's attitude to smoking, as it was found to be strongly associated not only with the cancers, but a range of respiratory and cardiovascular disease. The subsequent major reduction in smoking has been associated with significant reductions in deaths from these conditions (see below, WHO 2005).

Initially early studies found no clear association between ALS and smoking.[73] Moreover there appeared to be evidence that smoking was `protective' against Parkinson's disease.[74] This seemed counterintuitive as tobacco smoke contains many elements responsible for oxidative stress, an etiological factor associated with ALS and a biological process that is detrimental and damaging to motor neurons.[75] However this and other studies were based upon quite small samples. Nelson et al revisited the issue and found a distinct statistical association between smoking and subsequent ALS.[76]

1.3.1. ALS and Smoking in Washington state and New England

Kemal et al in a case-control study in New England found a strong association with people who had smoked and subsequently developed ALS.[77] The authors identified 109 cases of ALS during 1993-96 and contrasted them with 256 matched controls, examining respondents' smoking habits, ranging from never smoked, former smoker to current smoker. Amongst their cases 61% were male, with more college graduates amongst the non-smoker controls. Amongst their cases 6% had a familial history of ALS and relatives of cases had three times the ALS rate than relatives of controls, reflecting the FALS type and genetic susceptibility. However they found that having smoked cigarettes some time increased the risk of ALS by 70%.

From the other side of continental America, Nelson et al undertook a population based control study in Western Washington State.[78] Over a 4-year period they identified 161 cases of ALS and matched with 321 controls. The cases were initially differentiated between lower motor neuron syndrome (progressive muscular atrophy) and progressive bulbar palsy, as the two clinical sub-types of classical ALS but excluded any cases of only upper motor neuron involvement i.e. primary lateral sclerosis. Similar to Kamel et al they found more cases amongst less well educated people, while both research teams had examined any association with alcohol but found none. Nelson et al also found an almost two fold increase in risk of ALS in people who had ever smoked, but differentially they found a three-fold increase with ALS and current smokers. However Nelson made no comment on their gender distribution which was 55% male and 45% female cases. One wonders whether this finding may in part account for the North West to South East gradient of ALS cases in the USA noted by Noonan et al.[79]

Before offering a brief critique of the above two papers, we turn to an ingenious prospective study of Weisskopf et al, which utilized a case-control approach to analyze data from the prospective Cancer Prevention Study II cohort.[80] The study was based on more than one million people aged 45+ who had been enrolled in the CPS study, starting in 1982. Unfortunately ALS mortality was only codified beginning in 1989 but they have a complete data set up to 1998 of

people within the cohort who died from ALS. These results were juxtaposed against a whole series of demographic and behavioral information, including smoking habits.

Similar to Kamel and Nelson's studies, the ALS cases were less likely to have graduated college. Weisskopf et al's was also found no link between the risk of developing ALS and alcohol consumption. However, the study did find a significantly increased risk of ALS among current women smokers. The odd thing about this study, based upon 50 states, was that there was a higher ratio of female to male ALS cases (47% to 53%) than the textbooks would lead us to expect.

The current estimate of ALS annual mortality rates in the USA, based upon our extrapolation of Noonan et al data, is 18.25 per million.[81] The 621 ALS cases observed over the 10 years in the Weisskopf study was based upon a final total of 1.098 million people, suggesting to us a rate of 57 deaths per million per annum. Bearing in mind how recent the Weisskopf et al results are, might this be an inadvertent indicator of recent increases in ALS death rates in the USA? Or conversely, could this increase reflect some feature of the American Cancer Society's cohort recruitment practices?

Our critique of these three important studies concerns more or less the same factor, namely that current and previous cigarette smokers are in a nutshell more often less educated people. In Britain, smoking is almost a surrogate indicator for social class and all three papers seemed to avoid exploring in depth the occupational history of cases and controls. In view of all three studies mentioning pesticides, chemical and neuro-toxins possibly contained in cigarettes, another interpretation might be that, in addition to being more likely to smoke, people from the less advantaged socio-economic groupings might also be more likely to be exposed to deleterious environments than people with a college education.

Nonetheless, we can probably agree with Nelson's conclusion that "cigarette smoking is consistent with current etiological theories that implicate environmental chemicals and oxidative stress in then pathogenesis of ALS." [82] As it is clear that the range of toxic substances contained in tobacco smoke is considerable - as many as 3,800 have been identified.[83] While we can not account for the `protective element' linked with Parkinson's, we note Weisskopf's comment that - "although the concentrations of individual organochlorine pesticides in tobacco have declined dramatically since 1970 [though not fast enough perhaps for people over 45] cigarette smoke may remain a source of exposure to other agricultural chemicals that could directly injure motor neurons." Common sense, which at times can be misleading, suggests that if the person lives in a relatively degraded social environment, smoking becomes an additional personal pollutant, compounding whatever genetic and environmental factors affect them and increase the risk of ALS. However with both Nelson and Kamel's study, around 30% of ALS cases never smoked. Yet another example, if one is needed, of the complexity that surrounds the etiology of ALS.

2. Epidemiology

Before exploring epidemiological factors we need to bear in mind that mortality statistics over time may well cover significant periods of environmental, social, and economic change, which in effect may mean that we are examining populations of differing origins, even though they were born in the same country and region. Both in North America and the European western countries, in terms of diet and food and life styles there were be no or very few food preservatives pre-1950, and the type of air and other pollutants people would be exposed to were markedly different prior to the 1960's. During the period lead was in and out of petrol, and petro-chemical pollutants changed in nature and intensity. There were also changes in the background electro-magnetic fields to which people were exposed - for example there would be few televisions in every home until after the 1960's, no computers in every home until the late 1990's, not to mention the whole range of electric gadgetry that has appeared in recent years. As we saw in the Plato et al studies, even in relatively tight populations such as the island of Guam, there were major changes in the background and the type of lives that people led.[84] Reduced rates of ALS were reported in Guam, whereas over the past decade there have been reports of rising incidence and increases in deaths from ALS around the Western world.[85] We now turn to examining these changes.

2.1. Identifying ALS epidemiology

The big debate has been and still is: are the increases in ALS mortality due to the changing environment and/or genetic factors? Or are they due to the interaction of new exogenous factors upon people with a genetic susceptibility? Or is the rising incidence an artifact due to more accurate diagnostic techniques? We can deal quickly and easily with the argument that the increases are due to `diagnostic' factors. ALS at the end–point of death is relatively easy to diagnose and even major `critics' of an etiological hypothesis tied to the changing environment accept that the increase in ALS deaths is not an artifact of diagnostic techniques..[86] However, based upon the relatively narrow time period of International Classification of Disease [ICD] 9th edition, which covers the years 1979-1998, there is evidence that proportionately, throughout most of the Western world, there were more deaths from neurological causes in recent years than ever before.[87] Included in these categories were ALS and the other degenerative conditions, such as Parkinson's Disease, although the WHO does not report specifically on either condition. These results therefore raise the issue to what extent are the changes due to new or increased exogenous factors, the environment, or mainly related to increases in potentially susceptible populations living longer?

Riggs analyzed the USA adult mortality rates from 1900-1986.[88] He argued that the emerging changes were not so much a factor of environmental influences but rather reflected the rising proportion of people living longer, and brought to the fore the Gompertzian hypothesis that there is an inherent `competition' between causes of death. Consequently new patterns of mortality are essentially a "manifestation of the competitive nature of human mortality."[89] Thus as the

prevalence of diseases such as infections decreases and the quality of public health and lifestyles improves, people are succumbing to late-onset diseases to which they are genetically susceptible, such as ALS and cancers, which in previous times they would not have lived long enough to develop.[90] This seemed to be confirmed by other researchers looking at data in England & Wales, Italy and Spain.[91]

The key to this perspective is that there is a chain of potential underlying genetic problems the effects of which will only manifest themselves once a person reaches a certain age – an achievement which may be prevented by other, early-acting forms of disease. There is self-evidently some common sense to this, for example a child born in the Victorian era faced infant mortality rates similar to what occurs in many parts of Africa today; if children were endowed with a tendency towards developing familial ALS, proportionally fewer children would live long enough to develop the condition.

However there are a number of weaknesses in this approach, not least the way in which some people have interpreted Riggs work to mean that ALS and Parkinson's Disease are the inevitability of longevity – a conclusion which has produced a degree of inadvertent ageism. This line of thought can lead to an attitude that `we don't need to bother because its part of old age' and as such, ALS and other late-onset neurological disorders are `natural'. This ignores the fact that medicine should and always has challenged what is `inevitable' and `natural', and at the same time crucially forgets that even conditions which affect those who are genetically predisposed to them very often need an environmental trigger.

Sir Walter Bodmer, the eminent geneticist and former Director of the Imperial Cancer Research fund neatly summarized this in regard to cancer: "despite the genetic predisposition, the tumor requires an environmental [trigger] --- as we have known for 200 years that chemical abuse leads to cancer."[92] Hence just because there may be more people carrying a genetic susceptibility to ALS, it does not mean we have to ignore exogenous factors. We agree with Riggs & Schochet that there has been a major reduction in ischemic heart disease and stroke, but disagree that "there is no need to invoke intrinsic etiological alterations in the environment," as comforting such a position might be for some people.[93] There are a number of flaws in Riggs position. For example Riggs used the Gompertzian analysis to account for the rising cancer mortality in the USA between 1962-87 and argued that this was "evidence against environmental causation." Yet he virtually ignored the fact that since the 1960's, quite apart from the major improvements in the treatment of cancers and the extended five-year survival rates, the incidence of malignancy in younger adults, under 30 has been rising in most Western countries.[94] This trend continued until we saw the impact of reduced smoking positively influencing the prevalence rates.[95] Nonetheless, if you attend a chemotherapy center you will find that one in seven patients is less than 35 years old, which was unprecedented 30 or more years ago.

We may being unfair to the Gompertzian approach, but some use it as a defeatist position in regard to ALS and the other degenerative diseases, and it is an over-simplification of the surely redundant argument of `nature versus nurture.'[96] Moreover, the time period that Riggs examined ALS data is, in genetic terms, quite a short period and the Gompertzians make no attempt to account for this apparent speeding up of the genetic influence in the national gene

pool. Certainly in the field of cancer development and treatment, the simplistic Gompertzian paradigm has been challenged. Retsky et al showed that their results in regard to breast cancer did not fit the approach, and Bru et al demonstrated that tumor growth had different dynamics than would be predicated by Gompertzian analysis.[97] Equally, Riggs' position still leaves us with the questions about what exogenous factors influence genetic susceptibility, for after all familial ALS still only accounts for 10% of cases. The Gompertzian position assumes that there is as yet an undiscovered genetic factor. However, though Riggs' analysis acknowledges that longevity does extend the period of time over which an organism is exposed to possible accumulative `insults' to which the genetically predisposed are vulnerable, Riggs virtually ignores the variation between the genders that have been found both in regard to incidence of cancer and neurological disease.[98] Indeed Riggs paper arguing that changes in cancer was a demonstration of the Gompertzian trends, in regard to England & Wales was simply wrong, as shown by the massive change in incidence and prevalence of cancer in women under the age of 65 years.[99]

If there were few or little environmental factors impacting on ALS and the other neuro-degenerative diseases, then there would be little difference in the male:female ratio of the incidence of neurological disease. Yet in nine of the ten major Western countries - Australia, Canada, England & Wales, France, Germany, Italy, the Netherlands, Spain and the USA (Japan is the exception) - there are statistically significant and disproportionate variations in the increased rate of female neurological morbidity and mortality.[100] Following Riggs, when we juxtapose neurological morbidity with that of the malignancies, we again find a marked gender change within as short a period as 30 years: cancer deaths among women were considerably lower than those among men in the 1960's, but the gap between the two genders narrowed in the succeeding decades.[101] Indeed, by the 1980's we found that in England & Wales, for the first time since records began, we had more women with cancer then men and an even far bigger increase amongst under 34 year old women compared to men- this does not fit the Gompertzian analysis.[102] Why this should be so for either the cancers or the neurological disorders is unknown, but it is self-evident that women's life styles have changed much more than men's during the period studied, as women are now almost equally present in the general work force, which was simply not the case barely 30 years ago.[103] So the issue of the epidemiology of ALS per se is still highly pertinent and is not a statistical artifact. Even though ALS mainly affects citizens over the age of 60 years old, this is not a reason to disregard this terrible condition.

2.2. Counting ALS Mortality in the USA 1969-2000

The major and key epidemiological population cohort study of ALS in the USA is that of Noonan et al who explored 30 years epidemiology of ALS.[104] Focusing only on ALS mortality, the authors examined the period between 1969-1998, by geographic region of the USA, and by age, ethnicity and gender. This provides a clear indicator of the underlying ALS morbidity, especially when considering that the majority of patients die within an average of 3 years post diagnosis.[105] Noonan and colleagues used the term Motor Neuron Disease [MND]

because the illness will progress to include both upper and lower motor neurons and will be ultimately diagnosed as ALS. Moreover, MND is the sub-code in the International Classification of Diseases [ICD], which was the basis for their analysis of mortality files from the USA National Center for Health Statistics [NCHS], with all codes relating to ALS corresponding to MND.

Age-adjusted death rates were calculated for sex, ethnicity, age and birth cohort and place of death over the thirty-year period. This enabled the authors to contrast rates over time in a defined North, Middle and Southern regions of continental USA, based broadly upon lines of latitude. These varied a little to incorporate the states which over-lapped somewhat, in particular California was included in both the middle and southern regions. The Northern states were those above the 41st and 42nd parallels and the Southern states were those below the 37th latitude, while longitude measures divided the country into West, Mountain, Central and Eastern areas.

Noonan et al.'s study, an analysis of a total population of multiple-cause mortality files, merits in-depth methodological analysis as its results indicated increases in ALS deaths over the study period for most age bands and ethnicities, and also for both genders. By utilizing multi-cause death data, Noonan et al. overcame the problematic issue of accuracy of death recordings by including in the cohort any deaths that which mentioned ALS. It spanned the ICD 8 (1968-78) and the ICD 9th edition (1979-1998), which might be considered problematic as not all the codes across the two periods match up. However, their multi-causal mortality approach they overcame this problem, while Pritchard et al had to stay within the ICD 9 framework [1979-98] because of their focus on international data.[106] In regard to the accuracy of the diagnosis Noonan et al were dealing at the end of the process, which is less problematic in the late stages. Even in the late 1960's or early 1970's, because of the relative rarity of the disorder it would be highly unlikely for a specialist neurologist *not* to be involved, so Noonan's figures must be considered diagnostically sound.

Noonan et al. were able to extrapolate data for White and African Americans throughout the 1968-1998 period but only from 1992 onward for American Hispanics. The states of New Hampshire and Oklahoma had to be excluded, as they did not collect data related to people of Hispanic origin. Over the period they identified 105,318 ALS deaths, an average of 3,511 deaths p.a. For every recorded mortality there are moat likely another ten other family, friends, and relatives involved, meaning that almost a million US citizens were affected by this disease during Noonan's study period. And it is easy for researchers to forget that our statistics are about people and medicine needs remember the distress that families feel, especially with such a condition and its intractable prognosis, and theories of both simple Mendelian and more complex inherited susceptibility.

The ALS mortality rate for the first five year period 1969-73 was 16.1 per million for men and 9.5 per million for women, but by 1994-98, these rates had risen to 21.7 and 14.8 respectively. This is a significant increase, equivalent to rises of 35% in men and 56% for women over the period. Tables 1a & 1b are extrapolated from Noonan and show the number and rates of ALS deaths per million.

Table 1a. Annual U.S. ALS deaths by gender

	Average Annual Deaths			TotalDeaths
	M	F	p value	
1969-73	1295	956		11258
1979-83	1825	1536		16804
Ratio 1969-83	1.41	1.61	<0.02	1.49
1994-98	2255	1999		23074
Ratio 1979-98	1.24	1.30	-	1.37
Ratio 1969-1998	1.74	2.09	<0.0002	2.05

Our calculations show that women's ALS deaths went up significantly more than those of their male counterparts between 1969-83; women's ALS deaths also increased more than men's in the following period, but this difference between genders was not statically significantly so. Over all between 1969 and 1998 female ALS deaths rose significantly faster than male deaths ($p<0.0002$). It is important to note the variation in rates and numbers of men compared with women. Noonan et al failed to highlight that in their analysis, but changes in the gender distribution of deaths is an important feature when considering the arguments that stem from the Gompertzian perspective.

Table 1b shows the rate changes that the above numbers represent. It is interesting to note how the rates fluctuated over the three five -year end points. Noonan focused on ten-year age bands of both men and women combined, mainly 45-54, 55-64, 65-74 and so on, but even the under 45 year old age band showed a small but significant increase moving from 0.9 per million to 1.2 per million (a 33% change.) It should be noted however, that throughout the study period and for each age band, men have always had higher rates of ALS than women. Regrettably Noonan did not separately analyze the age bands by gender. It is suspected that this would be because the under 55 year old rates would be quite small. However, as will be seen, meaningful information can be obtained when extending the analysis along the age/gender axis.

In regard to ethnicity the biggest proportional increases were in White American women, then White men followed by combined African American at 29% and Hispanic people having virtually half the increases of White Americans.

Table 1b. Annual average ALS death rates in the U.S. by gender (rate per million)

	Male	Female
1969-73	16.1	0.95
1979-83	20.2	12.6
Ratio 1969-83	1.25	1.33
1994-98	21.7	14.8
Ratio 1979-1998	1.07	1.17
Ratio 1969-1998	1.35	1.56

Nonetheless they did increase, albeit over a shorter period. In all groups, the older age bands had higher rates of ALS deaths, with particularly large jumps between the 55-64 and 65-74 age bands, but declining in the 85+ group, which is a fairly typical finding in other countries and continents and is at variance with the Gompertzian hypothesis.[107]

Geographically, there was a Southeast to North-west gradient of ALS death rates - the highest death rates were recorded in the Northwest at 22.2 per million between 1989 and 1998, while in the Southeast rates were 15.7 per million over the same time period. In both regions, these rates were higher than those in the pre-1989 period (17.0 per million and 11.9 per million, respectively.)

Noonan et al were very circumspect in their interpretations of this important paper. They addressed the issues of the Gompertzian hypothesis, showing that this is addressed by direct age-standardization rates, which cannot explain the observed increases over time for the specific age groups.[108]

Another critique of evidence that ALS and other neurological diseases are increasing is that greater professional awareness of ALS, with improved technical sophistication means better and more frequent ALS diagnoses, thus artificially distorting the reported ALS rate.[109] Noonan et al.'s results very neatly rules out this possibility, as the highest rates of ALS were in the Northwest U.S., which had the smallest numbers of neurologists per population. The greatest proportion of neurologists are in the Southeastern states of the U.S., which have the lowest rates of ALS diagnoses.

Noonan et al. were stringent in emphasizing that ALS remains a "disease of unknown etiology" but mention a range of environmental risk factors sometimes found to be associated with ALS, ranging from heavy metals, solvents, agricultural chemicals, and pesticides to physical activity, trauma and smoking. However, the authors argue that their results cannot say which, if any, of their observations give weight to these causal hypotheses. What they are confident is that they have shown that there has been an increase in ALS deaths in the USA in both genders and across the major American ethnic groups, and that both genetic and environmental factors are implicated. Their study provides support "for the need for rigorous hypothesis driven research to examine environmental and/or behavioral for ALS in combination with factors of genetic susceptibility."

Some epidemiologist might be a little unhappy at Noonan et al conflating ICD 8 and ICD 9, which infers that they de facto accept a degree of plasticity in the diagnosis, rather than taking the later El Escorial criteria which of course was only established relatively recently.[110] They might have answered this critique by looking at the recent NCHS review of the revision between ICD 9 and the new ICD 10 edition which WHO and the USA have utilized since 1999.[111] Unfortunately we know of no equivalent analysis for any differences between the ICD 8 and 9 editions. However, Noonan et al. appear to have resolved this problem by their multi-causal approach of taking any death certificate, which mentions ALS type syndromes and including them in the rates over the period.

For us the biggest criticism was that when the authors produced numbers and rates for the range of age bands they combined data on men and women, which obscured a major socio-cultural finding of the disproportionate increase in female ALS deaths. Throughout the entire Western world countries the life styles of women have changed considerably. In effect, women have been living `more like men' and when this happens they `begin to die like men', i.e. they come into the workplace and the wider environment and disproportionately are exposed to new environmental factors and new family planning systems.[112]

Another issue with the Noonan study is that the authors did not seek to explain the flattening or even decreasing rate of ALS deaths among men from 1984-88 to 1994-98, nor did they investigate why the biggest increases in ALS deaths occurred between the 1974-78 and 1979-83 period for both men and women. Whether this was because of the changes in the ICD 8 and 9 recording is unsure, though of course there were

steady, albeit slight increases over each 5 year period up to 1994-98 for both gender and all age bands.

It should be remembered that people born after World War II will have had a very different exposure to changing environmental factors, than those born before the war. How, if at all, this change might have affected ALS deaths we simply do not know. Nonetheless, this socio-historical factor should not be ignored when trying to grasp the subtlety of these changes and the unquestioned rises in ALS deaths, not all of which can be accounted for by the Gompertzian hypothesis.

Noonan et al perhaps wisely were very cautious regarding any further interpretation of their data but it can be argued that they have failed to consider some implications of their results. For example while all the three ethnic groups have seen increases in ALS death rates, these increases have been disproportionately experienced by White Americans. Bearing in mind the varied genetic susceptibility associated with ALS, there is long standing evidence that White Americans generally have higher incomes and richer life styles than African and Hispanic Americans.[113] Thus, though it is more likely that Americans from lower socio-economic groupings will have been exposed to different environments, such as pesticides and heavy metals, might this mean that some of the etiological factors linked to rises in ALS is related to more affluent life-styles? On the other hand however, smoking does appear to be a risk factor related to ALS, and being a smoker is almost a surrogate category for `working class' and therefore this is more likely to affect less affluent Americans.[114] This is yet another example of the complexity involved in understanding the etiology of ALS. If, for example, there is a lower genetic susceptibility to ALS among African Americans, then the smoking factor may be mediated by this variation in genetic susceptibility. Certainly, it is known that some white Europeans have a very different genetic bias that of the USA.[115]

In brief, the Noonan study has established beyond a reasonable doubt that - despite the Gompertzian hypothesis and the problems of recording deaths over time and across regions of the continent of the United States - there were very real increases in the mortality and morbidity of ALS disease throughout the twentieth century.

2.3. An Opportunistic Experiment: ALS in Gulf War Veterans

Taking a public health and epidemiological approach, there is merit in exploring the de facto opportunistic experiment of reported increases of ALS and other neurological diseases in American and British Veterans of the 1991 Gulf War.[116] It is recognized that this is somewhat controversial.[117] However, the claimed outcomes of increased ALS in predominately young men under the age of 45 years, is a valuable opportunity to explore possible environmental and genetic interactive influences over a relatively short period of time.[118]

Coalition troops serving in the Persian Gulf in 1991 were exposed to a cocktail of anti-bacterial and viral vaccines and counter-chemicals in order to protect them from the feared threats of bio-chemical, chemical and nuclear weapons; these troops may also have been exposed to chemical and radioactive battlefield debris.[119]

While American and British forces undoubtedly were exposed to apparently `friendly counter measures,' many of the actions had never been used before in emergency and battle situations. Perhaps as is usual with military activity, there was and is a particular mixture of secrecy and confusion over the exact nature of what substances our troops received, apart from any chemical and radiological debris to which they might have been exposed. What did emerge was an unprecedented level of amorphic symptomatology in particular conditions apparently related to chemicals which, amongst others signs, produced apparent neurological outcomes.[120] The cases of ALS identified amongst Gulf War veterans raise the questions of whether these other atypical and often inexplicable health problems are part of a continuum of pathology of which ALS is the extreme example.

The first of two important papers which may inform the wider debate about the etiology of ALS is that by Haley.[121] Haley makes the point that almost by definition active service personal will be outside the usual age bands in which we might expect ALS to be found, predominately under 45 years old. The author asked the pertinent question of whether cases of ALS in this young group might have been "caused or triggered prematurely, in genetically susceptible persons by unusual environmental exposures during the war?" Haley undertook a comparative case-controlled type survey by identifying the observed numbers of cases of ALS in the Gulf War population and comparing them with the expected frequency in the age and gender related general population. He obtained his data on military personnel who had received a diagnosis of ALS and who had seen active service from the US Department of Defense. In 1999 the Veterans Administration service identified 40 cases of ALS but these cases were not made available to Professor Haley and consequently he sought cases via ALS and Veterans associations, and having permission to obtain medical records, was able to establish the ALS caseness of each person identified in a four year follow-up. He applied stringent criteria and determined ALS case status based on the El Escorial criteria.[122] Haley's study covered the period from1991 to 1998, and therefore may not account for all cases which have or will someday arise in Gulf War Veterans. The baseline for the study was the USA ALS rate for the 1979-1998 period in the 20-44 year old age group. NCHS provided the data on 3,460 cases, averaging 152 per year.

Haley identified 20 cases of ALS in former Gulf War personnel in people under 45 - 19 men and one woman. Strikingly, not a single one of the "20 patients have a family of ALS or other neurodegenerative disease. Thirteen developed symptoms of the `Gulf War syndrome' (undiagnosed illness) during or soon after returning from the Gulf War and years before developing the first symptoms of ALS."[123]

Haley lists the years patients in which patients were diagnosed and they increased from 1991, the highest, five, being in 1998. The Standard Mortality Ratio [SMR] of age-matched people was 2.27, whereas the Gulf War ALS cases rate was 5.72, statistically significant at the <0.006 level. Does this indicate possible environmental triggers or is this simply a false cluster? Even though the base population was in excess of 696,000 service personnel serving in the Gulf, the relatively small numbers of ALS cases raises the question of whether Haley's result might be a statistical fluke. It is not thought so, but before critiquing this study, a parallel study by Horner et al will be examined; Horner was able to obtain access to all the 40 ALS cases identified earlier.[124]

Horner led a team of fifteen most eminent researchers involving neurologists and statisticians from across the USA, in effect undertaking a population controlled study. Their study resolved one problem that Haley did not address, namely whether military personnel are `typical' of the general population, for the case can be made that they are not. Horner et al had access to information on the deployed military personnel in the Gulf War (696,118 people) and compared them with contemporaneous non-deployed personnel, (1,786,215 people) and the differential ALS rates between the two.

Horner et al.'s tudy had been initiated in response to expressions of concern by the Chief of Research in the Department of Veteran Affairs; earlier reports of excess ALS cases were initially disputed, hence the prestigious panel of experts set up led by Professor Horner.[125] A nationwide epidemiology case ascertainment study design was used to identify all new ALS cases amongst Gulf War veterans. The authors were able to study all service personnel, including the Reservists and National Guard and were able to confidentially assign them to those 696,166 people who were deployed in the Gulf during the war and the 1.786 million military personnel who did not serve in the Gulf. Like Haley, the authors used the El Escorial criteria to diagnose ALS, which excluded 15 people who had a range of rare but quite severe subsequent neurological disease (e.g. demyelinating disease, spinocerebellar degeneration, various neuropathies etc).

Out of the original sample of 516 people suspected of having a neurological condition, initially 40 were unlocatable, 313 were screened as ineligible or refused to participate and of the central 163 screened eligible, they lost a further 41 who either refused or were not reviewed, yielding an effective 107 ALS verified cases, with 15 neurologically damaged persons found not to have ALS, determined either by death certificate or medical records.

The authors were also able to differentiate which services the 40 ALS cases came from, namely Army 53%, Air Force 23%, Navy 18% and Marine Corps 8% and contrasted these results against the 67 ALS cases that emerged from the non-deployed military personal. Those deployed in the Gulf had an almost two-fold greater risk of developing ALS than the non-deployed and "a significantly elevated risk occurred for those who were active duty military, Air Force and Army. Elevated but non significant relative risks were found for deployed Reserves/National Guard, Marine Corps and Navy personnel."

In effect, there was an overall ALS incidence of 57.5 per million among `deployed' troops versus 37.5 per million among non-deployed troops. Among the 107 cases of ALS, 101 were aged 44 and under. Out of the combined 107 ALS cases, 6 had a familial history of ALS (6%) (two of whom were in the deployed group), which matches the familial rate often cited and provides a degree of external validation for the integrity of the cohort.[126] The low rates of familial ALS among the cohort studied emphasizes the special nature of the `sporadic' ALS category, and suggests that multiple external factors may have contributed to the excess of ALS observed in these people. The authors concluded that "potential etiologies must be viewed to include genetic predisposition and a range of possible environmental exposures. Although we found significant elevated risks in the two service branches – Air Force and Army – this may not enable researchers to narrow the array of possible risk factors." Thus both Horner and

Haley come to the same conclusion: that the etiology of ALS is still unknown but involves both genetic and external factors. These two studies are a useful counter weight to the way Riggs' use of the Gompertzian factor has been interpreted.[127] The null hypothesis in both these studies can conclusively be rejected as there was an excess of ALS cases amongst young adults who were exposed to a possibly interactive and unique changed environment.

We can extrapolate across papers and place Horner et al alongside Noonan et al in order to determine relative rates of ALS by age. [128] Unfortunately Noonan does not provide gender rates separately for the age bands but these can be estimated by determining a ratio of male: female rates on Noonan's all age data for the 1994-98 period (a ratio of 1.47:1 male to female ALS cases.) Horner provides us with the total ALS rate for both deployed and non-deployed personnel, but did not separate out the data by gender.

If we correct for the male predominance in the USA national rates as given by Noonan and multiply the observed rate by this ratio (1.47) we obtain a conservative calculation of male rates of ALS in the American general population. The total cohort of Horner et al rates are compared against the estimated male general population rate. The deployed versus non-deployed risk ratio in Horner ranged from 1.92 to 2.5 for those an active duty. To obtain a conservative estimate of active Gulf Vets rates per age, we mulitiply the total military cohort by 1.92. Conversely to correct for this and obtain a rate for the non-deployed by age we divide by 1.92, the results are seen in table 2 below

Even with conservative estimates, the total military cohort had virtually twice the general male US population rate in the three age bands. When we correct and estimate the deployed and non-deployed rates by age, we see that the former Gulf War ALS rates are almost 4 times than ALS rates of American males. Thus the Gulf Vets under 45 had 4.9 times the ALS rate than the estimated general USA male population. For those aged between 45-54, it was 3.8 times and for the over 55's it was 4.4 times the general population. All this strongly indicates environmental factors in the etiology of ALS in people under 64 years old.

These two studies, despite having different

Table 2. Estimated Comparative ALS rate U.S. General Male v. Military & Gulf War Veteran Populations (rate per million)

	<45 years	45 - 54	55 – 64
General population x1.47	1.76	25.28	60.27
Total Military Cohort	4.5 [2.34]	49.9 [25.98]	138.4 [72.1]
Ratio: Military: General Population	2.56:1 [1.32:1]	1.97:1 [1.03:1]	2.29:1 [1.20:1]
Gulf War Veterans x1.92	8.6	95.8	265.7
Ratio: Gulf v. General Population	4.9:1	3.8:1	4.9:1

Source: Data extracted from Horner 2003, Haley 2003. Rates in brackets are corrected estimates from total Military Cohorts.

controls, general population, and other non-deployed military personnel, have established that there was an excess of ALS patients emerging from among those who saw active service in the Gulf War cohort. However, the weakness of both studies is that they did not explore the socio-economic and psychological background of the service personnel.

At one level the fact that the military personnel were deemed fit to serve, means that to a slight extent they are not typical of the general population. With military personnel there is also a greater likelihood of their being recruited from less affluent socio-economic groups, demonstrated in the fact that 20.3% of all military personnel were designated as black Americans, which is above the portion of African Americans in the general population. If members of the military are disproportionately recuited from less affluent economic groups, this could also mean that military service is disproportionately associated with less advantaged life styles and with greater exposure to industrial pollution or agricultural chemicals prior to military services.

As was shown earlier there is evidence that smoking is associated with increased risk of ALS, which itself is associated with belonging to the lower socio-economic groups, indeed in Britain smoking is almost a surrogate indicator for belonging to the working class.[129] Hence, members of a military cohort are more likely than the general population to have disproportionately experienced a range of socio-economic and environmental disadvantages. This in turn may have made military personnel predisposed to be affected by any additional external factors they may have become exposed to in the Gulf War.

Another feature of military life which may have been a predisposition to developing ALS is the fact that a number of the personnel would have been exposed to weapons and other chemicals in their routine working world. Military personnel are also traditionally more physically active than their age peers; prior research has suggested that extreme physical exertion may influence the risk of developing ALS.[130] While there is little doubt that there is an excess of ALS patients amongst Gulf War veterans, it should also be remembered that the non-deployed group cohort of military personal *also* had slightly higher rates of ALS than those found in the U.S. male general population.[131]

The Haley and Horner papers did not consider was the psychological impact of the extremes of military conflict. Although there has been an inference that Gulf War veterans have been inadvertently encouraged to consider themselves ill, *a la* Gulf War syndrome, this has largely been successfully refuted in recent years.[132] In earlier years, service personnel were encouraged not to speak about their experiences. As a former active serviceman, the author of this review knows that some conversations can only be had with other ex-service personnel. Today, however, the reality of post-traumatic stress disorders following conflict is now appreciated and becoming better understood. Kang et al noted the excess levels of PSTD and 'Chronic Fatigue Syndrome' among servicemen; chronic fatigue syndrome is itself another ambiguous and problematic condition which theoretically has bio-physical origins, albeit of unknown etiology.[133] The question that no paper has yet asked is whether the `shock' of war itself might be an additional trigger for developing ALS in those already susceptible to familial or sporadic ALS. Animal modeling is very common in efforts to understand ALS, it may be that the single extreme `fright' that some species are exposed

which then provide a life-time conditioning reflex, might be a another feature in the complex array of exogenous factors in ALS.[134]

What can be said with confidence is that the Gulf War studies on ALS indicate multiple environmental factors contributing to the etiology of ALS at least in relatively young and early middle-aged people (20-44 years).

2.4. Degenerative Neurological Disease in the USA and other Major Western countries.

To seek to place U.S. ALS results in a wider epidemiological context and to discover whether the changes found in the U.S. were mirrored elsewhere, we sought to obtain comparable ALS data from a range of other countries. Unfortunately, because of the relative rareness of ALS, it is not separately reported upon by the WHO. Apart from ALS data from England & Wales, it was not possible in the time available to obtain specific data on ALS in more countries. However in view of the association of other degenerative neurological conditions with ALS we will explore all neurological deaths over the ICD 9 period (1979-98), and will also juxtapose neurological mortality against all other major causes of death in the U.S., contrasting the results with Canada and England & Wales.

2.4.1. ALS Deaths in England & Wales

ALS deaths in England & Wales are reviewed over the same period as the Noonan et al study by way of contrast, seen in Table 3a & 3b, though the designation is given in Britain is `Motor Neuron Disease' (MND.)[135] Unfortunately, age and gender-related MND data was not available until the 1979-99 period (all ICD9 to match the U.S. data), but we can report on the national figures for the 1969-73 period.

Compared to the equivalent rises in ALS in USA male of 35% and 56% for females, the Anglo-Welsh rates increases more than doubled over the period, 116% males and 106%, females. It should be noted however, that similar to Noonan's finding there was a flattening of male rates in the 45-64 year band and a slight decline in the females, the biggest rises being in the younger groups of 15-44 and the 65-74 year olds. However, both the USA and the British over 75 year old rates were actually lower than the younger 65-74 year olds. This trend is not what would be expected if the Gompertzian process were operating throughout the age

Table 3a. Average MND cases per million population, England & Wales. 1969-71, 1979-81, 1997-99.

	All Ages		15-44		45-64		65-74		75+	
	M	F	M	F	M	F	M	F	M	F
1969-73	13.0	10.0								
1979-81	19.2	14.9	1.6	0.7	35.3	27.4	93.0	63.0	96.0	47.0
1997-99	29.4	20.6	1.7	0.90	32.6	20.6	103	83	140	86
Ratio: 1969-73 v. 1979-81	1.48	1.49								
Ratio: 1979-81 v. 1997-99	1.53	1.38	1.06	1.29	0.92	0.75	1.11	1.32	1.46	1.83
Ratio: 1969-73 v. 1997-99	2.26	2.06								

Data Source: OPCS & ONS 1971-2001, ICD 8 1969-1978, ICD 9 1979-1999

Table 3b. Average total cases of MND, England & Wales. 1969-71, 1979-81, 1997-99.

	All Ages		15-44		45-64		65-74		75+	
	M	F	M	F	M	F	M	F	M	F
1969-73	364	295								
1979-81	461	376	17	7	190	155	187	163	86	88
1997-99	644	547	27	10	190	125	221	195	202	215
Ratio: 1969-73 v. 1979-81	1.27	1.27								
Ratio: 1979-81 v. 1997-99	1.38	1.44	1.59	1.43	1.001	0.81	1.18	1.20	2.35	2.44
Ratio: 1969-73 v. 1997-99	1.77	1.85								

Data Source: OPCS & ONS 1971-2001, ICD 8 1969-1978, ICD 9 1979-1999

bands.

Thus over the ICD 9 period, Anglo-Welsh male numbers of ALS deaths rose 38% and female ALS deaths rose 44%. Most notably, ALS deaths in the 15-44 band rose 59% and 43% respectively; the number of ALS deaths across all age in the 1997-1999 period were 77% and 85% higher, respectively, than those in the 1969-1973 period.

In terms of the Anglo-Welsh *rates* of ALS in the ICD 9 period, all age death rates rose 53% and 38%, while between 1969-83 and 1997-99 the death rates doubled in both genders. Between 1979-1981 and 1997-1999, there were increases in death rates among 15-44 year olds and 65-74 year olds, but the death rates in the 45-64 age band declined slightly.

Consequently, we can say with confidence that both in the USA and in England & Wales there have been substantial rises in ALS mortality over time – far greater than any increases that might be attributed to a greater awareness of ALS or a greater likelihood to diagnose ALS.

In view of the fact that the etiology of ALS remains uncertain, it seems appropriate to examine the specific ALS changes in the context of whether there are changes in other neurological conditions that may give us a clue to the nature of any possible exogenous factors related to ALS.

To examine international comparative data requires a common and uniform standardized method of collating data, hence the value of relying upon WHO annual mortality reports. WHO reports specifically on four `neurological' types of mortality in ICD 9 (1979-98): Epilepsy (225), Multiple Sclerosis (223), Mental Disorder Deaths (MDD), which includes dementia (21), and `Other Neurological Diseases (OND) which also includes Diseases of the Sense organs (221,222,224,229,23,24). It is noteworthy that while ALS and Parkinson's disease are subsumed in the OND; neither are reported on separately by the WHO.

Table 4 illustrates the changes over the ICD 9 period in Multiple Sclerosis deaths in the U.S. At the beginning of the period there were 1429 average MS deaths per year, while by 1998 this number had increased to an average of 2,382 per year, a numerical increase of 67%. Death rates from MS rose from a combined 6 per million in 1979-81 to 9 per million by 1998. It might come as some surprise that ALS death numbers and death rates of ALS (4254 per annum and 18.25 per million) exceed those of M.S. This suggests that ALS mortality merits being reported on

Table 4. U.S. Multiple Sclerosis Cases. 1979-81, 1996-98.

	All Ages		15-44		45-64		65-74		75+	
	M	F	M	F	M	F	M	F	M	F
Number of Deaths										
1979-81	540	889	132	198	164	264	93	165	30	63
1996-98	806	1576	211	415	209	344	187	334	101	258
1999-00	940	1808	245	461	238	438	197	351	130	326
Ratio: 1979-81 v. 1996-98	1.49	2.03	1.60	2.10	1.27	1.30	2.01	2.02	3.37	4.10
Ratio: 1979-81 v. 1999-00	1.75	2.03	1.88	2.33	1.45	1.66	2.12	2.13	4.33	5.17
Rate per 100,000										
1979-81	5	7	12	16	16	23	14	18	8	10
1996-98	6	12	13	24	20	30	23	33	18	26
1999-00	7	13	14	25	21	25	24	35	21	32
Ratio: 1979-81 v. 1996-98	1.20	1.71	1.08	1.50	1.25	1.30	1.64	1.83	2.25	2.60
Ratio: 1979-81 v. 1999-00	1.40	1.08	1.17	1.56	1.31	1.09	1.71	1.94	2.63	3.20

Data Source: WHO 1980-2005

separately by the WHO.

It should be noted that female rates and numbers of MS deaths increased significantly and the numbers of ALS rates were also significantly raised for women compared with men. It should be noted that the peak age band for MS deaths is 65-74 and in the earlier periods the 55-64 age band had higher MS rates than the 75+ age band.

The latest WHO data was published in February 2005 and takes the U.S. data up to 2000. The table shows the continued increase in this neurological disease in the USA up to and including the year 2000. While not reaching statistical significance, women's MS rates increased compared to their male U.S. counterparts. In the 55-64 year old age bracket male MS rates rose more than women's, though caution is required in interpreting this data as the rates are quite low.

2.4.2. Changes in Western World Neurological Deaths

What of changes in neurological deaths in the wider Western world? Pritchard et al produced an international comparison of the major two categories of neurological deaths, Mental Disorder Deaths [MDD] and `Other Neurological Deaths' (OND), the later including ALS and Parkinson's disease.[136] The Pritchard et al study covered Australia, Canada, England & Wales, France, Germany, Italy, Japan, Netherlands, Spain and the USA. The cut-off points for the study were that all countries with populations of 16+ million and had to also have a continuity of data from the WHO, which was the reason why former Warsaw Pact, African and Latin American countries were not included. The main focus will be upon OND mortalities because this category contains ALS; results for this category are shown in tables 5a and 5b for men and women separately.

Table 5a. Male 'Other Neurological Deaths' rate per million. 1979-81, 1994-96.

	All OND	45 - 54	55 - 64	65 - 74	75+
Australia					
1979-81	74	46	118	337	1079
1994-96	135	34	100	420	2274
Ratio	1.82	0.74	0.85	1.24	2.11
Canada					
1979-81	78	52	123	392	913
1994-96	170	49	132	536	2789
Ratio	2.18	0.94	1.07	1.37	3.05
England and Wales					
1979-81	104	48	116	343	1008
1995-97	147	41	124	391	1626
Ratio	1.42	0.86	1.07	1.14	1.61
France					
1979-81	183	55	153	624	2175
1994-96	172	51	141	486	2074
Ratio	0.94	0.92	0.92	0.78	0.95
Germany					
1979-81	95	51	136	370	795
1995-97	144	44	110	425	2073
Ratio	1.52	0.87	0.81	1.15	2.61
Italy					
1979-81	100	44	124	392	890
1995-97	170	44	141	469	1910
Ratio	1.70	1.00	1.14	1.20	2.15
Japan					
1979-81	44	31	86	222	392
1995-97	64	30	83	209	527
Ratio	1.46	0.99	0.97	0.94	1.35
Netherlands					
1979-81	107	50	116	455	1371
1995-97	126	44	115	432	1717
Ratio	1.17	0.89	0.99	0.95	1.25
Spain					
1979-81	81	49	118	310	826
1995-97	132	49	139	425	1366
Ratio	1.63	1.00	1.18	1.37	1.65
USA					
1979-81	83	53	133	355	805
1995-97	171	55	141	485	2485
Ratio	2.05	1.03	1.06	1.37	3.09

Table 5b. Female 'Other Neurological Deaths' rate per million. 1979-81, 1994-96.

	All OND	45 - 54	55 - 64	65 - 74	75+
Australia					
1979-81	61	32	84	208	639
1994-96	149	26	84	291	1866
Ratio	2.44	0.81	1.01	1.40	2.92
Canada					
1979-81	61	35	94	227	571
1994-96	216	36	109	380	2634
Ratio	3.55	1.04	1.16	1.68	4.61
England and Wales					
1979-81	105	33	83	238	796
1995-97	161	30	93	281	1235
Ratio	1.53	0.89	1.11	1.18	1.55
France					
1979-81	203	36	101	397	1803
1994-96	197	28	90	343	1693
Ratio	0.97	0.77	0.89	0.86	0.94
Germany					
1979-81	93	35	90	256	542
1995-97	172	26	76	289	1367
Ratio	1.85	0.74	0.84	1.13	2.52
Italy					
1979-81	87	32	80	264	693
1995-97	188	31	99	347	1585
Ratio	2.16	1.32	1.25	1.31	2.29
Japan					
1979-81	35	21	56	141	281
1995-97	57	19	56	151	357
Ratio	1.61	0.92	1.00	1.07	1.27
Netherlands					
1979-81	90	24	97	274	827
1995-97	159	33	105	294	1511
Ratio	1.76	1.34	1.08	1.08	1.83
Spain					
1979-81	71	31	70	207	611
1995-97	136	32	95	296	1132
Ratio	1.92	1.04	1.35	1.43	1.85
USA					
1979-81	68	37	92	214	498
1995-97	200	40	108	341	2127
Ratio	2/96	1.06	1.17	1.59	4.27

It is noteworthy that, apart from France, there were substantial increases in Male and Female OND deaths over the period 1979-97; this shift was significant at the all age level.

Male death rate increases ranged from 17% in the Netherlands to a doubling in the U.S. (105%) and Canada (118%.) Moreover, the U.S. rates increased in each age band for both genders, while in four countries the under 64 age bands actually decreased over the study period.

Initially the U.S. OND death rates for both men and women were the fourth lowest among the 10 countries studied, but by 1997 the U.S. death rate was the second highest. Interestingly, death rates were highest in the only other North American country on the list – Canada. Death rates among U.S. males rose from 83 to 171 per million and rose from 78 to 170 per million among Canadian men. Death rates among North American females also rose - from 61 to 216 per million in Canada and from 68 to 200 per million in the U.S.

Crucially, there were substantial increases in five female `younger age bands' 55-64, though as might be expected, the largest increases in death rates occurred in the 75+ age groups. The 45-54 age bracket combined population death rates went up an average of 4%, the 55-64 band increased an average of 12%, death rates in the 65-74 band increased 43% over the period, and death rates in the over 75+ more than doubled between 1979 and 1997.

Table 6a and 6b illustrate the change in Mental Disorder Death (MDD) numbers and rates. (The MDD category includes the dementias.) The exception in the general changing patterns of MDD was Japan, whose rates fell for men (-11%) but increased for women (35%). Apart from France. whose only substantial increase in MDD deaths was in the 75+ age band, MDD deaths and death rates in every other country rose substantially over the period studied. In particular, the death rate among males aged 55-64 rose the equivalent of 5% in the USA, 14% in Spain, 59% in Germany, 23% in Italy, and more than doubled in the Netherlands (146%). Among 55-64 year old females, MDD deaths increased 12% in the USA, 16% in Spain, 18% in Germany, 83% in Italy and 85% in the Netherlands. The remarkable changes observed in the Netherlands raises the question of whether there has been some major change in collating the data, but this could not be determined. The fact that such large increases in MDD death rates in the 'younger' age bracket of 55-64, means that in the countries studied, neurological conditions are not only increasing but are also starting younger.

Inn the 65-74 year age bracket, the increases in MDD rates can only be described as alarming. The traditional view of the dementias is that they predominately occur during the elderly years (75+) not during what modern society would describe as the `Third Age', a time of energetic and, historically, very healthy first-decade-of-retirement <74 year olds. Among male 65-74 year olds, MDD death rates increased 39% in Canada. 42% in the USA, 59% in England & Wales 59%, and doubled in Spain. For women, apart from Japan and France, there were substantial increases in every country, 90% for Canada, 94% for the USA and a doubling again in Spain. The female Canadian and Dutch MDD death rates had risen to a statistically greater extent than the male rates. The Italian and American female MDD death rates demonstrated a statistical trend that women's rates rose more than males. If, as might be argued, there is a greater willingness to report a death as being of a neurological cause, then there should have been little difference between the countries and little difference between genders.

It must not be forgotten however, that neurological deaths in Japan for both categories and for both genders in all age bands under 74, fell substantially over the period studied. Why this was so cannot be answered from this data. However it is worth while recalling Plato et al.'s study on the changing patterns of both ALS and the parkinsonism-dementia complex in Guam.[137]

Table 6a. Male Mental Disorder Deaths (MDD) (rate per million)

	All MDD	55-64	65-74	75+
Australia				
1979-81	63	92	219	956
1994-96	136	79	236	2045
Ratio 1979-96	2.16	0.86	1.08	2.14
Canada				
1979-81	68	158	249	770
1995-97	144	128	345	2480
Ratio 1979-97	2.12	0.81	1.39	3.22
Eng & Wales				
1979-81	46	31	111	691
1995-97	129	40	177	1662
Ratio 1979-97	2.80	1.29	1.59	2.40
France				
1979-81	160	299	498	1269
1994-96	192	218	309	2011
Ratio 1979-96	1.20	0.73	0.62	1.59
Germany				
1979-81	105	197	226	295
1995-97	178	314	288	798
Ratio 1979-97	1.70	1.59	1.27	2.71
Italy				
1979-81	20	40	37	52
1993-95	108	49	152	1122
Ratio 1979-95	5.40	1.23	4.11	21.58
Japan				
1979-81	27	43	92	352
195-97	24	27	38	300
Ratio 1979-97	0.89	0.63	0.41	0.85
Netherlands				
1979-81	23	30	60	320
1995-97	153	74	241	2972
Ratio 1979-97	6.65	2.46	4.02	9.29
Spain				
1979-81	31	58	89	271
1993-95	158	66	258	2683
Ratio 1979-95	5.10	1.14	2.90	9.90
USA				
1979-81	72	138	185	612
1995-97	137	145	262	1794
Ratio 1979-97	1.90	1.05	1.42	2.93

Table 6b. Female Mental Disorder Deaths (MDD) (rate per million)

	All Ages	55-64	65-74	75+
Australia				
1979-81	58	35	112	909
1994-96	179	34	137	2421
Ratio 1979-96	3.09	0.98	1.22	2.66
Canada				
1979-81	44	51	94	596
1995-97	219	45	179	3115
Ratio 1979-97	4.98	0.88	1.90	5.23
Eng & Wales				
1979-81	87	25	103	924
1995-97	230	25	129	2260
Ratio 1979-97	2.64	1.00	1.25	2.45
France				
1979-81	146	79	218	1326
1994-96	261	65	158	2684
Ratio 1979-96	1.79	0.82	0.72	2.02
Germany				
1979-81	49	68	80	220
1995-97	109	80	98	772
Ratio 1979-97	2.22	1.18	1.24	3.51
Italy				
1979-81	8	12	17	39
1993-95	126	22	120	1300
Ratio 1979-95	15.75	1.83	7.06	33.33
Japan				
1979-81	23	20	68	380
195-97	31	7	17	338
Ratio 1979-97	1.35	0.35	0.25	0.89
Netherlands				
1979-81	31	20	40	431
1995-97	365	37	253	4734
Ratio 1979-97	11.77	1.85	6.33	10.98
Spain				
1979-81	22	20	55	267
1993-95	271	24	188	3289
Ratio 1979-95	12.32	1.16	3.39	12.33
USA				
1979-81	53	43	77	547
1995-97	194	48	149	2367
Ratio 1979-97	3.66	1.12	1.94	4.33

In that study, the authors suggested this change had to do with the increased Westernization of diet and lifestyle over the past 40 years, which may reflect the social changes that have taken place in Japan. Conversely we can not ignore that there have been marked increases in serious neurological disease in the major Western world countries over the past 30 years. And U.S. death rate increases are amongst the worst in the Western world.

2.4.3 Combined Neurological Deaths in Canada, England & Wales & USA, 1979-2000

The above study took the analysis only up to 1997, whereas there data is available up to and including 2000. One problem is that in the 10th ICD the neurological categories have been changed – specifically, Alzheimer's Disease is now placed in the OND category. To allow for this change, Tables 7a & 7b give the combined MDD and OND rates for the end-point periods of 1979-81 and 1998-2000.

Between the 1997 data and that from only three years later, death rates in the Canadian male 65-74 age bracket rose 7%, while death rates among U.S. males rose 3% in the 55-64 year age bracket and 16% in the 65-74 year age bracket. While the overall 1979-2000 death rate among Canadian males doubled, against any expectation (Gompertzian or otherwise), death rates among 55-64 year old Canadian males continued to fall (an equivalent of 17% by 2000) and death rates among 65-74 year old Canadian males continued to rise (an equivalent of 29% between 1979-2000.)

Among U.S. males, combined OND & MDD death rates more than doubled from 1979 to 2000. Unlike the Canadian 55-64 year age bracket, death rates among U.S. males aged 55-64 rose a modest 9%. In the 65-74 year cohort, death rates in U.S. males rose 60%.The Anglo-Welsh rates from 2002 are given as a contrast - between 1997-2002 Anglo-Welsh death rates rose in all age brackets except the 55-64 year age bracket. Overall the differences between 1979 and 2002 were substantial, doubling for All Ages, and rising 24% and 50% in the 55-64 and 65-74 age brackets, respectively.

The combined female neurological death rates worsened both proportionately and in

Table 7a. Combined Male Neuological Deaths, 1979-81, 1995-1997, & 2000 in Canada, England & Wales, and the U.S. (rate per million and ratio of change)

	All MDD	55-64	65-74	75+
Canada				
1979-81	75	141	368	842
1995-97	157	130	441	2635
Ratio 1979-97	2.09	0.92	1.20	3.13
2000	195	117	474	3120
Ratio 1979-2000	1.24	0.90	1.07	1.22
Ratio 1979 -2000	2.30	0.83	1.29	3.71
Eng & Wales				
1979 – 81	75	74	227	850
1995-97	138	126	284	1644
Ratio 1979-97	1.84	1.70	1.25	1.93
2002	207	92	341	2409
Ratio 1997-02	1.50	0.73	1.20	1.47
Ratio 1979-2002	2.76	1.24	1.50	2.83
USA				
1979-81	78	136	270	709
1995-97	154	143	374	2140
Ratio 1979-97	1.97	1.05	1.38	3.02
2000	188	148	432	2727
Ratio 1997-2000	1.22	1.03	1.16	1.27
Ratio 1979-2000	2.41	1.09	1.60	3.85

comparison to males. OND & MDD death rates among Canadian women in the All Age and 65-74 age brackets rose at a statistically significantly higher rate than deaths among men in the same age brackets (4.10 and 1.74 versus 2.09 and 1.20, respectively). In the U.S., OND & MDD death rates were significantly greater in every age group examined, and highly significant in the 55-64 year cohort.

Self-evidently, the largest increases in death rates occurred in the 75+ age bracket, but the extent of the increases is remarkable. It needs to be remembered that we are comparing age cohort with the same age cohort across the genders; while initially we observed higher male death rates in 1979/81 in North America, by 2000, American women had higher combined neurological deaths for the All Age, 75+, and 55-64 year old age brackets. This latter result is quite stark demonstration of the change that has occurred.

Among U.S. 55-64 year olds, initially men were more likely to die of neurological disorders than women (a ratio of 2:1.) By 2000, however, this trend had reversed itself, with 1.03 women dying of neurological disorders for every 1 man

Table 7b. Combined Female Neurological Deaths, 1979-81, 1995-1997, & 2000/2002 in Canada, England & Wales, and the U.S. (rate per million and ratio of change)

	All MDD	55-64	65-74	75+
Canada				
1979-81	53	73	161	584
1995-97	218	77	280	2875
Ratio 1979-97	4.10	1.05	1.74	4.92
p value	+++	-	+++	++++
2000	285	95	307	3536
Ratio 1979-2000	1.31	1.23	1.10	1.23
p value	-	-	-	-
Ratio 1979 -2000	5.38	1.30	1.91	6.05
p value	+++	-	+++	++++
Eng & Wales				
1979 – 81	96	54	171	860
1995-97	196	59	205	1748
Ratio 1979-97	2.04	1.09	1.20	2.03
p value	-	-	-	++
2002	309	54	260	2879
Ratio 1997-02	1.58	0.92	1.27	1.65
p value	-	-	-	-
Ratio 1979-2002	3.22	1.00.	1.52	3.35
p value	-	-	-	++
USA				
1979-81	61	68	146	523
1995-97	197	78	245	2247
Ratio 1979-97	3.23	1.15	1.68	4.29
p value	-	-	-	-
2000	281	153	299	3250
Ratio 1997-2000	1.43	1.96	1.22	1.45
p value	-	-	-	-
Ratio 1979-2000	4.61	2.25	2.05	6.21
p value	+++	+++	+	++++

+ = p<0.05 ++ = p<0.01 +++ = p<0.0001 ++++ = p<0.00001 - = not significant

(a ratio of ratios of 0.48.) Something clearly has been affecting 55-64 year old U.S. women's susceptibility to ALS, be it genetic or environmental. Since the change has occurred too quickly to be due to genetic changes alone, this shift must largely be due to external factors.

When considering the underlying morbidity of this type of mortality, not only do these brain diseases appear to be increasing, but they are also starting earlier in North America, especially among women. North American women's situation has worsened significantly compared to men's. In addition, U.S. male 65-74 rates and U.S. female 55-75 and 65-74 rates are significantly worse over the study period than the equivalent rates in England & Wales ($p<0.02$, <0.001 and <0.04.) Thus, U.S. combined neurological deaths between 1979 and 2000 are amongst the worst in the world - clearly this can not be predominately due to genetic shifts in these age groups within such a short time span, but must include a major socio-environmental factor.

2.4.5. A Younger Perspective.

The focus thus far has been on people aged 55+ , since this is the age of onset usually associated with ALS.[138] Table 8 however shows the mortality rates for young adults (25-34) and middle-aged people (35-54) over the period studied, to determine any changes that have occurred in OND and MDD mortality in the U.S.

It can be seen that young adult OND male rates have risen an equivalent of 13% over the period studied, which may reflect the ALS increases identified by Haley and Horner et al, with very slight rises in the age bands from 35-54.[139] Changes in MDD rates, however, are much more varied, with decreases in rates among 25-34 year olds and increases in death rates among 35-54 year olds.

However, looking at changes in death rates disguises the fact that we are analyzing the numbers of people dying from these serious neurological disorders. Table 9 provides data on the total numbers of people dying from serious neurological disease in an attempt to determine possible prevalence. Apart from young (25-34) MDD numbers of deaths, every age band and in both gender saw a rise in the numbers of American people dying from serious neurological disease under the current life-expectancy. The OND deaths, which include ALS, rose in every age band and generally substantially so.

Taking these figures up to the latest WHO data, the final row shows the average numbers of combined MDD and OND deaths for 1999 and

Table 8. U.S. Young Adult & Middle-aged OND and MDD rates 1979-81 & 1996-1998 by gender (rate per million and ratio of change)

	25-34		35-44		45-54	
	M	F	M	F	M	F
OND						
1979-81	18	10	23	18	54	37
1996-1998	18	11	27	18	55	40
Ratio 1979-1998	1.13	1.10	1.17	1.00	1.02	1.08
MDD						
1979-81	38	12	65	22	110	36
1996-98	27	11	72	27	115	33
Ratio 1979-98	0.71	0.92	1.11	1.23	1.05	0.92

Table 9. Numbers of USA OND & MDD deaths among 25-74 year olds by gender, 1979-81 v. 1996-98

	25-34		35-44		45-54		55-64		65-74	
	M	F	M	F	M	F	M	F	M	F
M.D.D.										
1979-81	712	235	826	239	1199	426	1401	485	1253	682
1996-98	525	219	1583	594	1887	567	1463	540	2225	1618
Ratio 1979-1998	0.74	0.93	1.92	2.49	1.57	1.33	1.04	1.11	1.78	2.37
ON.D.										
1979-81	302	183	285	236	583	436	1352	1066	2508	1893
1996-98	346	210	584	377	695	680	1466	1590	4136	3568
Ratio 1979-1998	1.15	1.15	2.05	1.60	1.54	1.56	1.09	1.49	1.72	1.89
MDD & OND										
1979-81	1014	418	1111	475	1782	862	2753	1551	3661	2300
1996-98	871	429	2167	971	3644	1247	2931	2130	6361	3918
Ratio 1979-1998	0.86	1.03	1.95	2.04	2.04	1.45	1.06	1.37	1.74	1.70
1999-2000	889	457	2402	1055	3373	1544	3399	2106	7020	5915
Ratio 1979-2000	0.88	1.09	2.16	2.22	1.89	1.79	1.23	1.36	1.92	2.57

2000 among people aged between 25 and 74: an average of 28,160 people per year died from a serious neurological condition (OND and MDD) during this time period. Deaths in the 35-54 age bracket virtually doubled over the period studied and, apart from the decline in deaths among young males (25-34), deaths in all other brackets increased. Indeed, the average annual number of combined neurological deaths represents 4998 `extra' deaths among the 25-64 aged cohort and a further 6974 deaths among people aged 65-74, a total of 11,972 `extra' annual MDD and OND deaths in the USA over the period studied.

Considering younger adults as a whole (25-54), there were 5,341 USA neurological deaths in the 1979-81 period, while in 1999-2000 there were 10,308. More importantly, deaths among females aged 25-34 and 45-54 are significantly higher than those among males in the same age brackets ($p<0.007$ and <0.03, respectively), yet another indicator of the probable involvement of socio-environmental factors.

Bearing in mind that the progress of these diseases span an average of 3 years for ALS and 10+ for the other diseases, a conservative estimate to reach the numbers of people currently suffering from these diseases would be to multiply the numbers of deaths by 5, which would yield an extra 24,900 25-54 year old people and a further 34,870 `extra' cases in people aged 65-74 since the 1979-81 period. Of course, these are not all ALS deaths but an `extra' 11,972 serious neurological causes of death each year, which include ALS. How do these numbers compare with high profile HIV deaths? When the WHO first reported HIV deaths in 1987, there were 14,478 USA victims by the year 2000, the number was 13,468. Thus, the annual number of neurological deaths is more than double the annual HIV deaths, a ratio of 2.09. Indeed, HIV mortality is only slightly higher than all <54 year old neurological deaths (10,308) and the latter appear to be rising. Noonan et al noted that between 1969 and 1998, the number of ALS deaths rose from 2,252 to 4,615 per annum, an `extra' 2,363 people a year, suggesting that by 1998 there were an `extra' 7,089 people suffering from ALS.[140]

2.4.6. OND, MDD, and All Causes of Death

One final `contextual' analysis is to place these serious neurological deaths against the major

causes of death in the USA, and by way of further contrast, those in Canada and England & Wales, to highlight how different these OND and MDD rates compared to the rest of mortality. However, the analysis will only focus upon the 55-74 year old age bands in the OND category because it includes ALS, the MDD data and the results for the 75+ year olds provided to give a comprehensive picture.

Table 10 is presented to enable the reader to contrast every major cause of death in the USA with MDD and OND. Table 11 is a summary of any statistically significant differences between OND and the other causes.

The first point to note is that in many of the causes of death, unlike as in OND and MMD, there have been major and in some cases quite dramatic falls across all age bands, especially in the cardio-vascular diseases.[141] The second point to note is that `Other Respiratory Diseases' (which is another `catch all' WHO category) and Diabetes All Age are the only categories in the ALL Age band in which OND rates were not Highly Significantly greater ($p<0.00001$) than most of the other causes. Overall, there were major, significant differences between OND rates over the period and those concerning the broadly cardio-vascular diseases in all three age bands, All Cause, and 55-74. However in the 55-64 year old cohort only the women's OND rates were significantly higher than the All Causes rates, and in this age band there were fewer significant differences, with mainly male OND rates being greater than their male counterparts for Bronchitis and other digestive diseases. 'Other Sites of cancers' and Diabetes deaths in the 55-64 year old group were greater than OND rates.

When it comes to the 65-74 age cohorts however, increases in OND rates were invariably significantly greater than for most other causes – the only exceptions were male diabetes and OND. Generally therefore, while OND, with its relationship to ALS, had risen significantly in comparison with most other causes of death in the USA, it was related to increasing age though there were some differences between the genders.

Table 10. USA Average Mortality: Other Causes, All ages and ages 55-74 by gender, compared with MDD & OND (rate per million & ratio of change) [WHO ICD 9]

	All Ages		55 - 64		65 - 74		75+	
	M	F	M	F	M	F	M	F
All Causes								
1979 – 1981	9654	9031	18605	9409	40430	21031	104407	73601
1996 – 1998	8845	8508	13407	8101	31894	19686	91122	75480
Index	0.92	0.94	0.72	0.86 *	0.79	0.94 *	0.87	1.03
MMD	<0.0001	<0.0001	<0.005	-	<0.0001	<0.0001	-	-
OND	<0.0001	<0.0001	-	<0.03	<0.0001	<0.0001	-	-
Neoplasm								
1979 – 1981	2030	1608	5173	3599	10849	5889	18699	9736
1996 – 1998	2151	1890	4545	3415	10616	6791	19478	11581
Index	1.06	1.18 *	0.88	0.95	0.98	1.15 *	1.04	1.19
MDD	<0.01	<0.0001	-	-	<0.002	<0.0001	-	-
OND	<0.0001	<0.0001	-	-	<0.0001	<0.001	-	-
Breast								
1979 – 1981	-	304	-	788	-	1014	-	1351
1996 – 1998	-	309	-	647	-	955	-	1528
Index	-	1.01	-	0.82	-	0.94	-	1.13

MMD	-	<0.0001	-	-	-	<0.0001	-	-
OND	-	<0.0001	-	<0.02	-	<0.0001	-	-
Prostate								
1979 – 1981	207	-	248	-	1042	-	3641	-
1996 – 1998	253	-	203	-	993	-	3894	-
Index	1.22 *	-	0.82	-	0.95	-	1.07	-
MDD	<0.005	-	-	-	<0.001	-	-	-
OND	<0.0001	-	-	-	<0.0001	-	-	-
Other CA sites								
1979 – 1981	448	476	1160	978	2208	1774	3593	3061
1996 – 1998	485	526	1096	895	2236	1808	3798	3404
Index	1.08	1.11	0.94	0.92	1.01	1.02	1.06	1.11
MDD	<0.0001	<0.0001	-	-	<0.01	<0.0001	-	-
OND	<0.0001	<0.0001	-	<0.08t	<0.001	<0.0001	-	-
Leukaemia								
1979 – 1981	81	62	146	88	350	182	790	434
1996 – 1998	86	67	135	84	371	194	797	460
Index	1.06	1.08	0.92	0.95	1.06	1.07	1.01	1.06
MDD	<0.003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	-
OND	<0.0001	<0.0001	-	-	<0.006	<0.002	-	-
Other Lymph								
1979 – 1981	100	86	239	164	474	337	816	576
1996 – 1998	140	120	242	181	560	431	-	-
Index	1.40	1.40	1.04	1.10	1.18	1.28	-	-
MDD	<0.06t	<0.0001	-	-	-	<0.003	-	-
OND	-	<0.0001	-	-	<0.06t	<0.04	-	-
Other Blood Dis								
1979 – 1981	14	13	28	18	65	42	157	96
1996-1998	23	21	28	22	88	55	269	169
Index	1.64	1.62	2.0	1.22	1.45	1.31	1.74	1.76
MDD	-	<0.02	-	-	-	<0.07t	-	-
OND	-	<0.9t	-	-	-	-	-	-
Diabetes								
1979 – 1981	125	172	268	253	622	629	1436	1519
1996 – 1998	217	252	422	356	969	823	2086	1939
Index	1.73	1.47	1.57	1.41	1.56*	1.31	1.45	1.27
MDD	-	<0.0001	<0.003	-	-	<0.003	-	-
OND	-	<0.0001	<0.005	-	-	<0.03	-	-
Mental Disorder								
1979 – 1981	72	53	136	43	185	77	612	547
1996-98	144	214	140	47	252	158	1907	2603
Index	2.00	4.04 **	1.04	1.09	1.36	2.05 *	3.12	4.76 **
OND	-	-	-	-	-	-	-	-
M.S.								
1979 – 1981	5	8	16	23	14	18	8	10
1996-98	7	12	20	30	23	33	18	26
Index	1.40	1.50	1.25	1.30	1.64	1.83	2.25	2.60
MDD	-	<0.04	-	-	-	-	-	-
OND	-	-	-	<0.0001	-	-	-	-
O.N.D								
1979 – 1981	77	68	133	92	355	214	805	498
1996-98	175	208	140	109	499	349	2503	2209
Index	2.27	3.06	1.05	1.18	1.41	1.63	3.11	4.44 **
Circulatory								
1979 – 1981	4541	4097	8563	3418	20759	10503	61879	50095
1996 – 1998	3415	3671	5118	2338	12657	6997	42210	38843
Index	0.75	0.90*	0.60	0.68	0.61	0.57	0.68	0.78
MDD	<0.0001	<0.0001	-	-	-	-	-	-
OND	<0.001	<0.0001	-	-	-	-	-	-
Hypertensive								
1979 – 1981	124	166	264	169	567	449	1550	1717
1996 – 1998	136	183	288	143	481	361	1422	1848

Index	1.10	1.10	1.09 t*	0.85	0.85	0.80	0.92	1.08
MDD	<0.0002	<0.001	-	-	<0.001	<0.0001	-	-
OND	<0.0001	<0.0001	-	<0.06t	<0.0001	<0.0001	-	-
A.M.Infarction								
1979 – 1981	1712	1028	3869	1219	8332	3552	16843	10685
1996 – 1998	839	717	1546	592	3460	1703	9167	7140
Index	0.49	0.70 *	0.40	0.49	0.42	0.48	0.54	0.67
MDD	<0.0001	<0.0001	-	-	-	-	-	-
OND	<0.0001	<0.0001	-	-	-	-	-	-
Other Ischaem								
1979 – 1981	1177	1114	1927	620	5033	2401	18508	15235
1996 – 1998	977	963	1311	487	3645	1688	12927	10551
Index	0.83	0.86	0.68	0.79*	0.72	0.70	0.70	0.69
MDD	<0.0001	<0.0001	-	-	-	-	-	-
OND	<0.0001	<0.0001	-	-	-	-	-	-
Pulmonary Circ								
1979 – 1981	734	678	1365	595	3089	1700	9901	8102
1996 – 1998	804	888	1257	603	2808	1615	9510	7140
Index	1.10	1.31 *	0.92	1.01	0.91	0.95	0.96	0.88
MDD	<0.0001	<0.0001	-	-	<0.0001	<0.0001	-	-
OND	<0.0001	<0.0001	-	-	<0.0001	<0.0001	-	-
Cerebro Vascular								
1979 – 1981	728	846	752	558	2559	1876	11000	10941
1996 – 1998	474	712	511	377	1512	1191	6785	7755
Index	0.65	0.84 *	0.68	0.68	0.59	0.63	0.61	0.81
MDD	<0.0001	<0.0001	-	-	-	-	-	-
OND	<0.0001	<0.0001	-	-	-	-	-	-
Atherosclerosis								
1979 – 1981	99	149	64	33	301	176	2359	2377
1996 – 1998	45	73	34	17	123	75	776	913
Index	0.45	0.49	0.53	0.52	0.41	0.43	0.33	0.38
MDD	-	-	-	-	-	-	-	-
OND	-	-	-	-	-	-	-	-
Embolism								
1979 – 1981	116	60	209	63	652	198	1470	626
1996 – 1998	114	92	156	69	516	248	1340	869
Index	0.98	1.53 *	0.75	1.10 t*	0.79	1.25 *	0.91	1.39
MDD	<0.0003	<0.0001	<0.0001	-	<0.0001	<0.004	-	-
OND	<0.0001	<0.0001	<0.04	-	<0.0001	<0.05	-	-
Pneumonia								
1979 – 1981	230	203	243	113	728	329	4192	2709
1996 – 1998	298	345	210	131	759	432	4813	3989
Index	1.30	1.70 *	0.86	1.16 t*	1.01	1.31 *	1.15	1.47
MDD	<0.01	<0.0001	-	-	<0.02	<0.004	-	-
OND	<0.0001	<0.001	-	-	<0.001	<0.06t	-	-
Bronchitis								
1979 – 1981	123	56	223	109	734	241	1504	367
1996 – 1998	98	96	142	130	480	350	1088	661
Index	0.80	1.71*	0.64	1.19 *	0.65	1.45 *	0.72	1.80
MDD	<0.0001	<0.0001	<0.003	-	<0.0001	<0.04	-	-
OND	<0.0001	<0.01	<0.002 .	-	<0.0001	<0.0001	-	-
Other D. Respir								
1979 – 1981	314	146	519	229	1741	564	4192	1190
1996 – 1998	452	379	493	376	1948	1275	6270	3704
Index	1.44	2.60 *	0.95	1.64 *	1.12	2.26 *	1.50	3.11
MDD	<0.05	<0.05	-	<0.07t	<0.06t	-	-	-
OND	<0.05	-	-	<0.05	<0.003	<0.002	-	-
Ulcer Stomach								
1979 – 1981	31	24	57	28	136	67	403	262
1996 – 1998	18	19	25	14	62	38	219	196

Index	0.58	0.79	0.44	0.50	0.46	0.57	0.54	0.75
MDD	<0.001	<0.0001	-	-	-	-	-	-
OND	<0.0001	<0.0001	-	-	-	-	-	-
Liver Cirrhosis								
1979 – 1981	175	90	573	259	622	271	419	196
1996 – 1998	124	64	354	138	414	225	375	232
Index	0.71	0.71	0.62	0.53	0.67	0.83 *	0.89	1.18
MDD	<0.0001	<0.0001	-	-	-	-	-	-
OND	<0.0001	<0.0001	-	-	-	-	-	-
Other D. Digest								
1979 – 1981	136	143	267	169	562	386	1529	1430
1996 – 1998	137	173	206	151	480	391	1477	1609
Index	1.04	1.21	0.77	0.89	0.85	1.01 t*	0.97	1.13
MDD	<0.001	0.0001	<0.06t	-	<0.001	<0.0001	-	-
OND	<0.0001	0.0001	<0.05	-	<0.0001	<0.001	-	-
Nephritis								
1979 – 1981	77	69	104	73	309	196	717	717
1996 – 1998	93	95	92	70	301	217	957	957
Index	1.21	1.37	0.88	0.96	0.97	1.11	1.33	1.33
MDD	<0.02	<0.0001	-	-	<0.008	0.001	-	-
OND	<0.002	<0.0001	-	-	-	-	-	-
Suicide								
1979 – 1981	179	52	248	88	300	69	440	54
1996 – 1998	189	44	221	54	268	45	455	50
Index	1.06	0.85	0.89	0.61	0.89	0.65	1.03	0.93
MDD	<0.003	<0.0001	-	-	-	-	-	-
OND	<0.0001	<0.0001	-	-	-	-	-	-

Table 11. Comparison of U.S. OND deaths v. all other major causes by age and gender, 1979-1998 (p values only)

	All Ages		55-64 years		65-74 years	
	M	F	M	F	M	F
OND compared with:						
All Causes	<0.0001	<0.0001	-	<0.03	<0.0001	<0.0001
Neoplasm	<0.0001	<0.0001	-	-	++++	++++
Lung Cancer	<0.001	<0.003	<0.05	-	<0.001	<0.02
Breast	N/A	<0.0001	N/A	-	N/A	++++
Prostate	++++	N/A	-	N/A	<0.0001	N/A
Other Sites	++++	++++	-	<0.8T	++++	++++
Leukaemia	<0.001	<0.001	-	-	<0.06t	<0.002
Other Lymph	<0.01	<0.0001	-	-	<0.06T	<0.04
Diabetes	-	<0.001	<0.005	-	-	<0.03
Circulatory	++++	++++	++++	++++	++++	++++
Hypertension	++++	++++	-	<0.06T	<0.0001	<0.0001
Infarction	++++	++++	++++	++	++++	++++
Other Ischaemic	++++	++++	++++	++	++++	++++
Pulmonary	++++	++++	-	-	++++	++++
Cerebro-vascular	++++	++++	++++	++++	++++	++++
Embolism	++++	++++	<0.04	-	++++	<0.05
Pneumonia	++++	<0.001	-	-	<0.001	<0.06T
Bronchitis	++++	<0.01	<0.002	-	++++	++++
Other Resp. Diseases	<0.05	<0.05	-	<0.05	<0.003	<0.002
Stomach Ulcer	++++	++++	++++	++++	++++	++++
Nephritis	<0.002	++++	-	-	++++	++++
Liver Cirrhosis	++++	++++	++++	++++	++++	++++
Other Digestive Diseases	++++	++++	<0.05	-	++++	++++

++++ = p<0.00001 - = not significant

2.4.7. Gender and Mortality

Table 12 raises the interesting finding of how women's mortality has changed significantly more than men's over the period studied. For example, in All Cause deaths in all age groups the decline in male death rates was not matched by equal declines in female death rates. Also, women had significantly worse malignant neoplasm deaths across the three age bands and for Mental Disorder Deaths in the 65-74 age cohort, and even the `success ' of the declines in cardio-vascular deaths had less of an impact on death rates in women than those in men. This was also the case in the respiratory death categories. This variation in outcomes over the 1979-1998 period again strongly indicates the involvement of socio-environmental factors.

2.4.8. Are the patterns of USA relative mortality unique?

A reasonable question would be whether these patterns of major decline in cardiovascular and respiratory diseases and rises in neurological deaths only pertain to the U.S. In an attempt to put the American changes into broader international context, we have calculated MDD and OND comparisons with All Causes deaths and the major categories for Canada (to complete the review for North America) and for England & Wales as a European example,

Table 12. Comparison of USA Women's v. Men's Changing Death Rates, 1979-1998

	All Ages rate of change (p Value)		55-64 years rate of change (p value)		65-74 years rate of change (p value)	
	M	F	M	F	M	F
All Causes	1.06 <0.02	1.18	0.88 <0.02	0.95	0.98 <0.03	1.15
Mental Disorder Deaths	2.00 <0.0001	4.04	1.03 -	1.09	1.36 <0.02	2.05
Neoplasm	1.06 <0.02	1.18	0.88 <0.02	0.95	0.98 <0.03	1.15
Lung Cancer	1.03 <0.0001	1.89	0.82 <0.0001	1.38	1.01 <0.001	2.07
Diabetes	-	-.	-	-	1.56 <0.02	1.71
Circulatory	0.75 <0.0001	0.90	-	-	-	-
Infarction	0.49 <0.0001	0.70	-	-	-	-
Pulmonary	1.10 <0.02	1.31	-	-	-	-
Cerebro-vascular	0.65 <0.001	0.84	-	-	-	-
Embolism	0.98 <0.001	1.53	-	-	0.79 <0.0001	1.25
Pneumonia	1.30 <0.03	1.70	0.86 <0.0001	1.16	1.01 <0.01	1.31
Other Respiratory Diseases	1.44 <0.0001	2.60	0.95 <0.0001	1.64	1.12 <0.0001	2.23
Liver Cirrhosis	-	-	-	-	0.67 <0.06	0.83
Other Digestive Diseases	-	-	-	-	0.85 <0.07t	1.01

focusing on the key ALS age bracket of 55-74.

Canada has very similar patterns to the U.S., with the exception of Lung cancer, diabetes and Other respiratory disease in the 55-64 age group, MDD and OND increased to a statistically significant degree compared to the other categories including, for example, breast cancer which attracted so much public support. For England & Wales, the picture is similar, with the exception of `Other Sites of Cancer', Lung cancer, in women in the 64-74 year cohort, MDD and OND was significantly worse than the other mortalities. However, Other Respiratory Disease and Cirrhosis of the Liver had significantly worse outcomes than either MDD and OND, especially for women.

England & Wales, like the U.S. and Canada have a marked variation in women's outcomes, especially in the `ragbag' categories of `Other Sites of Cancer' and in the case of England & Wales, `Other Lymph and Blood' disease. This gives further emphasis that relative to men there has been greater change in women's life-styles and appears to be reflected in subsequent mortality. The Anglo-Welsh cirrhosis result probably mirrors marked changes in our pattern

of drinking alcohol as youngsters, especially females have started to drink earlier and more often wine and spirits, which was virtually unheard of a little more than twenty years ago.[142]

One feature worth recalling is that the gap between male and female rates of ALS have narrowed considerably in both the U.S. and England & Wales. Studies across the U.S. and on the East and West coasts have also shown a particular narrowing of the gender divide. It is sometimes forgotten that prior to the 1980's, the male: female ratio for cancer ranged from 2-1.5:1 but this later changed. Moreover, the success in the reduction of cancer deaths has hidden substantial increases in incidence, especially among women. In England & Wales for example, while young men's (15-34) cancers rose 45% in 20 years, young women's rose five fold.[143] And of course we saw women's relative position to men move in the same direction for ALS and the other neuro-degenerative diseases. So perhaps what might be happening in our wider environment, is affecting others disorders, not just the neurological. Bearing in mind the major reduction in death rates in the ALS onset years of 55-74, a brief resume of the those disease categories in which there were at least an equivalent of a 10% rise in deaths between 1979 and 2000, highlights that it is not only ALS and the other neuro-degenerative diseases that are rising. We extrapolate the appropriate data for Canada, England & Wales and the USA We list 24 major causes of death from the WHO categories and the above show that deaths other than the neurological increased for people within the 55-74 age bands. There are three points to note regarding this data. First, increases in the Other Lymph Disorders and Other Blood Diseases and the Other Respiratory Disease are common to all three countries. Second, women are more affected in North America than men, which is also generally true for all these category increases - 7 out of 14 in Canada, 14 out of 18 in England and Wales and 15 out of 18 in the USA. Thirdly, these common conditions, like the MDD and OND categories, are portmanteau categories. While conditions such as diabetes and the respiratory diseases seem probably associated with diet and end-product of smoking, the others also raise questions as to the possible bio-genetic and socio-environmental influence of the increases in these conditions. Time and space prohibits any further in-depth analysis here, other than to say, a Gompertzian explanation, i.e. end product of improved longevity, would not appear to account for these changes. There appears to be something happening in modern societies, and that changing patterns of disease are not just the by-product of better diagnosis, and though the USA did not have the problem of New Variant Crutchfeld-Jacobs Disease the socio-environmental as was the case in the United Kingdom, something very odd emerged in young British Mental Disorder Deaths shown in the table below.

Table 13. Deaths with a ratio rise of 1.10+ in 55-74 age bands, 1979-2000. Canada, England & Wales, U.S.

	All Ages		55-64		65-74	
	M	F	M	F	M	F
Canada						
MDD	2.34	5.79	0.76	0.88	1.44	2.15
OND	2.17	3.61	1.06	1.08	1.41	1.61
Lung Cancer	1.14	1.13	1.13	1.81	1.00	1.22
Prostate cancer	1.38		0.89		1.44	
Other Lymph Disorder	1.42	1.47	1.10	1.05	1.33	1.21
Other Blood Diseases	1.00	1.00	0.65	0.80	0.69	1.11
Diabetes	1.80	1.46	1.33	1.10	1.50	1.00
Multiple Sclerosis	1.44	1.47	1.12	0.95	1.18	2.17
Other Respiratory Diseases	1.88	3.25	0.78	1.26	1.19	2.22
Nephritis	1.58	1.69	1.07	0.94	1.29	1.06
England & Wales						
MDD	3.15	3.04	1.55	1.16	1.50	1.30
OND	1.42	1.55	0.93	1.00	1.04	1.15
Lung cancer	0.64	1.28	0.48	0.83	0.59	1.39
Prostate cancer	1.12		1.17		1.16	
Other Cancer Sites	1.36	1.29	1.15	1.44	1.53	1.58
Leukaemia	1.00	1.02	0.92	0.93	1.03	1.11
Other Lymph Disorder	1.33	1.40	1.14	1.19	1.30	1.33
Other Blood Diseases	2.60	2.30	1.20	1.00	1.89	1.58
Multiple Sclerosis	0.83	1.00	0.76	0.83	0.84	1.13
Other Respiratory Disease	3.15	5.37	1.81	2.61	2.34	5.33
Liver Cirrhosis	2.24	1.63	1.92	1.45	1.67	1.51
Other Digestive Disorder	1.28	1.40	0.91	0.98	1.02	1.13
USA						
MDD	2.00	4.04	1.02	1.09	1.30	2.05
OND	2.27	3.06	1.05	1.18	1.41	1.63
All Neoplasm	1.06	1.18	0.80	0.95	0.98	1.15
Other Lymph Disorders	1.40	1.40	1.01	1.10	1.18	1.28
Other Blood Diseases	1.64	1.62	1.00	1.22	1.35	1.31
Diabetes	1.73	1.47	1.57	1.41	1.50	1.31
Multiple Sclerosis	1.40	1.50	1.25	1.30	1.64	1.83
Pneumonia	1.30	1.70	0.86	1.16	1.03	1.31
Bronchitis	0.80	1.71	0.64	1.19	1.03	1.31
Other Respiratory Disease	1.44	2.60	0.95	1.64	1.12	2.26
Nephritis	1.21	1.37	0.68	0.96	0.97	1.11

This remarkable change can hardly be due to the Gompertzian ageing process and these rates represent a change of an average of 146 Mental Disorder Deaths per annum to an average of 831. This has not happened in the USA, but it is an indication of external factors impacting upon the serious neurological disease, but in this case we do not know what or why or whether NVCJD has any relevance to these figures, but certainly there appears to be a potential public health issue.

Our critique of the Gompertzian approach is that while our broad analysis gives some support to the emphasis such authors place on ALS and the other neurological degenerative diseases being associated with increasing age, it does not explain the fact that the majority of elderly people do not develop ALS, that it proportionately falls in the pre 85 year olds.[144] To assume a predominately `genetic susceptibility' triggered by increasing longevitiy ignores the nature of the environmental trigger. Moreover, Riggs was totally wrong about cancer, as the biggest incidence, not mortality, of the malignancies, was observed among the under 35 year old women.[145] Crucially, the Gompertzian position explains nothing about

the increasingly worsening situation of women across a number of conditions, but especially in ALS and the other neuro-degenerative diseases. The Gompertzian approach engenders a kind of passivity that medicine left behind more than 100 years ago. Today medicine seeks to understand the totality of the human phenomena, and considers it a challenge when its art and science cannot bring about either amelioration, cure or prevention.

2.4.8. Summary

What are the key findings from Part One concerning public health and epidemiological factors related to ALS and other serious neurological diseases? The results concerning Clusters of ALS, for example in Guam, the Kii Japanese peninsula and in parts of Europe and conjugal clusters, show that changes are occurring. There appear to be both genetic and external factors related to these changes, and because of the relative speed of these changes, the emphasis appears to be upon the socio-environment, for example conjugal clusters may be due to either shared infection or exposure to a noxious environment.

The public health issue of smoking should not surprise considering its effect on cancer and respiratory disease, though the fact that smoking tobacco may be a preventative factor for Parkinson's disease is intriguing and seems counterintuitive. Perhaps it is another reminder that the neural degeneration process in Parkinson's is different from that in ALS.

The influence of season of birth, place of birth and place of death on the risk of developing ALS again emphasizes the interactive nature of the disease, including the possibility that infections and virology may contribute to the development of the disease, with possible differential impact depending upon any genetic predisposition.

The epidemiological factors are fascinating and clearly demonstrate that in regard to ALS in the U.S. there have been continued increases in deaths from the disease between 1969 and 2000, although at a varied pace. Very importantly however, it is women's death rates which have increased the most, possibly reflecting the greater extent to which women's life-styles have changed compared to men's.

Moreover, while Canada and the U.S. have had amongst the largest relative rises in serious neurological mortality, and therefore an increase in underlying morbidity, with the exception of Japan these phenomena appear to be common throughout all the major Western countries. There have undoubtedly been increases in ALS death rates, along with substantial increases in other neurological disorder, in these countries, particularly in the under 74 and the under 65 year old age brackets.

The Gulf War veteran ALS studies are intriguing and, if nothing else, provide another argument against the Gompertzian hypothesis, not least because the latter approach ignores the implications of susceptibility and possible environmental triggers. The Gompertzian passivity also fails to ask the question of *why* neurological disease should increase substantially with advancing years, and ignores the fact that the majority of elderly people do not develop serious neurological disease. Most important is that the rate of ALS disease in relatively young people under the age of 45 clearly is predominately environmentally linked, although we will probably never know what amongst the `cocktails' of injections to protect against the fear of biochemical, biological and nuclear weapons, contributed to these increased rates.

So, ALS has risen substantially in the U.S., and in England & Wales, but when placed in the context of other serious neurological disease it appears to be one part of a worsening international pattern. (The USA, however, has seen the most serious increases.) Moreover, women have been affected more than men, although men still outnumber women in terms of the absolute number of deaths. The actual numbers of U.S. deaths from neurological disease is a stark reminder of how many people are now being affected – not only the patients, but also the family members, friends, and relatives impacted by each death. Conservative estimates suggest that increases in the number of neurological disease-related deaths have lead to more than 25,000 `extra' people a year under the age of 55 having a serious neurological condition. Placed alongside these increases in ALS and other neurodegenerative diseases, there have been substantial increases in deaths from `Other Blood Disorders', `Other Lymph Diseases' in the U.S.. Substantial increases in the incidenc of certain cancers have been masked by improved treatment, which has reduced the number of deaths. It would seem, therefore, that whatever is contributing to the increases in neurological deaths is contributing to increases in other types of disease like blood disorders and the malignancies.

Despite the limitations of the works explored, we can state with confidence that there are and continue to be significant changes in the epidemiology and public health-related aspects of ALS. These changes appear to be related to socio-environmental influences possibly, in a minority of cases, impacting on bio-genetic susceptibility. This is the context for our examination of the biological, the agri-industrial-domestic chemicals, and social factors, which are explored in the following two sections of this report.

PART II: GENETICS

Unlike more prevalent neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, ALS is a rapidly progressive disorder. There is a substantial body of research behind the epidemiology and public health factors of ALS, and it comes as something of a surprise, when addressing the minutiae of the disease's pathogenic factors, that the research is of comparable breadth and ingenuity, especially within the last decade or so. However, the literature concerning the etiopathogenesis of ALS remains divided and inconclusive; the pathogenesis of the disease is still poorly understood and why motor neurons are specifically vulnerable in ALS remains an enigmatic problem.[146]

Nevertheless, this is not to say that exciting advances in ALS research have not been made in the past decade. Increasing evidence is accumulating that injury caused by superoxide free radicals,[147] glutamate excitotoxicity,[148] gene regulation in spinal motor neurons,[149] the down-regulation of human cellular prion protein,[150] and diet[151] may be play significant roles in the etiopathogenesis of ALS. It is important to remark, however, that the majority of the research exploring pathogenic factors in ALS falls into one of two categories: first, investigations of the mutations of the copper/zinc superoxide dismutase (SOD1) and the enzyme's role as a sulfhydryl metalloenzyme that scavenges for toxic superoxide free radicals[152] and second, the examination of Ca^{2+} influx through AMPA-type (Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) glutamate receptors (iGluRs) and posttranscriptional RNA editing of the transcript of the GluR2 gene (located in the GluR2 subunit) that attempts to maintain intracellular Ca^{2+} homeostasis when edited correctly.

Sporadic and familial forms of ALS are clinically indistinguishable.[153] However, Jonsson et al maintain that about 10% of ALS cases are familial and in 20% of these cases the disease has been linked to mutations in SOD1.[154] Andersen et al concur with this figure and while estimating that 5 – 10% of all cases of ALS are familial ALS (or FALS) they suggest that the inheritance is usually as an autosomal dominant trait. SOD1 gene mutations are less frequent in sporadic ALS (or SALS) accounting for roughly 2.6% of these cases.[155] Additional support for SOD1 involvement in both ALS 'types' comes from Ferrante and colleagues, who examined markers of oxidative damage in the brains of

ALS cases and found evidence of increased oxidative damage in the motor cortex of both familial and sporadic ALS cases compared with controls.[156] Demonstrably, the damaging effect of superoxide free radicals play a role in the development of sporadic ALS as well as familial ALS, albeit with distinctively dissimilar levels of influence.

Glutamate excitotoxicity has been implicated as a mechanism of motor neuron death in both SALS and FALS and research has indicated that excessive activation of AMPA receptors, by excitatory amino acids such as glutamate, can lead to a large increase of intracellular free Ca^{2+}, which in turn triggers a cascade of events leading to structural damage and, potentially, apoptosis.[157] Although this 'excitotoxic theory' can account for selective motor neuron death in the motor cortex, the apoptotic process following glutamate excitotoxicity is not unique to motor neurons and has been well documented in human prefrontal cortex, striatum, and white matter in cases of Huntington's disease, Alzheimer's disease, and schizophrenia.[158] As previously mentioned, the process of GluR2 RNA posttranscriptional editing underpins Ca^{2+} AMPA permeability. So, while RNA editing may contribute to death and dysfunction in the motor cortex, RNA editing may be implicated in the pathogenesis of ALS and in the pathogenesis of other neurodegenerative diseases and mental illnesses.

Little is known about global gene expression patterns in ALS and whether SALS or FALS present with different gene expression profiles. Dangond et al addressed this challenge with "high-density oligonucleotide microarray technology that compared expression levels of approximately 6,800 genes in post-mortem spinal cord grey matter obtained from individuals with ALS as well as normal individuals."[159] To discern an ALS-specific signature they employed two techniques: Fisher discriminant analysis and leave-one-out cross-validation. Remarkably, the authors discovered many alterations in genes involving mitochondrial function, oxidative stress, excitotoxicity, apoptosis, cytoskeletal architecture, RNA transcription and translation, proteasomal function, and growth and signaling. Moreover, and perhaps critically, it was possible to distinguish between SALS and FALS gene expression profiles based on the post-mortem spinal sections.

Although Dangond et al's notable investigation sheds light upon a previously unknown distinction between the global gene expressions of SALS and FALS, the nature of the post-mortem samples were such that clear pathogenic pathways could not be discerned. However, Jiang et al have also analysed gene expression profiles and focused their efforts upon pathogenesis in an attempt to elucidate the causative pathomechanism of sporadic ALS only.[160] Using microarray technology combined with laser-captured microdissection, gene expression profiles of degenerating spinal motor neurons were isolated from autopsied patients with SALS and examined. Jiang et al. have shown that gene-regulation is a key concept in the pathogenesis of SALS: cell death associated genes in spinal motor neurons were up-regulated, as well as promoters for the cell death pathway (death receptor 5, cyclins A1 and C, and caspases-1, -3, and –9). However, while the effects of gene-regulation are now easily verifiable through gene expression analyses, the factors that promote atypical up- and down-regulation of genes still remain elusive.

In an effort to deliver an integrated and interdisciplinary perspective on both sporadic

and familial ALS, it is critical to appreciate that agents of exogenous origin are often just as important as those of endogenous origin. A common critique of some of the papers that consider AMPAR receptor physiology or the effects of SOD1 mutations is that they over-look or ignore the effect of exogenous influences, rather than considering the external context for the physiological changes. Recent research however posits that excitatory amino acids of dietary intake may play a crucial role in the pathogenesis of ALS, which self-evidently are `external.'[161] The disregulation of glutamate, the brain's primary excitatory neurotransmitter, has previously been mentioned as playing a major role in 'excitotoxic theory' where excessive synaptic levels of glutamate cause repetitive motor neuronal firing which results in a large increase on Ca^{2+} with drastic cytotoxic effects, and diet, can not be ignored, as it is a major source of exposure to glutamate and aspartate – both excitatory amino acids that could quite readily trigger the first steps in excitotoxic theory. Given the attention to oxidative damage in the pathogenesis of ALS it s necessary to consider the consumption of dietary antioxidants and their role in potentially preventing oxidative stress. The reduction in Guam ALS cases is in part, ascribed to the changed diet of the population and the concomitant reduction in glutamate in the food intake.[162]

1. An exogenous context for an endogenous effect: glutamate excitotoxicity

Lorene Nelson and colleagues have produced an exceptional piece of work on the association of nutrient intake with the risk of ALS.[163] Their primary aim was to assess diet as a predisposing factor in the development of ALS where macronutrients, excitatory amino acids, and antioxidant vitamins were of particular interest. A population-based case-control study was conducted in three counties of western Washington State from 1990 to 1994. Incident ALS cases (n = 161) were identified and individually matched on age and gender to population controls (n = 321). Nelson et al utilised a standardized questionnaire, which explored food frequency to provide a comprehensive assessment of nutrient intake. Previous studies have investigated case-control differences in diet, but only analysed the consumption of a few substances within the food items (e.g. solvents, heavy metals).[164]

The case definition of ALS required that all patients included in the study had a progressive motor neuron disease that affected both upper and lower motor neurons or to have one of the recognised clinical variants (progressive muscular atrophy, progressive bulbar palsy). Patients with primary lateral sclerosis were excluded, as well as patients whose diagnosis of ALS had changed in the year following their initial diagnosis. Two controls were matched to each case on gender and age (within 5 years). The food frequency questionnaire was completed by 161 (93%) of the cases and 321 (92%) of the controls. Data for the study was derived from two sources: an in-person interview and a self-administered food questionnaire. Professional interviewers collected information on: demographic factors, residential and occupational history, physical activity, height and weight, medical antecedents, and family history of neurodegenerative disorder.

Information about 98 food items was sourced from the semi-quantitative 'National Cancer Institute's Health Habits and History Questionnaire'. The 98 food items on the questionnaire were validated by a study that ensured that 90% of the nutrients in the US diet were included.[165] For each case, eating habits

were assessed for the year prior to the onset of the ALS symptoms via the food frequency questionnaire, which were then edited and coded by a nutritionist.

Carbohydrate intake was not associated with the risk of ALS (p for trend = 0.95). And even though the odds ratio for the highest quartile of protein intake was modestly elevated, No significant trends were observed for this macronutrient either (for >79.9g protein/day, OR = 2.1, 95% CI: 0.8, 5.4; p for trend = 0.27. Importantly all odds ratios were adjusted for smoking, education, and total energy intake. Significant trends were observed between quartiles of fat intake and the risk of ALS. Fibre intake was inversely associated with ALS risk; with the inverse relationship observed for water-soluble and insoluble fibre.

Because of the inverse relationship of ALS and fibre intake further adjustments were made when calculating the fat intake ALS risk association. Nelson et al reported that: "the positive association with fat intake was only marginally attenuated, and the trend of increasing risk with higher levels of fat persisted. The positive association with fat was largely explained by the contribution of polyunsaturated fats, saturated fats, and linoleic acid."[166] The association of ALS with monosaturated fats however, was weaker with no clear dose-response trend observed.

Glutamate intake and ALS was associated with an increasing trend across all quartiles of intake (p for trend = 0.02). And while no significant trends were observed for other excitatory amino acids tested, some of the odds ratios reported for the highest quartiles of intake were moderately elevated. The results from antioxidant vitamins were intriguing. When consumption of antioxidants from both food sources and dietary supplements were considered the total intake of vitamin A, vitamin C, and vitamin E were not significant.

Copper, zinc, iron, and calcium are all important factors for enzymatic activity and intracellular homeostasis. Nelson et al included these metals in their dietary investigation with the risk of ALS, but no associations were observed. Bergomi et al, in a similar population-based case-control study, analyzed the association between environmental exposure to trace elements and the risk of ALS in northern Italy, using a logistic regression analysis and supported Nelson et al's results.[167] Bergomi et al. also found no evidence of an association between ALS and the levels of copper, zinc, or iron in normal controls and ALS cases.[168] Furthermore, no associations were found with cadmium, manganese, selenium, chromium, cobalt, and aluminium exposure either.

The justification for introducing Lorene Nelson's paper on dietary intake is to provide a context for the etiopathogenic pathways that will be discussed in depth in this section of the paper. The significant observation of a dose-response relationship with fat consumption is worrying; and Keller & Mattson can further illuminate the fundamental problem of ALS risk association and lipid intake.[169] Although Nelson et al's paper reports two case-control studies of Parkinson's disease and dietary lipids;[170] Keller & Mattson implicate lipid peroxidation and lipid peroxidation products in a host of neuropathological conditions including ALS, Alzheimer's disease, and ischemia.[171]

Oxyradical-induced cytotoxicity can be explained as a process where whereby reactive oxygen species give rise to a number of 'oxyradicals' that produce 'peroxidation

metabolites'. Emerging evidence suggests that these metabolites underlie the extensive neuronal degeneration seen in conditions such as Parkinson's disease and ALS. Keller & Mattson suggest that chronic (or even acute) increases in the numbers of reactive oxygen species give rise to subsequent lipid peroxidation. The brain is particularly vulnerable to oxidative damage due to the relatively high concentrations of polyunsaturated fats that are especially prone to lipid peroxidation.[172]

By reacting with polyunsaturated fatty acids in the various cellular membranes, oxyradicals such as hydroxyl (OH.) and peroxynitrite (ONOO-) give rise to a variety of lipid peroxidation products, including 4-hydroxynonenal and malondialdehyde. Once formed, these peroxidation metabolites have been demonstrated to have relatively long half-lives within cells (minutes to hours), allowing for multiple interactions with cellular components. Since membrane levels of polyunsaturated fats are determined by dietary lipid intake, increased consumption could yield higher levels of lipid substrate and naturally increase the risk of neuronal degeneration.

Despite the prominence of the oxidative stress theory in the hypotheses of ALS etiology, Nelson et al found no inverse associations with the intake of dietary antioxidants, namely vitamin E, and also reported that up until 2000 there were no published reports of the positive effects of vitamin E on disease progression.[173] However, Desnuelle et al reported only a year later that "patients given alpha-tocopherol (the antioxidant vitamin E) were less likely to progress from the milder state A to the more severe state B (p for trend = 0.046) of the ALS Health State scale."[174] In addition, Ascherio et al have also very recently shown that: "regular use of vitamin E supplements was associated with a lower risk of dying of ALS."[175] However, Graf et al conducted an extremely stringent double blind, placebo-controlled, randomised, stratified, parallel-group clinical trial that attempted to determine whether vitamin E (5000 mg per day) may be efficacious in slowing down disease progression when added to riluzole.[176] From their results they were unable to determine whether the "megadose" of vitamin E had had any effect in slowing down disease progression; administration of this megadose did not seem to have any significant side effects in the patient population.

The observation that dietary fibre has an inverse association with ALS risk raises many more questions than answers. Diet is a potential source of exposure to toxins and excitotoxins that have been implicated in the development of neurodegenerative disorders, but the presence of dietary fibre could mitigate such threats. However, while it is tempting to hypothesize that dietary fibre prevents the "absorption of a dietary toxin associated with ALS," we are unsure. It is suggested that the complex causative pathways that free radicals follow in oxidative stress theory are far from being fully understood, and it would therefore be hasty to suggest that the preventative pathway could be reduced to the absorption of dietary toxins. Nevertheless, perhaps the inverse relationship with fibre is intertwined with the peroxidative pathway of (polyunsaturated) fats since Munakata et al have shown that fibre supplementation has been shown to decrease fat absorption in the bowel.[177]

Glutamate excitotoxicity (excitotoxic theory) is another prominent hypothesis explaining the etiopathogenic pathways in the progression of ALS. Therefore, the finding of a significant dose-response association of glutamate intake with

ALS (with more than a three-fold increase in risk at the highest quartiles) goes a long way in substantiating excitotoxic theory as a leading theory in the field. Pattern et al observed elevated levels of glutamate in serum, cerebrospinal fluid, and brain tissue of ALS patients; and clinical trials of riluzole (an inhibitor of glutamate release) prolongs survival in ALS patients, albeit only for some months.[178] However, Nelson et al make three extremely lucid criticisms of the role of dietary glutamate in ALS pathogenesis: (1) much of dietary glutamate is metabolised before it enters systemic circulation; (2) the amino acid carrier system for acidic amino acids within the blood brain barrier should maintain glutamate homeostasis; and (3) previous studies suggest that high levels of plasma glutamate must be attained to cause glutamate excitotoxicity in the brain.

On the other hand, glutamate is also produced endogenously as the premier excitatory neurotransmitter in the brain. Up till now we have characterised glutamate excitotoxicity as the chronic over stimulation of AMPA receptors leading to Ca^{2+} intracellular imbalances and eventual neuronal death. However, the fundamental debate within excitotoxic theory and AMPA receptor research lies with RNA editing, the GluR2 subunit, and its role within Ca^{2+} permeability.

The debate is outlined by a succinct investigation by Ludo Van Den Bosch and colleagues, in evaluating the role of excitotoxicity in the pathogenesis of ALS, comparing the sensitivity of motor neurons and that of dorsal horn neurons.[179] Of the three families of ionotropic glutamate receptors (AMPA-type, karinate acid-type (KA), and NMDA-type) AMPA and KA-type are studied alike in ALS research since both mediate excitatory synaptic signals. Van Den Bosch suggest that the increased susceptibility of AMPA and KA-type receptors "may be due to unusual Ca^{2+} permeability of these receptors on motor neurons". Akbarian et al elucidate: "The Ca^{2+} conductance of the AMPA class receptors…is determined by a single subunit polypeptide (GluR2) which is one of the four subunits that have been described for the AMPA receptor class (GluR1 – GluR4). It has been shown that the presence of a specific arginine (R) residue located within the second transmembrane region (TM II) of the GluR2 polypeptide is critical for a reduction in Ca^{2+} conductance."[180] In fact, Jonas & Burnashev maintain that the arginine residue's presence in the heterometric receptor complex drastically decrease the channels permeability for any divalent ions.[181]

Sommer et al and Verdoon et al observe that in all four AMPA subunit genes (including the critical GluR2 gene) the genomic coding for the Q/R (you've used the letter Q but don't think you have previously said what amino acid the letter Q refers to (you do a couple of lines below on 354), you have said what R is already on line 342 though) position on the TM II region contains a glutamine codon (CAG); therefore almost all AMPA receptors have a glutamine (Q) in this region of the TM II.[182] However, in posttranscriptional editing of the GluR2 RNA, an exchange of a single nucleotide causes the replacement of the CAG codon for glutamine by a CGG codon for aginine.[183] The fundamental principle of GluR2 physiology is that: the edited and highly prevalent GluR2 subunit has a drastic decline in its permeability to Ca^{2+}; conversely, the unedited and comparatively rarer GluR2 subunit has a considerably amplified Ca^{2+} permeability.

It is clear that several groups have investigated

the presence of the GluR2 subunit in motor neurons but results are inconsistent. While many studies found evidence for GluR2 presence most suggest the confusing correlation of extremely low expression of GluR2 with selective vulnerability of motor neurons.[184] To overcome the problem of selectivity, Van Den Bosch et al cultured motor neurons and dorsal horn neurons in identical conditions and then compared the sensitivity of the two cell types to KA-induced excitotoxicity (toxicity that is clinically comparable to glutamate excitotoxicity). Critically, they found that motor neurons were selectively vulnerable to KA-induced cell death as compared to dorsal horn neurons; and that this cell death was Ca^{2+} dependent and selective for motor neurons containing Ca^{2+} permeable AMPA receptors. They sought to answer whether the presence or absence of the GluR2 subunit was the factor determining the selective vulnerability of the motor neurons.

Van Den Bosch et al cultured motor and dorsal horn neurons in identical conditions on a glial feeder tray. At 8 days in vitro, the cultures were exposed to KA in the presence of MK801, an inhibitor of the NMDA receptor, to ensure only non-NMDA receptors were activated for KA uptake.[185] They observed a dose-response motor neuron death; while the dorsal horn neurons were not affected by treatment with KA, even at higher concentrations (for 100μM (KA), $p = 0.001$; and for 300μM (KA), $p = 0.0005$). The motor neuron death could be blocked by CNQX, an antagonist of AMPA and KA receptors (i.e. the non-NMDARs); where 0μM (CNQX) vs. 25μM (CNQX); $p = 0.007$ for % neuronal survival of control culture.

KA-induced cell death was undetectable in the absence of external Ca^{2+} and was dose-dependently enhanced by the presence of extracellular Ca^{2+}. In contrast, Na^{+} entry through the receptor channel did not appear to contribute to selective neuronal death. Furthermore, 1mM NiCl was utilised as a blocker of voltage operated Ca^{2+} channels but did not affect Ka-induced cell death. This indicates that Ca^{2+} entry via voltage-operated Ca^{2+} channels does not contribute to the KA-induced motor neuron death. Van Den Bosch et al's paper also manages to unequivocally demonstrate involvement of the AMPA receptor and to exclude a role for the KA receptor – which further implicates GluR2's involvement (Madden states that: KA receptors are homo- or hetero- oligomers of the subunits GluR5 – GluR7, KA1 and KA2 and thus do not include GluR2 in their receptor complex.)[186] They studied the effect of the selective AMPA antagonist LY300164, and observed as the antagonist dose-dependently inhibited the KA-induced motor neuron death (p for this trend = 0.001). Altogether, the above data indicates that Ca^{2+} entry via Ca^{2+} permeable AMPA receptors is responsible for KA-induced motor neuron death.

To correlate the sensitivity of motor neurons to the presence or absence of the GluR2 subunit, motor neurons were stained for GluR2. Van Den Bosch et al's results indicated that motor neurons with Ca^{2+} permeable AMPA receptors express a high amount of GluR2 subunits. Therefore, they do not support the idea that the absence or low abundance of the GluR2 subunit is responsible for Ca^{2+} permeability in AMPA receptors in motor neurons.

Thus, yet again we are left with something of an enigma. It is expressly clear to Van Den Bosch that it is Ca^{2+} influx through AMPA receptors that is responsible for selective motor neuron death. However, the blame cannot be placed on the absence or scarcity of the Ca^{2+} permeability

controller, the GluR2 subunit, since it is expressed in high amounts throughout the motor neuron AMPA receptors. Now that we have exhausted all other plausible explanations, we turn to RNA editing efficiency of the GluR2 gene.

Kawahara et al maintain that the RNA editing of the GluR2 subunit is the key to neuronal survival.[187] Brusca et al observed the premature demise of mice that have defective GluR2 mRNA editing is caused by neuronal death, but the neuron can be rescued by restoration of the RNA-editing function; thus indicating to Kawahara et al that "this GluR2 modification is crucial for neuronal survival."[188] Their experiment involved extracting RNA from single motor neurons isolated with a laser microdissector from five individuals with ALS and from five normal control subjects. Editing efficiency was calculated by measuring the difference in digestion patterns of nested GluR2 mRNA products from the polymerase chain reaction. The editing efficiency in cerebella Purkinje cells was also quantified for individuals with ALS and the results were compared with those of the normal subjects.

The frequency of GluR2 mRNA positivity was not significantly different between the ALS and the control groups (two-sample test for equality of proportions, $p > 0.05$). However, the editing efficiency varied between 0% and 100% in the motor neurons from each individual with ALS, and in 44 of the motor neurons (56% of the sample) editing was incomplete i.e. 56% of the sample lay between 0% - 99.9% editing efficiency. All of the 76 control motor neurons showed 100% editing efficiency. The efficiency of the editing in the Purkinje cells was virtually complete in the ALS and control groups. Kawahara et al conclude that motor neurons may be are specifically vulnerable to defective RNA editing; and thus suggest that ALS: "may turn out to be a disease of RNA processing". However, there is no published study that investigates the etiopathogenic pathways that lead to defective RNA editing.

Kawahara et al confusingly cite Feldmeyer et al to justify their conclusions.[189] They suggest that Feldmeyer et al found that transgenic mice with GluR2 subunits, which were made artificially permeable to calcium ions, developed motor neuron disease later in life. Conversely, Feldmeyer et al actually reported that: "calcium-triggered neuronal death was not observed"; and only a variety of neurological dysfunctions were noticed including epilepsy and deficits in dendritic architecture? We have previously established that if RNA editing in ALS patients was as deficient as reported in the Kawahara et al paper then Ca^{2+} permeability in the GluR2 subunits (in the AMPA receptors) would be expected to be high enough to allow a large increase of intracellular calcium. This is turn has been shown to trigger a cascade of events leading to selective cell death.[190] The Feldmeyer et al study specifically declared that calcium-triggered death was not observed. 56% of the motor neurons of the sample in the Kawahara et al study operated with an editing efficiency of below 99.9% and in order to properly illustrate the ramifications of this figure we must turn an earlier study of Akbarian et al, who produced a report on editing efficiency in the AMPA receptor subunit GluR2 in the human prefrontal cortex and striatum and its roles in Alzheimer's disease, Huntington's disease, and schizophrenia.[191] They suggest, along similar lines to Kawahara et al, that disturbances in the editing efficiency of the GluR2 subunit could lead to neuronal dysfunction. Their study determined the proportions of edited GluR2 (low Ca^{2+} permeability) and unedited GluR2 (high Ca^{2+} permeability) in the prefrontal cortex

of brains from patients with Alzhiemer's disease, in the striatum of brains from patients with Huntington's disease, and in the same areas of brains from age-matched schizophrenics and control subjects.

They showed that in the human brain the RNA for the GluR2 subunit polypeptide is present in both edited and unedited isoforms. In the normal human prefrontal cortex and striatum, 99.9% and 99.8% respectively, of all GluR2 RNA molecules were fully edited. The high percentage of the editing efficiency is comparable to the results found in normal human motor neurons in the Kawahara et al study that found 100% editing efficiency. However, Akbarian et al observed that there was a significant elevation in the ratio of unedited to edited GluR2 molecules in the prefrontal cortex of Alzheimer's patients and schizophrenics, and in the striatum of Huntington's patients (in comparison to age-matched controls). In each disease group, approximately 95% of all the GluR2 RNA molecules remain edited, whereas normal controls have a figure of approximately 99.9%. Their conjecture is that the several fold increase in the unedited form (normal controls vs. disease group patients) may have serious neurotoxic consequences.

To clarify, Akbarian et al report that in the unedited GluR2 isoform, there is an increase in relative distribution from normal patients to the disease group (from ~0.01% to ~5% of all the GluR2 subunits); and that this small increase aided the neural degeneration seen in these conditions. Kawahara et al reported that they had observed that 56% of all the GluR2 subunits (of the motor neurons) in their sample had remained unedited – a relatively massive difference in unedited GluR2 subunits.[192] Fundamentally: if Akbarian et al believe that the 5% of their (prefrontal and striatum) sample that contained unedited GluR2 contributed to neural degeneration; it truly reinforces the Kawahara et al believe that the 56% of their (motor neuron) sample that contain unedited GluR2 lead to neuronal death.

To add an additional perspective: Van Den Bosch et al suggested that receptor complex stoichiometrics will dictate that a tiny sub-population of AMPA receptors on the motor neuron surface will be GluR2-deficient naturally and that this miniscule number of receptors would be sufficient to induce cell death. Greger et al also warn us not to overlook the powerful influence that receptor subunit stoichiometry holds.[193] They suggest that naturally occurring, unedited GluR2 subunits are incorporated into functional AMPA receptors and then transported to the cell surface far more efficiently than their edited GluR2 counterparts. Akbarian et al theorize that a small population of neurons could show dramatic changes in the proportions of edited and unedited GluR2 subunits;[194] Van Den Bosch cite evidence that motor neurons selectively target Ca^{2+} permeable AMPA receptors.[195] And since aggregated and unedited AMPA receptors could therefore hypothetically be selectively targeted on the cell surface, sporadic ALS could be caused by unfortunate receptor stoichiometry in an individual with fully effective and functional posttranscriptional RNA editing!

In sum, Nelson et al revealed in the first fully competent assessment of nutrient intake that glutamate intake was associated with an increasing risk of ALS across all quartiles of intake. They also showed that fat intake was positively associated with ALS risk and that clear dose-response curves for polyunsaturated and saturated fats were observed. The additional finding that fibre intake was

inversely associated with ALS risk was intriguing and provoked the formulation of various hypotheses; but ultimately remained inexplicable.

Keller and Mattson's observations were discussed in concordance with the finding that fat intake was positively associated with ALS risk since they maintain that: reactive oxyradicals react to produce peroxidation metabolites; and in turn these metabolites react with polyunsaturated fats to produce lipid peroxidation products.[196] The suggestion is that these peroxidation products engage in a host of cellular interactions which ultimately positively influence neuronal degeneration. Munakata et al have shown that fibre supplementation may to decrease fat absorption in the bowel.[197] This observation may contribute to an explanation of why fibre has an inverse risk association with ALS.

We have characterised 'glutamate excitotoxicity' as the chronic over-stimulation of AMPA receptors leading to Ca^{2+} intracellular imbalances and eventual neuronal death. Van Den Bosch et al investigated AMPA receptors to discover that: (1) chronic increases in Ca^{2+} influx results in motor neuron death; (2) this effect is not due to the scarcity/absence of Ca^{2+} permeability controller, the GluR2 subunit; and (3) Ca^{2+} permeability in AMPA receptors is perhaps best explained by the lack of efficient RNA editing.[198] Kawahara et al, in a sample of 78 motor neurons from ALS patients, found that RNA editing was incomplete in 56% of them at the crucial Q/R site of the GluR2 subunit. They provide additional support for a Ca^{2+} influx initiated neuron degeneration and also suggest that ALS may turn out to be a disease of RNA processing.[199]

However, Greger et al warn against ignoring the powerful effects of receptor complex stoichiometrics since unedited AMPA receptors are far more easily transported to the cell surface than their edited counterparts.[200] Van Den Bosch et al suggest that these unedited subunits are selectively targeted and therefore a small population of aggregated and unedited AMPA receptors situated in the cell surface could initiate an apoptotic condition.[201]

2. Mutant SOD1 and the toxic gain of function

Having discussed and highlighted many of the central and peripheral arguments in excitotoxic theory we finally turn to the cytosolic free-radical scavenging metalloenzyme: the copper/zinc superoxide dismutase theory and a discussion of its role in ALS etiopathogenesis.

The SOD1 gene consists of five exons each consisting of 150 – 200 nucleotides interspaced with four larger introns; the small size of the exons and the absence of longer repetitive sequences have facilitated diagnostic genetic studies of the gene.[202] As previously discussed, 105 different SOD1 mutations have now been published worldwide specifically with patients of ALS. These mutations are predominantly single amino acid replacements, apparently randomly scattered throughout the structure of this homodimeric 32,000-Da metalloprotien. From Andersen et al we extrapolate that of the 105 mutations: 19 mutations occur on exon 1, 13 on exon II, 6 on exon III, 39 on exon IV, and 28 on exon V.

Jonsson et al maintain that about 10% of all ALS cases are FALS and in 20% of these cases the disease has been linked to mutations in SOD1.[203] Epidemiological studies have looked at the frequencies of the SOD1 mutations in FALS and SALS and observe that: "The frequency of SOD1 mutations in FALS patients is reported to be 23.5 % in Scandinavia, 23.4% in the USA, 21% in

Great Britain, and 14.3% in France". In SALS, the frequency of SOD1 mutations is much lower: "accounting for 7% in Scotland, 4% in Scandanavia, and 3% in England."[204] Yet it is fair to point that the SOD1 mutation only accounts for approximately one fifth of familial ALS cases worldwide; consequently 80% of FALS cases remains to be explicated.

Peter Andersen and colleagues at the Umeå University Hospital (Umeå, Sweden) deserve particular recognition in the field of SOD1 mutations. They were able to detect mutations in the SOD1 gene through an analysis of patient files at the Neurogenic DNA Diagnostic Laboratory at Massachusetts General Hospital. They report that this laboratory, in association with Athena Diagnostic Laboratories, have preformed routine clinical testing for SOD1 mutations since February 1995. In the event of positive identification of a SOD1 mutation, pertinent medical, genetic, and genealogical records were reviewed; and in this way they discovered 16 (of the 105) novel SOD1 mutations.

In Andersen et al's investigation 2240 blood samples were referred to the Massachusetts General Hospital between February 1995 and January 2003.[205] Of the 2240, only 2045 were eventually analysed since some samples were duplicates, and were sent for presymptomatic testing or fetal diagnostic testing. After sequencing of all the five exons in all the 2045 ALS patients, 148 (7.2%) of the patients had a disease-associated exonic SOD1 point mutation. A single patient revealed a silent mutation on exon V (A140A) and a further 21 patients had intronic mutations of unknown significance. The most common mutation was A4V, present in 61 (41%) of the individuals. However, it was not possible to obtain medical records for all the 2045 ALS patients so the frequencies of FALS and SALS could not be calculated.

A notable finding of the Andersen et al study is that 41% of the patients with an A4V mutation were heterozygous for the mutation. This concurs with earlier studies of the FALS pedigree used for genetic linkage analysis that revealed that A4V is the most prevalent SOD1 mutation in the USA.[206] The participants included in this study were recruited throughout the United States so this comes as no surprise. However, outside North America the A4V mutation has only been found in a single FALS family in Sweden and two families in Italy.[207] Indeed Andersen et al observe that the high prevalence of the A4V mutation is contained to North America – the D90A mutation is recognised as the most prevalent mutation in Europe where the A4V mutation features in almost complete absence. [208]The D90A mutation is of special interest since it is relatively very common in Northern Scandinavia.

Five patterns of inheritance have been reported in ALS associated with SOD1 gene mutations: dominant inheritance with complete penetrance, dominant inheritance with reduced penetrance, recessive inheritance, recessive inheritance with compound heterozygosity, and de novo mutations.[209] Andersen et al note that reduced penetrance within families can often present as SALS; gene tests can reveal SOD1 mutation and further genealogical investigation demonstrate reduced penetrance.

Andersen et al and Cudkowicz et McKenna-Yusen both recognize the tendency for the transmitted infected individual (in families with reduced penetrance) to be female.[210] Murakami et al identified a novel missense mutation that coded a transition of T to C, resulting in a substitution of leucine 126 to serine in exon 5

(mutation L114S)..[211] They observed that: "The family had very unique clinical features of extremely mild severity only in the legs of two male patients with onset of 42 and 52 years old, and their mothers did not develop any symptom even after reaching the age of 80 and carrying the same mutation." With the increase in male susceptibility in cases of incomplete penetrance the question of why females in particular remain asymptomatic cannot be ignored. And while X-linked recessive inheritance is an all too tempting prospect to neglect, so far the literature is not forthcoming with any conclusive answers.

Spinal and bulbar muscular atrophy (SBMA) and ALS are truly representative motor neuron diseases where selective neuronal death occurs. However, SBMA is an X-linked neurodegenerative disease caused by the expansion of a CAG repeat in the first exon of the androgen receptor gene (Sobue 2001). With such similarities in symptomology one wonders whether it would be plausible to consider additional and interactive X-linked factors in the inheritance of ALS. Yaguchi et al (2003) recently compared the golgi apparatus of spinal anterior horn cells in five patients with SBMA and five with ALS? The different frequency of fragmented Golgi apparatus between SMBA and ALS observed however, adds another finding to the host of pathogenic differences of neurodegeneration between these two disorders.[212] However likely or unlikely it is that the L114S mutation presents with incomplete penetrance because of X-linked recessive inheritance has yet to be properly substantiated.

Returning to the distinctive D90A mutation, as already mentioned the D90A-SOD1 mutation deserves special attention because in Northern Scandinavia (where approximately 1 in 100 people carry the disease) people from more than 20 families have developed ALS from D90A mutations; and it the mutation also shows a unique pattern of inheritance. Dr. Parton reports that these people with ALS in Scandinavia are all homozygous for the mutant gene: both their chromosomes carry D90A. None of their relatives (who carry one D90A gene and one WT) have ever developed ALS. Furthermore, those who are homozygotes all have the same pattern of disease, starting in the legs before slowly becoming more progressive.[213] More than 80 cases homozygous for the mutation have been found – most of these cases are from Northern Scandinavia – where the allele frequency of the D90A mutation reaches 2.8%.[214] Elsewhere the mutation is rare and a figure of 0.03% was found in the U.K.[215]

The consistency of symptom progression in Scandinavian homozygotes is highly unusual as, in SOD1 or FALS in general, members of the same family often differ in both how their ALS begins and subsequently develops. Confusingly, as well as the affected homozygotes, 15 people heterozygous for D90A have been found with ALS, some of whom show inheritance of the disease while others do not.[216] The D90A mutation also reveals a phenotypic continuum when expressed in the homozygous form characterised by slow disease progression; mean survival time calculated by Turner et al at 14 years.[217] The D90A mutation expressed in the heterozygous form shows no such continuum and the resulting phenotype is more comparable to the classic Charcot-type of ALS. Therefore, it seems that the D90A complex has an extremely complex means of inheritance: in some it only causes ALS in recessive inheritance; while in others it causes ALS when only one copy of the gene is present (sometimes referred to as dominant inheritance). No other disease is known to have two separate means of inheritance. However, the most intriguing debate is of how people with two copies of the

disease-causing gene have a less aggressive form of ALS than those expressing one copy.

Andersen et al and Al-Chalabi et al have conducted haplotype analysis of the dominant and recessive D90A families from around the world and the results suggest that all the recessive cases share a common founder.[218] However, those individuals with a single copy of the gene (where the disease had developed) were descendants of at least two separate ancestors. The foremost explanation of how the D90A produces a phenotypic continuum is characterised by Jonsson et al (2002): "one explanation for the conundrum could…be that the recessive haplotype carries a genomic difference tightly linked to the Cu/Zn SOD locus that modifies the toxic effect of the D90A mutation". This factor would not only diminishes the toxic effect as advertised, but only those individuals with two copies develop ALS – their heterozygous relatives are protected from the disease.

While reminding us that SOD1 constitutes about 0.5 – 1% of all soluble protein in the human brain, Pardo et al also prompt us to consider the metalloprotein's biological importance; the ubiquitous expression of the enzyme in the spinal cord, motor neuron axons and dendrites, cell bodies, and in a subset of other neurons.[219] Andersen et al maintain that, "despite a decade of extensive studies, no common biophysical or biochemical denominator has been found for all the mutations in the…SOD1 gene."[220] No null mutations have been reported which suggests to Andersen et al that the mutant SOD1 polypeptide subunit is essential for the cytotoxic action. Therefore, we review a number of theories from prominent papers attempting to explain how the effects of a mutant SOD1 lead to neuronal death.

There is robust evidence that mutant SOD1 exerts its deleterious effects through a toxic gain of function as opposed to a loss of superoxide dismutase activity.[221] The major hypotheses for the toxicity of mutant SOD1, which are not mutually exclusive, include: alterations in the handling of intracellular free radicals leading to an increased formation of damaging hydroxyl radicals and peroxynitrite derivatives; and misfolding of the mutant protein leading to the formation of intracellular SOD1 aggregates.[222] Kirby et al (2005) assert that ALS patients with a mutated SOD1 experience motor neuron injury caused by a toxic gain of function. They postulate that the precise mechanism(s) remains unclear and therefore suggest that mutant SOD1 alters the motor neuronal transcriptome. They investigated the effects of mutant SOD1 on cells modelling the vulnerable cell population by using the established cellular model for SOD1-associated FALS: NSC34 (neuroblastoma x spinal motor neuron cell line).

By utilising gene expression profiling, 268 transcripts were expressed in the presence of mutant human G93A SOD1 – a mutation found on exon IV. Of these, 74% (197) were decreased demonstrating that the presence of mutant SOD1 leads to a marked degree of transcriptional repression. Critically, amongst the repressed transcripts were a group of: (1) antioxidant response element (ARE) genes encoding phase II detoxifying enzymes; and (2) antioxidant response proteins, which are regulated by a transcription factor called NRF2. Kirby et al provide evidence that the disregulation of ARE genes and NRF2 "represent significant and hitherto unrecognised components of the toxic gain of function of mutant SOD1."[223] In addition, genes involved in protein degradation, cell death/survival and the heat shock proteins all experienced significant alterations after mutant SOD1 exposure.

The association of mutant SOD1 and mitochondrial dysfunction has only recently been tackled as a pathogenic factor in ALS. After conducting a small- scale literature search an elucidative paper by Menzies et al which clarified the key issues in the debate. They begin by observing that in both subtypes of ALS (familial and sporadic) there is a possibility that mitochondrial damage may contribute to age-related motor neuronal injury. There are important properties of mitochondria relevant to the pathogenesis of neurodegenerative diseases including: the generation of intracellular ATP, the buffering of intracellular calcium, the generation of intracellular free radical species, and the involvement in the initiation of apoptosis.[224]

Immediately we see Menzies et al's point concerning mitochondria's importance since the linchpin of excitotoxic theory is the build up of intracellular Ca^{2+}. Menzies et al also note that mitochondrial proteins and DNA are shown to be particularly susceptible to oxidative stress and that free radicals are known to inhibit the activities of specific mitochondrial enzymes.[225] Alterations in the activities of respiratory chain complexes have been described in SALS, showing increased activity of complexes I,II, and III in the frontal cortex of patients with SOD1-related ALS, and decreased activity in complex IV in the spinal cord. The aims of the Menzies et al (2002) study were clearly stated: (1) to investigate whether morphological mitochondrial abnormalities occur at expression levels of mutant SOD1 close to physiological levels; and (2) to determine whether the presence of mutant SOD1 causes abnormalities of the mitochondrial respiratory chain function and changes the cellular bioenergetic parameters in motor neuron cells.

The NSC34 motor neuron cell line was utilised and with it Menzies et al demonstrated that the presence of mutant SOD1 results in the development of abnormally swollen and pale staining mitochondria. They also observed that, "these morphological changes are accompanied by biochemical abnormalities with specific decreases in the activities of complexes II and IV of the mitochondrial electron transfer chain". Interestingly, these are the same complexes that are inhibited when control NSC34 cells are subjected to oxidative stress induced by serum withdrawal. The motor neuron cells expressing mutant SOD1 showed increased apoptosis when exposed to oxidative stress by serum withdrawal, whereas normal SOD1 initiated a protective effect. From their observations we see how the mitochondrial changes associated with mutant SOD1 clearly had adverse cellular bioenergetic consequences as shown by increased cell death in the presence of pharmacological inhibition of the glycolytic pathway.

Menzies et al demonstrated, for the first time, that the expression of mutant SOD1 results in biochemical dysfunction accompanied by changes in mitochondrial morphological changes.[226] This dysfunction was followed by functional changes in cellular energy metabolism, demonstrated by the reduced ability of the cells to withstand inhibition of glycolysis. Critically, motor neurons in vitro are particularly vulnerable to cell death in the face of chronic inhibition of complexes II and IV – a direct result of abnormal mitochondria.

Since Menzies et al's investigation, a slew of similarly based studies were conducted with concurring results. Fukada et al recognised that SOD1 mutants caused alterations in the post-translational modifications of the voltage dependent anion channel 2 (VDAC2).[227] This is

relevant since VDAC2 plays a hand in regulating mitochondrial membrane permeability and activating apoptotic pathways. Gonzalez de Aguilar et al and Dupuis et al implicate dysfunctional energy homeostasis in increasing motor neuron vulnerability.[228] They incriminate mitochondrial dysfunction, occurring in skeletal muscle, as well as in motor neurons, as a process leading to a hypermetabolism that may, by itself, represent an additional driving force involved in increasing motor neuron vulnerability. Rizzardini et al have also experimented on the proliferation of mutant SOD1 cells and their effect on mitochondria.[229] After an analysis of membrane potentials they recognised strong mitochondrial depolarisation and also observed that mutant SOD1 cells maintained higher levels of reactive oxygen species.

Choi et al have revealed that SOD1 is a major target for oxidative damage in the brains of Alzheimer's and Parkinson's disease patients.[230] They have identified that there exist four isoforms of the superoxide dismutase and their investigation suggests that the total level of SOD1 isoform is significantly increased in both Alzheimer's and Parkinson's disease. Furthermore, and more crucially to ALS studies, Choi et al state that SOD1 forms proteinaceous aggregates that are associated with amyloid senile plaques in the brains of Alzheimer's disease patients. It is enticing to try and forge some common overlapping pathogenic mechanism between the major neurodegenerative disorders as it would help to clarify some of the research and clinical inconsistencies; and Choi et al predictably suggest that their work could potentially realise a similar therapeutic strategy for PD, AD, and ALS. Nevertheless, Andersen and his colleagues from Umeå also profess to a similar predilection and suggest that data derived from autopsied ALS patients "clearly points to [beta amyloids] in the motor neuron"; that "the basic mechanism in ALS can…be compared to that of Alzheimer's disease". They suggest that when the mutated proteins are broken down they release minor deposits burdening the motor neuron. Initially, they are dissolved, but gradually the region of these deposits turn into a "toxic dump" surrounding the motor neuron. The long-term burden apparently accounts for the latency of ALS and the eventual cell death.

The fleeting resemblances between the major neurodegenerative disorders will continue to persist; yet the reality is that we are still wandering in the dark, both arms firmly thrust out in front of us hoping to uncover more of an enigmatic common pathogenesis that may not even exist for Alzheimer's, Parkinson's, and ALS.

The evidence that suggests that mutant SOD1 exerts its deleterious effects through a toxic gain of function is quite substantial. A number of recent studies, thwarted by the 105 SOD1 mutations that provide no clear functional or structural clues to the underlying disease mechanism, have turned to investigating the SOD1 structural characteristics. They suggest that it is not the mature mutant Cu/Zn SOD holo enzyme that it is cytotoxic, rather, it is during the folding of the polypeptide subunits and the formation of the metal-free SOD apo protein (with the stabilizing intrachain disulphide bridge) that the mutant apo proteins are much more prone to misfold than wild type SOD apo protein and therefore become cytotoxic. Lindberg et al began to look for "folding-related defects by comparing the unfolding behaviour of five ALS-associated mutants with distinct structural characteristics: A4V at the interface between the N and C termini, C6F in the hydrophobic core, D90A at the protein surface,

and G93A and G93C, which decrease backbone flexibility."[231] They found that all the mutants share a pronounced destabilization of the metal-free apo state: the higher the stability loss, the lower the mean survival time for ALS patients carrying the mutation. Thus, there are a number of observations that support the belief that it is the apo state, and not the holo state, that accommodates a cytotoxic function.

Subramaniam et al investigated how critical a role copper-mediated oxidative damage plays in the pathogenesis of SOD1-linked FALS. To this end they ablated the gene encoding the copper chaperone for SOD1 in a series of FALS-linked SOD1 mutant mice. Through metabolic ^{64}Cu labelling they observed that the copper chaperone for SOD1 is necessary for the efficient incorporation of copper into SOD1 in motor neurons; and therefore the eventual production of copper-laden mutant SOD1 was markedly decreased. This however, did not modify the onset and progression of ALS in SOD1-mutant mice. Their conclusion was that deletion of the copper chaperone in transgenic animals with (G37R, G85R, and G93A) SOD1 mutations did not prevent motor neuron degeneration. Copper was shown to be non-essential for cytotoxic action; mutant polypeptides in the apo state still successfully actualised their cytotoxic intentions.[232]

Okado-Matsumoto & Fridovich, in line with Menzies et al, have previously reported the existence of SOD1 in the membrane space of mitochondria; this effect is seen in wild type (WT) or mutant SOD1.[233] They demonstrate that whereas partially or wholly demetallated SOD1 is taken into the mitochondria, the holo enzyme is not. This effect was seen in wild type and FALS-associated mutant superoxide dismutases. However, the most thought-provoking observations were seen when WT and mutant SODs were treated with cytosolic fractions of heated-shocked cells. The cytosol from heat-shocked neuroblastoma N2A cells inhibited the uptake of demetallated SOD1 into the mitochondrial membrane. Critically, "whereas the uptake of mutant SOD1 is blocked by the cytosol from heat-shocked cells, the uptake of WT SOD1 was much less affected". This observation indicated that some heat-shock proteins preferentially bind to mutant SOD1s and thus prevent mitochondrial uptake of the mutant SOD1s. What, however, are the implications of such a finding?

Motor neurons are particularly prone to the results of oxidative stress since their axons are very long and slow axonal transport makes turnover of the damaged macromolecules a slow process.[234] This proapoptotic condition is usually opposed by the antiapoptotic effect of the heat-shock proteins (Hsps); however, the presence of any abundant misfolded mutant SOD1 protein binds the Hsps. In humans (with FALS by caused mutant SOD1) we would therefore expect the membrane spaces of mitochondria to be deficient in SOD1, because of the blocking of the uptake of SOD1 molecules by their binding with Hsps. The mitochondria would therefore be denied the protective effect of SOD1 and thus be selectively prone to oxidative damage. The theory suggested is one of cumulative risk: decreased levels of Hsps increase the likelihood of establishing an oxidative stress-induced proapoptotic condition; and decreased levels of SOD1 in the mitochondrial membrane enhances the likelihood of inducing oxidative stress.

Andersen et al cite Tiwari et al in the final observation of holo vs. apo state cytotoxicity.[235] Mutations that truncate the SOD1 polypeptide will inevitably place additional stress on the intrachain C57 – C146 disulfide bridge and interfere with its structural integrity. Tiwari et al

found the disulfide bridge to be more susceptible to reduction in the mutant apo state than WT SOD1.[236] Ogawa et al have observed that the integrity of the disulfide bridge may play a crucial role in cytotoxic action leading to the elevation of thioredoxin in erythrocytes from patients with truncating SOD1 mutations.[237]

The misfolding of mutant SOD1 is clearly the fundamental feature of the oxidative theory, whether or not the polypeptide is in an apo or holo state.[238] Calcium-permeable AMPA receptors are clearly the key in excitotoxic theory.[239] Thus, we asked the question: is there any evidence that Ca^{2+}-permeable AMPA receptors promote misfolding of mutant SOD1? Such a study would perhaps be overlooked since it would be controversially attempting to interweave the two key features from the two most prominent etiopathogenic theories: oxidative stress theory and excitotoxic theory. Remarkably, Tateno et al have produced such a paper. It suggests that AMPA receptors and GluR2 expression significantly effects SOD1 morphology.[240]

To evaluate the contribution of motoneuronal Ca^{2+}-permeable AMPA-type glutamate receptors Tateno et al generated data on -GluR2 mice with significantly reduced Ca^{2+} permeability and crossbred them with G93A SOD1 transgenic mice.[241] The crossbreeding led to marked delay in disease onset (19.5%) and mortality (14.3%). Subcellular fractionation analysis revealed that unusual (misfolded) SOD1 species had accumulated in two fractions dense with neurofilaments/nuclei and mitochondria before disease onset, and then concentrated into the former fraction by the time of disease onset. Nevertheless, the overexpression of GluR2 significantly delayed unusual SOD1 accumulation. Tateno et al conclude that, "Ca^{2+}-influx through atypical motoneuronal AMPA receptors thus promotes a misfolding of mutant SOD1 protein and eventual death of these neurons"; perhaps his observations suggest that the direction of future research should be simply to simplify – and attempt a more integrated perspective?[242]

In a final examination of the effects of SOD1 it is appropriate to look to the cells of the anterior horn of the spinal cord. In a paper by Sasaki et al, the axon hillock (AH) and initial segment (IS) of the anterior horn was examined for the first time by electron microscope.[243] Little is known about the impairment of axonal transport of the fast and slow component in mutant SOD1 transgenic mice. Six transgenic mice were killed at ages ranging from presymptomatic to symptomatic stages. In the transgenic mice, 95 AH and IS directly emanating from normal-looking anterior horn cells were seen (in comparison to 91 AH and IS in the control mice). Increased neurofilaments and mitochondria were also observed in the AH and IS relative to the non-transgenic mice. To Sasaki et al this suggested that in mutant SOD1 transgenic mice, the slow axonal transport of neurofilaments and fast axonal transport of mitochondria are impaired even before the onset of the disease. This finding helps elucidate the commonly-observed primary feature of ALS, which is anterior horn neuronal cell dysfunction and death.[244]

In sum, Jonsson et al state that 10% of all ALS cases are FALS; and 20% of all FALS cases directly result from an SOD1 mutation.[245] The infamy of the SOD1 mutation is perhaps undeserved since it still leaves the majority of FALS cases unexplained. Andersen et al have discovered there to be at least 105 SOD1 mutations. However, the frequency of SOD1 mutations in cases of ALS differs between countries e.g. 23.5% of FALS cases in

Scandinavia SOD1 from SOD1 mutations, whereas the incidence is only 14.3% in France.[246] Internationally, the two most notorious SOD1 mutations are the A4V mutation and the D90A mutation; however their fame is suffered by two very distinct populations. Outside North America only three families in Europe have been detected as suffering from A4V SOD1 mutation instigated ALS. However, the A4V mutation's fame endures almost complete absence in Europe where the D90A mutation is the most prevalent.

In Scandinavia 1/100 people carry the more intriguing D90A mutation. Andersen et al have observed that: (1) individuals who are homozygous and heterozygous for the mutation can still exhibit ALS; (2) the D90A mutation presents with a phenotypic continuum of symptoms and as a result produces a type of ALS with a massively elongated survival time; and (3) D90A has a somewhat unique pattern of inheritance since with homo- and hetero-zygous sufferers it has two methods of inheritance.[247]

SOD1 exerts its deleterious effects through a toxic gain of function as opposed to a loss of superoxide dismutase activity.[248] In this report we have outlined three of these maladjusted functions. Kirby et al observed that mutant SOD1 represses antioxidant response and detoxification gene transcription;[249] Menzies et al showed how mutant SOD1 initiated morphological changes in mitochondria which resulted in a decrease of activity in complexes II and IV, ultimately which lead to an increased cell death vulnerability;[250] and Andersen et al postulated that when mutant SOD1 is broken down it leaves beta amyloid deposits in the motor neurons which build up over time and slowly begin to exert a toxic effect.[251]

There is also a debate as to how the SOD1 metallo-enzyme applies a toxic function: is it in an apo state (metal free) or a holo state (metal burdened)? Subramanium et al showed that the SOD1 in the apo state can still fulfil its cytotoxic intentions;[252] Okado-Matsumoto & Fridovich observed that the apo state SOD1 binds cytosolic heat-shock proteins which disables them in future antioxidant function;[253] and Tiwari et al showed that truncating mutations in SOD1 disrupt the anti-cytotoxic action of the disulphide bridge present in the SOD1;[254] mutant SOD1 therefore has a increased toxic gain of function. We can conclude that the evidence cited in this report suggests a toxic gain of function in the apo state of SOD1.

3. Peripheral theories: roles for nitric oxide, vascular endothelial growth factor, and human cellular prion protein in ALS

Nitric oxide (NO) is a gas sometimes found in the CNS and is most commonly associated with nueroendocrinology, since even though it is produced in a neuron it does not communicate via the traditional synaptic mechanism. Instead, this gas diffuses through cells, affecting changes to the neurons that may not have a synaptic connection with the secreting cell. This novel messenger is produced by NO synthase, but may enter the CNS via simple diffusion from outside sources, among other ways. The role of NO in SOD1 related motor neuron injury has been particularly controversial. Theoretically, there are arguments to suggest that NO may exert an important influence in motor neuron injury, but there is relatively little directly experimental support for this hypothesis.[255]

Ciriolo et al state the claim that a "NO challenge to human neuroblastoma cells ultimately results in apoptosis. They have linked increased NO levels and increased levels of mutant SOD to increased incidence of neuronal apoptosis. They

noted that elevated levels of WT SOD seemed to protect the neuron from NO mediated apoptosis, and in WT cells exhibiting a down regulation of SOD there was an increased incidence of apoptosis. They conclude that resistance to NO-mediated apoptosis is strictly related to the level and integrity of the SOD1. [256]

Separately, Menzies et al maintain that upon initiating conditions suitable for elevated levels of oxidative stress NO levels increase.[257] They have also observed that NO reversibly inhibits mitochondrial complex IV activity and completes with oxygen binding sites. Superoxide is thought to potentially inhibit complexes I and II and the presence of superoxide and nitric oxide generates peroxynitrite.[258] This extremely reactive species is thought to aid further inhibition of complexes I and II; thus increasing the vulnerability of motor neurons to cell death.

Cookson et al conducted a study to examine the potential role for NO in mutant SOD1 motor neuron injury. Using an NSC34 culture they examined the effect of initiating serum withdrawal to induce oxidative stress. They found that "the expression of both the normal and mutant SOD1 decreased the measured extracellular superoxide release but had divergent effects on the measured release of NO." Normal SOD1 increased the measured NO release whereas mutant SOD1 cells released less NO (release of superoxide and NO were measured in real time using microelectrode biosensors). However, co-administration of two different NO synthase inhibitors (L-NAME and L-N-methyl arginine) did show some neuroprotective effect. Cookson et al conclude that "NO is likely to contribute to motor neuron injury but this does not fully account for the cellular toxic effects of mutant SOD1" and in doing so echo the attitudes of the literature investigating the effects of NO in ALS.[259]

Vascular endothelial growth factor (VEGF) is a key signal in the induction of vessel growth (a process termed angiogenesis). Storkebaum et al report that a decade ago VEGF was considered to be an endothelial cell-specific factor, but more recent findings reveal that VEGF also has direct effects of neural cells.[260] Lambrechts et al have previously reported that reduced expression of VEGF caused ALS-like motoneuron degeneration in VEGF (delta/delta) mice.[261] Their current study involved a meta-analysis of over 900 individuals from Sweden and over 1000 individuals from Belgium and England. There exist certain VEGF haplotypes (-2, 578A/-1, 154A/-634G or –2, 578A/-1, 154G/-634G) in the VEGF promoter/leader sequence that lower circulating VEGF levels and reduce VEGF gene transcription. Individuals in the meta-analysis who expressed these haplotypes had a 1.8 times greater risk of ALS (p for trend = 0.00004). Moreover, G93A SOD1 transgenic mice crossbred with VEGF (delta/delta) mice died even quicker than mice expressing just the VEGF dysfunction due to severe motoneuron degeneration. Reduced VEGF levels in humans may therefore promote motor neuron degeneration by limiting neural tissue perfusion and VEGF-dependent neuroprotection.

Grzenkowicz et al have investigated the effects of SOD1 on the synthesis of VEGF. Hydrogen peroxide and NO are both important regulators of angiogenesis. Therefore, VEGF synthesis cannot only be enhanced by gene transfer of VEGF but also by the overexpression of NO synthase genes. Examined in the Grzenkowicz et al study was the possibility that gene transfer of the SOD1 augments VEGF production. Overexpression of the (normal WT) human SOD1 in NIH 3T3 fibroblasts increased SOD activity, enhanced generation of hydrogen

peroxide, and significantly stimulated VEGF production as determined by increased VEGF promoter activity, VEGF mRNA expression and VEGF protein synthesis. Grzenkowicz et al investigated the effects of SOD1 on VEGF production since their aim was to realise a method to stimulate angiogenesis; and overexpression of human SOD1 has proved to be a feasible method.[262] However, the question not clearly addressed in the Lambrechts et al report: was whether mutant SOD 1 alone will cause a cytotoxic gain of function, but also provoke decreased levels of VEGF production.[263] The realization that VEGF's mazy have a neuroprotective ability is a new one, and the research is still in early days. These insights have primed widespread interest in developing VEGF-based therapies for degenerative diseases; raising hope for the successful treatment of ALS.)

The literature concerning ALS and human cellular prion protein is tentative at best. Dupuis et al's paper is a notable exception and investigates human prion protein's links with the SOD1 mutation G86R. They observe that in a transgenic model of ALS the overexpression of G86R SOD1 led to the specific repression of PrP(C). Analyses revealed that PrP(C) was down-regulated in the early asymptomatic phase of the pathology and occurred in those tissues primarily affected by the disease (spinal cord, sciatic nerve, and gastrocnemius muscle). Dupuis et al report that the down-regulation was not accompanied by refolding of the aberrant PrP(Sc) isoform, which is the agent which caused that causes transmissible spongiform encephalopathies. Since PrP(C) has been shown to play a part in the protection against oxidative stress, it is proposed that PrP(C) down-regulation may contribute at least in part to ALS pathogenesis. Kovacs et al (2002) have also investigated the effects of PrP(C) in human degenerative disorders. Using immunohistochemistry for PrP(C) they evaluated the immunoreactivity (IR) of prion protein's immunoreactivity (IR) in a number of degenerative disorders including ALS. From their investigation they found that PrP(C) IR neurons may contain abnormal tau or alpha-synuclein aggregates. However, intriguingly they observed a loss of PrP(C) IR in anterior horn neurons in ALS.[264] Kovacs et al suggest that the expression of PrP(C) reflects a general response to cellular distress rather than specific co-operation of aggregations of proteins.[265]

4. Virology and ALS: Searching for the acute or Latency Trigger

The final area of this bio-genetic inquiry are studies which have sought to identify a potential acute or latency trigger to make the exposed person vulnerable to either any external factor that leads to ALS or make them vulnerable to any dormant genetic predisposition.

In the late 1980's, a major British study demonstrated a temporal and statistical parallel between poliomyelitis and ALS. They analyzed all notifiable diseases by geographical distribution from 1931-1939 in all areas of England & Wales, focusing especially on the 6463 notifications of polio. These were juxtaposed against 25 major causes of death, including ALS in people aged 55-74 and by gender. There were no correlations for poliomyelitis infections and any causes of death except ALS, with a correlation of 0.42. Apart from breast and ovarian cancer, with correlations of 0.27 and 0.23 respectively, all other causes had negative correlations. This statistical association was the same for men and women. Interestingly, polio is a viral infection, which appears to have a predilection for more affluent places and families, which the ALS

results matched in that there was a significant correlation with male ALS deaths belonging to social class 1,2 and 3, and with a significantly lower rate of ALS deaths in classes 4 and 5, i.e. semi and unskilled manual workers. Because of the differing social circumstances of those times it was not possible to accurately ascribe the Registrar General's occupational social class to women.[266]

It is difficult to fault this study, other than the perennial question about the accuracy of death certificates. It raises the question of whether there is an etiological viral link with subsequent development of ALS. Furthermore, it poses the question of early infection and later onset and the issue of latency as it is recognized that a range of viruses can remain life-long latent, while viruses are known to infect and destroy cortical motor neurons.[267] Moreover, the poliovirus being so destructive of neural tissue theoretically would be a good candidate for a viral etiology of a number of neurodegenerative disease such as Multiple Sclerosis [MS] but also including ALS.[268] Of course, since the 1950's there has been almost universal vaccination against polio, to the extant that it has been virtually eradicated in the West. Martyn et al predicted therefore that any rises in AlS might continue for twenty years, until people born after 1950 have reached the age for classical onset of ALS (2018), and then the rates of ALS should decline.[269]

However, a number of subsequent studies that has sought to clarify what if any viral infection is linked to ALS have proved to be inconsistent with markedly different results. For example a number of studies showed no association with either herpesvirus or echovirus7.[270] Conversely Girard et al found an excess of enteroviral sequences in post-mortem sample of Japanese ALS patient's spinal cord, matching an earlier finding of a British study in a small case-control study.[271] In 8 of 11 sporadic ALS cases, and one of two FALS, and one case of poliomyelitis and ALS the enterovirus was detected in subject's spinal cord but none in the small number of matched controls.[272]

The hypothetical viral link has been given further impetus from single case studies, where people have had polio or a detected abnormal level of viral infection.[273] Also, a number of papers report single case or small groups of people infected with HIV virus and subsequently producing ALS symptoms, which after antiretroviral therapy have either fully recovered or shown marked improvement.[274] All intriguingly suggesting a possible viral trigger. While Calza et al highlight that HIV positive patients have a 27 fold greater frequency of serious neurological disorders, including ALS-like illness. They point out with the changes in antiretroviral therapy there have been marked improvements in stemming the ravages of HIV and report on a young infected male, who had transiently reversal of ALS symptoms after protease inhibitor antiretrovival therapy, but it was only transient.[275] But, Sola et al exploring the HHV-6-8 viruses associated with Kaposi's sarcoma fond No serological data in ALS patients.[276]

The most recent case-control study we could examine came from Italy. Cermelli et al explored the risk of sporadic ALS and the herpesvirus and echovirus-7 on 20 newly diagnosed ALS and 20 control cases. On a range of tests ALS patient's had greater frequency of herpesvirus 6-8 and echovirus-7 both neural and IFA than controls, non reached a level of statistical significance. The herpesvirus 8 showed in terms of odds ratio and observed frequency a statistical trend [p<0.07t and 0.09t] above the controls, but with such numbers, little

conclusive can be determined.[277]

One intriguing study worthy of note in this section came from Japan as Kira et al explored allergic disorders and subsequent `common neurologic diseases'. The strength of this study was that it was a two-year prospective study on 3,113 out-patients referred to a neurology department. Based on self-report questionnaire, 2152 neurological patients responded. They found that myelitis patients had a highly significant earlier atopic dermatitis, while patients with lower motor neuron disease had a significantly higher previous or current asthma and a marginal increase in concomitant bronchial asthma. Unfortunately this study's presentation is somewhat confused as the authors use lower MND and MND interchangeably, while the numbers of patients they report upon are inconsistent. Nonetheless the 6 patients related to a motor neuron disorder represent an annual rate of 139 p.m., yet another intriguing and teasing result.[278]

A recent study from Hungary brought together viral and radiogenic lower Motor Neuron Disease (LMND), which may provide an early example of an interactive model of the etiology of neurological disorders. The Hungarian team focused entirely upon LMND (which would be described a progressive muscular atrophy in the USA) in a meta analysis of this rare distinct condition which followed radiotherapy for cancer, the most common being testicular cancer, hence the predominance of 7.8:1 male to female cases, hence categorized as `Radiogenic LMND' (RLMND). They clearly established that the LMND was subsequent to radiotherapy and was in no way dose related and that it appeared to have a degree of randomness in that RLMND emerged between 9 and 276 months after radiotherapy, the mean being around 48 months. Crucially RLMND differed markedly from `Classic Radiogenic Myelopathy (CRM) which was invariably fatal. In view of the apparent natural history of RLMND which displayed relative benign and long lasting characteristics similar to viral LMND, similar to post-poliomyelitis syndrome, they hypothesized that RLMND was consistent with a previous viral infection which was probably the origin of the subsequent RLMND. This is the first paper which postulates a sound theoretical rational of specific interactive risk factors for a neuro-degenerative disease, other than the genetic mutation SOD1 in FALS, of viral infections and irradiation. While radiotherapy is self-evidently dealing with markedly different radio frequencies, it is suggestive that the EMF impact may well be linked to a previous sub-clinical viral infection, which may make people more vulnerable to develop ALS.[279]

Thus, though Martyn et al.'s classic study has not been repeated, there are sufficient indicators that suggest that for some ALS patients, particularly men of the sporadic type, there may well be a previous viral link / susceptibility along the ALS continuum of neurodegenerative disorders. Hence, this could be a meeting of other risk factors, which with or without a previous viral infection, which would account for the variation in the various inconsistent results on the etiology of ALS.[280]

5. Animal Equivalent of ALS

It may not be well known but ALS is not exclusive to human beings but also is known to affect horses and dogs. Motor neuron disease as ALS is invariably described in the veterinary literature, can as in humans, affect either both upper and lower or just lower motor neurons.[281] The lower motor neuron disease occurs mainly in dogs, and reports we have found suggest that it occurs within the same litter, indicating a genetic link.[282] Equine ALS has been described

over the years and it is suggested that it is increasing, but there are strong indications that it is linked to deficits in the diet.[283] This might be due to either extreme grazing condition and an absence of vitamin E. Polack et al found copper concentrations abnormally high in the spinal cord of affected horses, leading to an oxidative injury hypothesis, again tied in with vitamin E.[284] All suggestive of similar patterns to what occurs in humans. We could find no research which suggestion a possible cross over, or allergic response to dogs, cats or horses, but the clusters of horses affected pointed to an environmental trigger.[285] Unlike New Variant Cruchfeldt Jacobs Disease in parts of Europe, there is no suggestion of the pathology crossing between species. Thus while transgenic studies on rats and mice give useful experimental data to test the various biological mechanisms, as yet there is nothing of concern to human of in ALS in animals.

6. Twin Studies and Heritability

The debate and complex research findings along the SOD1, Cu2 and VEGF axes all relate to the strength of a genetic predisposition. Ideally the field is crying out for a proper case-controlled study of ALS in twins. In view of the incidence of ALS it is not surprising that such an approach has not been tried from the Danish and Swedish data bases, instead we could find only one such study, Graham et al from Britain who explored the genetic contribution to ASL in a matched twin study, to include possible environmental factors.[286]

Between 1979 and 1989 they examined 10,872 death certificates and extrapolated any relating to ALS. All twins in the cohort were identified and their status was determined via the NHS Central Register. Diagnosis of ALS was verified via the co-twin, relatives and medical records, and their zygosity was confimed using Magnus et al standardized questionnaire, which has a 97.6% accuracy with both twins or 96.1% if it is only possible to interrogate one twin.[287] Heritability was calculated as twice the difference between monozygotic (MZ) and dyzogotic (DZ) correlations and tetrachoric correlations calculated.

They identified 128 pairs of twins, in which at least one died from ALS and were able to trace 58 living, 31 adult deaths, 29 infant deaths. Ignoring the co-twin who died in infancy they had a final case-control of 91 probands, unfortunately there was no information on 10 of the same sex twins, out of the final 58 pairs there were 25 MZ and 30 DZ probands. To offset their lost twins and improve the rigor of the study they excluded 2 MZ who had a family history of ALS. This yielded 2 MZ pairs who were concordant for ALS out of 21, with no DZ twins found to be concordant for ALS. This yielded a concordance rate of 9.5% or binomial exact 95% CI: 1.2%-30.4% that is 2 out of 21 pairs of MZ twin. They estimated that heritability, and after allowing for a zero results in the DZ group, heritability lay somewhere between 0.38 to 0.85 in sporadic ALS.[288]

They also explored possible environmental factors but only found the following to yield levels of statistical significance for people involved in car maintenance [<0.006] crafts using chemicals [<0.024] paints used in job [0.002] and `Do-it-Yourself (i.e. a range of crafts about the home stemming from painting, plumbing, electrical work etc]) [<0.05]. They reported a number of negative environmental findings based on odds ratio, which included, surgical operations, head injury, heart disease, malignant and endocrine disease.[289]

Graham et al. recognized the limits that the small numbers in the final sample produced,

and advised all environmental results to be interpreted conservatively. Also there were problems in regard to the loss of twins only 45.3% of co-twins had been traced, hence having to depend upon proxy information and the perennial question of the accuracy of death certificates. Nonetheless, this is a vitally important study the pity is it is based upon such small numbers. It strongly suggests that based upon MZ twins concordant for apparently sporadic ALS, there are probably underlying genetic factors contributing to the development of the disease. They always sought balance and acknowledged the problems of trying to determine environmental factors, and acknowledged the "likelihood that exposure to industrial chemicals has a significantly causative role, as men are more likely to carry out activities using noxious chemicals. More subtle or hidden exposure could be sufficient to cause disease but it is difficult to determine…..ALS exhibits increasing incidence with age, any environmental influence on its development is more likely to be of a chronic nature…Alternatively the ageing nervous system may become more sensitive, particularly the motor neurons to environmental assaults…"[290] This excellent balanced paper, which eschewed the sterile argument about `nurture versus nature', gives further impetus to the genetic predisposition meeting environmental triggers hypothesis in some cases of sporadic ALS.

Yet it is fascinating to appreciate that only 2 out of 21 MZ twins were concordant for ALS, though theoretically they share 100% genetic inheritance. It needs to be re-stated that people are not machines, that our DNA reminds us that we are bio-genetically unique, which probably accounts for the degree of variance found in all the studies were have consulted. This is demonstrated when considering the range of biochemical tests, using system of international units (SI). For example assumed normal clotting times range from 70-180 seconds, IgG antibodies from 0-15, neutrophils0.40-0.70; lymphocytes 0.22-0.44; haptoglobin 0.16-1.99 g/L' platlet s 150-350 $X10^3$, Immunoglobulin IgA 0.60-3.09 g/L; calcintonin 0-35 kU/L and thyroxine fT4 10.3- 35 pmol/L.[291] Yet clinically, individual patients will fall within these ranges and for some they will produce symptoms of various diseases, other will be straightforwardly normal, reminding us that we need to treat people not the disease.

However, when Graham et al.'s levels of estimated heritability are compared with other disease known to have strong genetic foundations, the numbers begin to look quite low. [292] Though not with challenge even in the psychiatric disorders, which do not have such a tight circumscribed diagnostic criteria as ALS, MZ concordant rates are higher as are estimates of heritability in schizophrenia, and psychiatric proneness.[293]

It seems clear that there are bio-genetic etiological factors in ALS, and possibly subtly not yet demonstrated in so-called sporadic ALS, the three molecular biological theories ascribing the cellular process that lead to neural degeneration, require external triggers to produce the full-blown disease, and it is the nature and type of these external factors, which create the varied pathways to ALS and other neurodegenerative diseases.

Are there lessons to be learned from Parkinson's Disease (PD) research? The issue of genetic susceptibility in ALS is problematic as the ALS twin study produced a lower concordance rate than might have been expected, hence the temptation to consider the genetic base for other neurodegenerative disorders. However, apart from Huntingdon's chorea which has the

strongest loading, and Alzheimer's, the most common neurodegenerative disorder, Parkinson's Disease (PD), appears to have even less strong inheritability than ALS. Earlier Duvoisin et al found no concordance for PD in their 12 monozygotic (mz) twins.[294] Conversely in Germany Vieregge et al found 3 of 9 mz and 3 of 12 dyzogotic (dz) twins were concordant for PD.[295] Utilizing the formulae to estimate inheritability, yields 0.32, as opposed to the Graham et al estimates of between 0.38 0.85 for ALS.[296] However in the largest recent twin study on PD we could find, Wirdefeldt et al screened 14,082 pairs of twin, born before 1950, the majority being aged 65 and over. There were 247 twins with confirmed PD and 517 twins with PD symptoms. Only two pairs of twins were concordant for PD, both female and dz. They concluded that there was little inheritability in PD., as the inheritability formulae yielded a negative result.[297]

Despite those studies which find overlaps between ALS and PD and which found a slight familial link for Parkinson's, genetic susceptibility can only be described as relatively slight, with strong implications of a range of environmental influences.[298]

7. "Wen folks get ole an stricken" - is it just Ageing?

A uni-dimensional approach to ALS would be to state with more than 85% certainty, that ALS, like PD, is predominately age related, and therefore part of the `natural order' of things, its just ageing. Allam et al however makes the point that, "Perhaps one of the most important questions posed by the neurobiology of ageing concerns the pathogenic mechanisms in Parkinson's Disease," a point which surely must be true for ALS.[299] However the question should be even wider reflecting medicine's greatest challenge, to slow down the ageing process, which exposes the organism to a range of pathologies, including the neurodegenerative. While Allam et al acknowledge there are major environmental factors in PD, they argue for a Gompertzian like argument, but changes throughout the Western world are at odds with such a position.[300] Firestone et al recently found that occupational pesticide exposures (including herbicides) are consistent with a PD link but not home-based exposures, suggesting a gradient of occupational exposure.[301] Perhaps the last word on the endogenous / exogamous debate comes from the recent Mayo Clinic family study of PD.[302] They undertook a historical cohort study of PD proband's relatives and relatives of matched control, with two times periods 1976-95 and 1996 through to 2000 of people from Minnesota and people referred to the Mayo clinic. Interestingly, they almost are a mirror image of ALS results, the younger PD onset <66 the greater the Relative Risk (RR), whereas relatives of PD probands with later onset had no increased RR, indicating to us, that PD is predominately a degenerative disorder associated with ageing and the environment, with only slight familial contributions.

Support is given for this interpretation by some fascinating recent results on clusters of PD. For example, Sydney, Australia has one of the highest incidences in the developed world, seven times that of New York.[303] But Alabama welders have a prevalence of PD almost nine times the rate of the city of New York as shown in a recent American study![304] Thus, for PD at least, socio-environmental factors are the dominant explanation of the increases in PD in the USA and the major Western countries. This provides a degree of tangential support for the importance of socio-environmental influences in ALS.

PART THREE: ALS & SOCIO-ENVIROMENTAL FACTORS

Social & Occupational Factors: EMF, Heavy metals, Pesticides and Solvents

A key feature in seeking the etiology of ALS is to avoid `atomizing' the problem and to include all relevant research so that we seek to integrate the bio-genetic *and* the socio-environmental factors. Otherwise, we will fail to obtain an accurate and total picture of how the different facets interrelate (if at all.) The classic problem is as Sir Karl Popper taught the scientific world: the researcher can inadvertently bring a bias into his work, and runs the risk of ascribing the solution to the problem with the hypothesis they are researching. The field of ALS study is made for such confusion, yet there is always a desire to simplify in an effort to contain within one paradigm a theory that explains everything. As must now be clear, there appears to be a range of bio-genetic-environmental etiological factors that influence the development of ALS, and in this section we explore those social factors, which bring together those external factors that impact upon the biological. Despite our desire to take an interactive and integrated approach, for heuristic reaons we explore the issue schematically.

1. Occupation & ALS

Since the advent of a Public Health approach, the importance of people's occupation and the health risks of injury, infection or toxemia, etc, have become well understood. The classic case of occupational health hazards is the `Mad Hatter' of the 19th century, who showed signs of neural damage due to the mercury used in the manufacture of top hats in Victorian England. In this section, we explore possible etiological factors such as exppsure to heavy metals, pesticides and solvents, linking them schematically to occupation.

Brooks reviewed risk factors for ALS and identified a number of risk factors, including electrical injury and prolonged exposure at work and at home to agricultural chemicals, pesticides, and herbicides, but felt that any link with heavy metals had not been established.[305] Interestingly, Brooks found an higher risk of developing ALS among pilots and navigators, and one wonders about a possible connection with the electro-magnetic field experienced by people in those occupations. Brooks reports on the creation of a longitudinal register recently set up on 1800 patients in North America, which is already yielding invaluable clinical information in the

care and treatment of ALS patients, with future promise for developing into an element of prospective study.[306]

An important American study is that carried out by Schulte et al., which focused n particular occupations associated with neuro-degenerative diseases, including ALS, through a population study of death certificates between 1982-1991 in 27 of the US states.[307] The study focused on four conditions: Pre-senile dementia, Alzheimer's, Parkinson's disease (PD) and ALS, and analyzed the persons known occupation, contrasting the neurodegenerative diseases against other causes of death and occupations. Excess rates of neurodegenerative disease for all four categories over-lapped into a number of occupations. The authors identified clusters of occupations for the four disorders; of the 130,420 neuro-degenerative deaths, 9,435 (7.2%) were ALS deaths, based upon ICD 9 categorization. Ratios were calculated comparing the proportion of deaths within a specific occupational group with the proportion of deaths from that cause in all occupations. The authors noted that agricultural workers clustered around Parkinson's disease, pre-senile and ALS was linked to electrical workers; pre-senile, PD, and ALS were all related to construction workers, with a common pattern for lawyers, judges and archivist for Alzheimer's, PD and ALS.

Schulte and colleagues list the occupations for the four conditions and there are some very pertinent findings.[308] The authors plot ALS related deaths to occupations by ethnic grouping, Black and White males and females and there are about 9 different jobs for each group. Schulte et al however, makes the point that "relatively early death may be a clue that an environmental factor is involved in the etiology," and pointed in particular to firefighters, janitors, military personnel, teachers, evacuation machine operators and vetinarians, amongst others. This is an interesting point, and was perhaps a forerunner of the findings on the Gulf War studies which showed young onset and early death among ALS patients.[309] However, there are a number of problems, which Schulte recognizes when he says, "the findings however need to be viewed as leads, not as confirmed associations." [310]

The first problem with such a study is of course `historical' in that variations in occupations among ethnic groups and between genders have changed considerably since the early 1990's. Secondly the `pre-senile' condition is now being incorporated into Alzheimer's statistics, though that is less relevant to our ALS focus.[311]

Another problem is that Schulte and colleagues have so much data that it is not fully used - in part because it is so diffuse. For example, looking at the occupations related to ALS, there is no overlap between Black and White males. Even given the socio-economic and historical context of the time, this is surprising, since at lease some degree of over-lap might have been expected even in 1982-1991. Does the dichotomy found between the two groups imply different trigger mechanisms of ALS or different susceptibilities? It is possible to make guesses about likely exposure to the possible etiological links of heavy metals, pesticides and solvents, but this defeats the specificity sought by specifically analyzing occupations.

Furthermore, there was no cross-over between Black or White men and women's

occupations, and the only link among women was that of teachers, which in part reflects the disproportionately larger number of women in this occupation. The most frequent occupation for Black men and ALS were cleaning and building service occupations (arguably an occupation in which employees might be exposed to an incalculable range of toxic products,) and the next highest was excavating and road machine operatives. White male Veterinarians, followed by Geologists and Geodesists, and then Power Plant operators were most strongly linked to ALS. For Black women, teachers were the most common category linked to ALS, then Hairdressers and Cosmetologists and for White women, also Teachers, followed by Financial Record Processors and Designers.

As can be seen, the data and implications of the study are a bit diffuse. There was a slight overlap of ALS with other conditions and occupations, but these were not specifically mentioned by the authors. Significantly for us, the overlap lay with Black and White women, viz. teachers with PD and Alzheimer's, but White women alone for Teachers, Designers, Writers and Artists with Alzheimer's and PD.

The problem, of course, is how to interpret these associations. The authors concede the biggest weakness of their study is that they could only look at occupations around death or possibly the longest occupations people were in – thus, there was no way of knowing the extent to which the level or intensity of being exposed to any potential experience deleterious to motor neurons occurred within those occupations, or the possible time needed for any effects to emerge. Nor can the authors evaluated whether their subjects had been exposed to other theoretically noxious materials in previous occupations.

Consequently, it can be cautiously concluded that there are patterns of occupations associated specifically with a range of neurodegenerative disease, which provides clues to the environmental etiological axis of the genetic-environmental interface that leads to ALS.

2. Electro-Magnetic-Fields [EMF]

Since 1983, there has been a growing interest in the possible connection between ALS and chronic exposure to electromagnetic fields (EMF).[312] Schulte et al had noted that White male ALS deaths were associated with power plant workers, which raised further interest in this area.[313] In an early U.S. study, Sidofsky noted how previously healthy individuals (all men) developed ALS symptoms after receiving a severe electric shock at work, and were most likely to have symptoms in the limb which was most affected by the shock.[314]

In 1997, Davanipour et al hypothesized a link between ALS and EMF exposure and undertook a small case-control study, but with only 28 and 32 control cases.[315] Another study on electricity utility workers found no association for PD, but a possible raised risk for Alzheimer, with an odds ratio of 2.0 O.R for ALS, which worsened with increasing exposure times.[316] This study was particularly useful in that it explored the concept of cumulative exposure. Savitz and colleagues followed this study with an analysis of US death certificates and occupational coding, and found odds ratios for Alzheimer's, PD and

ALS at 1.2, 1.1 and 1.3 respectively.[317] However the authors noted that there was marked variation within industry and type of job; some occupations were associated with odds ratios of 2-5: 1 for ALS.

Subsequent studies have yielded mixed results. For example a Swedish study gave some support to a link between prolonged EMF exposure and ALS but noted a number of confounding issues, including the need to determine type, length and intensity of exposure.[318] One advantage of this latter study was that the authors originally intended to explore the possible connection between EMF and cancers. In the end they found little firm evidence for a connection between EMF and cancers aside from childhood leukemia. Johnsen et al also explored any association with EMF and cancer rates and found nothing of note, but did find a slight ALS link.[319] Cruz and colleagues from the West Washington team of Nelson and McGuire explored physical trauma and family history of ALS in a population based control study, but found only a very weak association with the electrical industry, indeed the only significant odds ratio concerned major electric shocks.[320] As mentioned previously, they did find a de facto social class association in which more cases were observed among less educated people and people who smoked.

An especially valuable study came from Denmark by Johannsen & Olsen.[321] The authors utilized the employment records of all Danish people of the Danish Supplementary Pension Fund register, which holds all records of people aged 18 to 66 employed since 1964. Johansen & Olsen tested the hypothesis that a higher risk of ALS would be associated with men who had worked in the electrical utility industry since the inception of the register. A total of 26,135 men were identified who had been employed at least 3 months in a Danish electrical utility company. With great ingenuity, Johansen & Olsen had devised a measurement of average exposures to EMF from a combination of jobs within the industry, ranging from 50-HZ which in turn provided levels of background exposure from low (0.1-0.29 uT) to high (>1.0 uT). Unfortunately, because of incomplete records, 4267 cases had insufficient information to be included in the study viz. levels of EMF exposure, leaving the final cohort at 21,236. This cohort study provided data to explore any of the subsequent deaths of these men, from 1974 to 1993, with information on cause specific deaths from the Danish National Register and EMF exposure.

The size of the cohort was equivalent to 303,000 person-years, with an average of 14.3 years; 3,540 deaths were observed, whereas the expected frequency based on national data was 3,709 `all cause' deaths. However, the observed frequency of ALS deaths was 14 compared to an expected frequency of 6.9, yielding an overall SMR of 2.0. There was a non-statistically significant association between risk of developing ALS and time since first employment in the electrical utility industry, with a significant increase in deaths related to accidents with electricity.

When medium and high levels of exposure were combined, the SMR rate *did* reach statistical significance, "equivalent to one extra case per 30,000 person-years." In this very cautious and sound cohort study, within the 14 ALS cases they found only 1 person with a familial link with ALS (7%) , but two people who had been exposed to quite severe electrical shocks.

One of the benefits of this study was that the authors were primarily exploring associations between malignancies and the electrical

industry; their ALS focus was, as it were, a bi-product of their national study. One problem of the study, which they readily acknowledge, is that some of the death records may have been less than complete; subsequent to the authors' first examination, they returned to the data and found only one further ALS-associated case, who died of other causes and also had a malignant neoplasm. Another weakness of the study is that the authors may have under-estimated the link between ALS and EMF in that the cohort are not yet complete - there may yet be future ALS-type deaths. A perennial weakness is that despite having estimates of average length EMF, individuals might easily have had considerably more or less exposure than would be expected from their job titles. Perhaps the most serious weakness is that the authors lost more than 4,000 of their original cohort due to insufficient details on job activity. However, what is attractive in this study is that its primary focus was not ALS and therefore did not have a previous position to `defend' in an area where results have historically been contradictory.

Two examples of studies which present contradictory findings come from Feychting et al in Sweden, who studied ALS deaths between 1981 and 1995, and Noonan et al from the State of Colorado, who explored ALS deaths over the 1987-1996 period.[322] The Swedish study was based upon 4.812 million people and sought to determine any link between neuro-degenerative disease and EMF exposure between 1970-1980. The authors found that the Relative Risk (RR) for Alzheimer's Disease was significant at 2.3, with what they described as `early onset' (<75 years) following moderate to high exposure. They found no association, however, with ALS – if anything, there appeared to be a reduced risk related to exposures of >0.3 uT for both men and women. The authors did find a slight link between increased risk for ALS among male welders and railway men, and if involved with an electric accident, among radio and television assemblers and repairmen. Furthermore they found no links with EMF exposure and Parkinson's disease in either gender.

Conversely, Noonan and colleagues *did* find a link between EMF exposure and ALS. They explored all death certificates in Colorado; since 1980, 98% of Colorado death certificates have included the person's occupation. This case-referent study took one case of ALS to 4 referent cases for male deaths in Colorado, and utilized three methods of determining EMF exposure. Unlike the Feychting study, Noonan and colleagues found no association between EMF exposure and Alzheimer's but an odds ratio of 1.5 for Parkinson's disease [PD] and a ratio of 2.3 for ALS in people with a history of electrical occupations (although this was not related with the job-exposure matrix.)

The Feychting et al (2003) team included Ahlbom, whose earlier study had found a slight link between EMF and ALS, and they belong to the prestigious Karolinska Institute in Stockholm.[323] How, therefore, to account for their negative finding, especially when based on such a major population cohort? Feychting and colleagues' critique of other studies suggested an inadvertent bias in case selection viz. inaccurate certification. Though the Feychting study did acknowledge the quality of such studies as Johansen & Olsen, it did not seek to account for their variant findings.[324] Conversely Feychting and colleagues cited a number of studies in which there were missing death certificates and neuro-degenerative diseases. Feychting et al admitted there may have been problems in the quality of the `Causes' of death in their registry, but this would not appear to account for such a null finding compared with

others.[325] However, it may be that the key reason for the difference was the period of employmen (only from 1970-1980) and the fact the cohort covered two ICD periods of registration, i.e. ICD 8 (used up to 1986) and then ICD 9. Also, Feychting's method of determining EMF exposure and employment was based on measuring average possible exposure amongst a number of occupations of a job-exposure index. They did, however, measure electricians separately as they are the most often susceptible to electric shock accidents.

Also Feychting et al.'s cut-off point for Alheimer's cases was <75, which in terms of clinical experience, is somewhat early.[326] Another Swedish study by Hakansson, based upon a large cohort, 537,000 men and 180,000 women, did find a positive association between Alzheimer's Disease and EMF exposure, and demonstrated links with ALS but not PD.[327] This matches a subsequent study by Johnson, who like all the research teams found ALS more strongly linked if electric shocks were involved.[328] Looking at the Feychting et al methodology, there is no immediate apparent reason why this study was at odds with so many others.[329] It may be however, because they started with the largest population data-base, or it may be that the greater number of possible genetic carriers for ALS in Sweden's population masked any EMF affect.[330]

The key strengths of Noonan et al.'s approach are the tightness of mandatory occupation reporting on death certificates and Noonan's three-rponged approach to measuring exposure: primary life-time occupation, and jobs likely to have high exposures, the second combing occupation and types of industry. Then, similar to Feychting, a job exposure matrix based large number of EMF measurements undertaken in studies in the USA, the Nordic countries and New Zealand. A very important added measure was that of education, which we think is a surrogate measure for social class, though Noonan et al assigned this at three levels.[331]

Fascinatingly, when Noonan et al looked at exposure by electrical occupations, only when combining both approaches did they find a statistical connection with Alzheimer's, whereas for ALS there was an "elevation in risk using both methods 1 and 2….No elevated risk for ALS was observed when the job-exposure matrix was used to assess the exposure." Thus in effect job title were less relevant, it was "due to electrical or electronic technicians and engineers" which appeared to make the difference. While PD had an association, the link for Alzheimer's was different from that found in Feychting and in that sense does not support the Alzheimer-EMF association. Both Noonan and Feychting accept that the strength or weakness of their results stands or falls by the validity and reliability of accurate recording on death certificates and on their measurements of EMF exposure.[332]

On balance, taking into account the Schulte et al and Savitz et al studies, the `electric shock' aspect found by Jafari et al in France, and placing them alongside that of Johansen & Olsen, the Feychting et al study raises questions, but on balance, Noonan et al and the others, allow one to conclude that there is an association between ALS and electric shocks and some people working in the electrician and electrical technicians areas.[333] How and why this occurs is still unclear.

It may be that the EMF affects people of a greater, albeit as yet unmeasured susceptibility. Or conversely, the `at-odds' results of Feychting might be due to possible inherent differences in

the populations explored. Thinking theoretically about electric shocks, we sought to discover whether there had been any research in regard to Electro-Convulsive–Therapy [ECT] and subsequent risk of developing of ALS, but found nothing. Bearing in mind the extent of the use of ECT in the 1950's and well into the 1970's, the absence of any paper investigating this possible correlation was a little surprising,

It is difficult to determine whether the EMF road to understanding the etiology of ALS is a cul de sac or whether there is further distance to travel. What should be noted however, is that in the wider `environment' of people living in the 20th and 21st centuries, we are being exposed to a range of relatively rapidly changing backgrounds. These interactions may enhance any impact EMF may have upon ALS.

3. Heavy metals and ALS

In a brilliant review, Dr. JD Mitchell explores the history of toxins and the environment, especially exposure to heavy metals and its possible link with occupations and diet, starting with the Roman Pliny (AD 23-79) who warned "to cook with lead-lined vessels could result in dangling paralytic hands." Mitchell identified some of the earliest medical concerns of lead poisoning from a British 1836 edition on the nervous system and a slightly later 1853 German *Lehrbuch der Nervenkranken*. Mitchell's review takes us up to 1999. While lead is obviously toxic to humans, there is no direct evidence that it is a causal agent in ALS, though in both human and animal studies higher concentrations in the cord and damage to anterior horn cells are reported.[334] However these findings might just as well be secondary phenomena as primary, as diseased or damaged cells preferentially take up lead.[335]

Mouse studies have shown that mercury take-up damages the anterior horn cells but like lead, the evidence could suggest either primary or secondary factor to the development of ALS. Unlike lead, mercury has been associated with occupation and raised levels in ALS patients.[336] However, this was not confirmed in subsequent case control studies, nor noted in more recent case-control studies concerned with occupation, though one of the latter studies had serious methodological flaws.[337] Incidentally, Gunnarsson et al in their case-control study reported upon the mean level of dental amalgam fillings, an average of 10 per person in their 92 ALS case group, compared with a mean of 12 per control group (n=372.)[338] In passing this negative finding of dental amalgam's with their mercury vapor not being statistically associated with ALS might be considered reassuring in the intermittent controversy about neurological risk and dental amalgam, though Mutter et al were less sanguine in regards to possible oxidative stress and links to MS.[339] As Tacitus reminds us "when knowledge fails, wonder [hypotheses] grow" it could be that for the susceptible, the dental amalgam was the trigger, we just do not know.

Another heavy metal believed to be linked with ALS was selenium as it is recognized that there is some oxidative stress associated with a substance when in excess is toxic to humans.[340] Perhaps the most interesting and revealing study emerged following accidental long-term exposure to selenium in drinking water in a region of Italy. In effect Vinceti et al had a naturalistic study when it was discovered that between 1972 and 1988, 5,182 people had

inadvertently regularly been using drinking water with 7 times the level of natural selenium in water. Vinceti obtained all cases of ALS in the region and was able via addresses to determine which of the people had been part of the prolonged selenium exposure. There was in effect a 4 times the rate of observed to expected frequency of ALS cases over the period, and we calculated that the affected area had an annual ALS case rate of 48 per million, considerably higher than the overall `general population' rate. Despite small numbers, the researchers were able to confirm that all the cases were of the sporadic ALS type.[341]

The link between copper and ALS and or zinc, appears to be related to possible interactions with SOD1 mutations, which may suggest different pathways to ALS and/ or a greater susceptibility of damaged neurons to take up or store the metals noted by earlier researchers.[342] There has been some conjecture that free radicals are linked to presence of metals, which induces oxidative stress.[343] An interesting findings analyzing the presences of heavy metals, in this case copper and zinc, found No association with ALS but with a lowered SOD1 activity. The researchers Vinceti et al asked whether SOD1 is a marker or causal action, or is secondary to a confounder at the disease onset itself.[344]

A case-control study from Italy that explored the risk of ALS in the presence of trace elements, briefly mentioned above, added to the non-specific association.[345] The authors took a series of toenail samples from 22 ALS case people and compared them against matched 40 controls. They noted that the progress of the disease was not associated with levels of trace elements found in the samples. They acknowledged that toenail samples as a marker for zinc, copper and selenium are limited in terms of determining chronic exposure. However they found no association with ALS toenail content of cadmium, lead, copper, zinc, magnesium, chromium, selenium, ion or aluminum, all concentrations expressed as ug/g, except for cobalt expressed as ug/kg. Interestingly they analyzed by type of concentration and in most cases of the ten elements examined by a 3 way level of 25% percentile, median and 75% percentile. In a possible 30 cases v. controls, controls had higher rates in all but five comparisons, though none of the results began to approach statistical significance. This is a small but ingenious study reminding us that sometimes negative findings are just as important as positive results. [346]

Thus, apart from the obvious toxic impact of heavy metals, the latter do not appear to be an unequivocal factor in the etiology of ALS unless, as in the accidental poisoning in Italy, people are exposed to excessive amounts.[347]

4. OCCUPATIONAL EXPOSURE

4.1. Solvents

Exposure to solvents has been linked to the development of ALS. In a case-control study from Scotland, Chancellor et al found that a combination of solvents and chemicals was overly represented among cases contained with the Scottish MND register - 103 patients, with odds ratios of 3.3:1 associated with occupational

exposure.[348] The authors found no socio-economic or childhood links with subsequent ALS in their analysis. In a previously-mentioned British MND twin study, a `solvent' link was noted in regard to people working with vehicle maintenance and occupational paint usage, with quite high and significant odds ratios of 7.0:1 and 3.75:1 respectively.[349] However, unlike Gunnarssen et al, the authors did not attempt to calculate a hereditability/ environmental ratio for these cases.[350] More recently, in examining ceramide and its role in neural apoptosis, Satio et al found links with neural death, which was significantly increased in Alzheimer patients but not detected in ALS or control brains.[351]

Two studies whose results were contradictory are those of Gunnarsson et al. and Gait et al. from Britain.[352] The Gunnarsson team's case-control study covered nine Swedish countries and concerned occupational and medical data on 92 ALS cases and 372 well matched controls. As mentioned previously, the authors did find increased risk of ALS associated with working in the electrical industry (6.7:1), welding (3.7) and in regards to solvents `impregnating agents' (3.5:1), though the confidence interval spread was much narrower for the solvent finding.

The authors also explored the association with heritability, i.e. whether or not the occupational associations were stronger in people with FALS rather than SALS. They found a distinct strengthening of these associations in FALS, especially in relation to males and exposure to solvents, an odds ratio of 15.6 with CI of 2.8-87. However they noted that there was variation between the `types' of ALS, with environmental links being more prominent in spinal rather than involving the pyramidal tracts and subsequent bulbar paralysis

As already noted, electricity work and shocks were high especially for cases starting under 59. This age of onset and analysis of chemicals was particularly useful, with pesticide and impregnating agents having an odds ratios of 2.8 and 4.2 respectively for the under 59 age group. These findings point towards stronger environmental influence in younger ALS patients, reinforcing the findings of Haley and Horner et al. and suggesting that solvents may be a particular trigger for people with a familial susceptibility to ALS.[353]

In general, the Gunnarsson study appears very sound study except for the lack of clarity on how the authors determined familial ALS links. There was no indication of whether they differentiated between 1st and 2nd degree relatives with either ALS or other neuro-degenerative disorders. Their respondent rate of 85% and 75% cases to controls were quite good, but they noted there was a relative lack of agricultural and forestry workers compared with rates in the general population, and they felt that these groups might have been under-estimated and the risks of chemical estimates too high.

The British paper of Gait et al was somewhat disappointing, bearing in mind it came later and could have benefited from a better research design. Gait et al explored the work and mortality histories of 22,526 people who had worked for a major engineering company, and examined the differential exposure rates to metals and solvents and the risk of subsequent ALS.[354] Their study covered the period from 1967 to 1997 and examined death certificates held in pension fund archives. The case group was composed of records that mentioned ALS or progressive bulbar palsy, and these cases were juxtaposed against randomly obtained controls taken from the same database. Since the majority of the workforce was male, the authors

excluded women from any subsequent analysis. Occupational data was obtained by searching personnel records.

Unfortunately, and surprisingly from a centre of such prestige, the Gait team could only report on 22 out of 65 male cases and 206 out of 402 eligible controls! This was apparently due to the occupational records being lost. Based upon their truncated cases for analysis, the authors found no significant correlation between exposure to solvents or heavy metals and ALS, indeed the heaviest exposure personnel came from the control group. Apart from the loss od data, the paper was weakened by the fact that the occupational data was collated by a company occupational hygienist, albeit blind to case or control file. Furthermore, half the research term came not from the University but from the company involved in the research. From today's medical pharmaceutical research we have learned to have a degree of cynicism and are aware of the dangers of inadvertent vested interest. This study would raise eyebrows in any weekly evidence-based research club. However, we are not suggesting for a moment any deliberate bias, but the loss of more than half their database undermines the validity of the study.[355]

Another weakness was that the authors reported 67 cases, of which 65 were male, yielding a mortality rate of 2.97/ 1,000. The authors quietly acknowledged that this was higher than the national rate by a ratio of 1.53. Our calculations indicate that this would suggest an annual death rate of 99 per million per annum. We have no information on the age bands of their ALS cohort, but this 99 per million rate is certainly higher than the Anglo-Welsh male rate observed in 1969 and 1979, though it does approach the level of ALS deaths observed in 65-74 year olds in England and Wales by 1998.

Overall therefore, although in terms of size of effect the link between solvents and ALS does not appear to be extensive, there does appear to be a link, especially in relatively younger onset in men, which may be a trigger in neural apoptosis. Moreover, the solvent axis again points to the interaction of hereditability and environmental triggers.

A study that explored the impact of workers exposed to herbicides, in particular 2,4-dichlorophenoxyacetic acid (2,4-D), which was feared to be carcinogenic, was undertaken by the company's epidemiological team.[356] The study covered the period 1945 up to 1994 and contrasted employee's deaths with that of national rates, controlled for same locations, a total of 330 out of 1,517 people. Out of 24 listed forms of malignancy only 2 observed frequencies exceeded expected frequency and neither was significant. However in terms of All Causes, the observed rates exceeded the expected frequency, with risk ratios of between 1.07 and 1.12. When time and rate of exposure was examined, risk ratios ranged from 1.05 to 1.20, though unexpectedly the low but prolonged rate of exposure had the highest relative risk (1.20.) Our focus is on ALS, but this research study undertaken by the company does remind us of the problem of potential vested interest: our interpretation of their results does not match their sanguine presentation. However, unexpectedly and outside their original hypothesis, the authors found significantly higher than expected ALS deaths - 6 in a cohort of 1,517 people, yielding an equivalent annual rate of 79 per million and a RR of 3.45 (CI 1.10-11.11). These workers had been employed between 1947-51 and 1968-86, so the annual rate is higher than would be expected compared to the general U.S. rate.[357] There was no attempt to determine whether these ALS deaths were familial or sporadic.

4.2. Pesticides & Agricultural Chemicals

There is growing public concern about pesticides and agricultural chemicals - not only in terms of their impact on people living and working in rural areas, but also in terms of their impact on overall human health. One British repsonse to this concern has been the establishment a Royal Commission on Pesticides, which drew together a very eminent and distinguished panel of experts. Conversely, some might say the size and longevity of such a commission is intended to `bury the issue', especially when such commissions focus very narrowly upon particular pesticides and specific outcomes. While this can be justified as good science, the tendency is for caution to `favor' defendants rather than appellants. This is to the frustration of such campaigning organizations as Pesticide Action Network, who have active groups throughout the world (e.g. PANNA - PAN North America – panna.org, and, PAN International, pan. international. org.) These groups are a reflection of the realization that despite the undoubted effectiveness of pesticides in improving food supply, residual levels of pesticides are at rates which some consider beyond levels of safety. On the other hand, the `campaigners' can over simplify and sometimes rely too heavily on anecdotal evidence, rather than good science. Yet the latter groups do raise important issues, stimulating a range of research, such as research on the impact of organophosphates on depression and suicide. London et al showed that in animal studies organophosphates disturb serotonin levels, which are linked to both depression and increased risk of suicide.[358] Another example is that of the bioaccumulation in fish of pesticides and chemicals. Sethajinanin et al examined fish contaminants from the lower Willamette River in Portland Harbor in Oregon, which a British observer would assume to be pristine.[359] The authors found 25 PCBS (polychlorinated biphenyls) and 15 organochlorines (OC) pesticides, plus mercury in fish collected in 2000. While the levels of contaminants varied depending upon the fish site in Portland Harbor, the range of PCBS and OC is a clear indicator of the extent of the usage of the chemicals and pesticides on land and their ability to enter the human food chain. It would be legitimate to wonder whether such relatively recent changes may have been involved in the rise in the neuro-degenerative diseases in North America and England & Wales.

The obvious occupation involved with pesticides and agricultural chemicals is that of farming. A number of studies have explored the frequency of ALS and other neuro-degenerative disease among farmers. For example, a study from the University of Athens found an over-representation of farmers with ALS in a particular region of Greece. Was this a genetic or environmental cluster, or an interaction of the two?[360] Chio et al conducted an Italian case-control study comparing 512 cases of ALS with 512 Other neurological diseases. The authors found that there were more agricultural workers amongst ALS cases, and more exposure to chemicals, with greater than expected frequency of gastric ulcers and mechanical injuries amongst the ALS group. The latter may have been due to earlier neurological problems, leading to instability and therefore making them more vulnerable to accidents in a workplace that is particularly problematic. An intriguing finding was that the women affected by ALS had later menarches and earlier menopause, indicating a shorter reproductive period. Chio speculated whether this was an indication that female hormones might have an etiological role in ALS.[361]

There have been a few studies responding to the fact the gender preponderance of men over

women but little clarity has emerged from these studies. Mititello et al found no differences in serum levels of DHEAS in a small study comparing blood serum levels of 35 ALS people and 57 controls. However, free testosterone was significantly decreased in the ALS group, but this might well be reactive or secondary, though the authors raise the question of whether this hormone might play a part in the pathophysiology of ALS.[362]

As already indicated, Gunnarson et al. found clusters of ALS between 1961-1990, almost in an `epidemic'-like pattern, with an excess of agricultural chemical workers amongst the ALS group.[363] This was similar to McGuire et al.'s case-control study in Washington State. The latter study examined 174 ALS cases and 338 controls, and found higher than expected frequency of ALS amongst male, but not female, workers in the agri-chemical industry, with an odds ratio of 2.1 (CI 1.1-3.8).[364] However, just how complicated occupation-based case-control studies can be is seen in an American study on waste water workers that explored a possible association between that occupation and malignancy and neurological deaths. Betemps et al divided the workforce into migrant (i.e. born outside the USA) and non-migrant workers, and found significant differences between the two for certain malignancies and some neurological disorders, but not for ALS.[365] This adds another twist to the methodological problems associated with matching cohorts in regard to place of birth and main lifetime occupation.

Nonetheless, we can conclude that while there are statistical associations between ALS and `external' triggers, the extent of the roles which environmental processes and genetic predispositions play in the etiology of ALS remains unclear. One feature is clear: the previous "distinction between SALS and FALS is increasingly blurred."[366] The evidence that there are environmental triggers in ALS, often linked to the work place, seems reasonably well established. But how this links to the SOD1 hypothesis or the VEGF hypothesis is problematic. It has to be remembered that only a proportion of FALS have a defective SOD1 mutation, and that this mutation sometimes, but not often, also occurs within SALS patients. We would agree with Mitchell that, "there may also be a multiplicity of factors at least some of which are environmental in SALS. [While] ALS may occur in genetically susceptible subset of individuals, who may have defective detoxification mechanisms, which results in the degenerative process, this might be precipitated by different agents." [367] Even in the theoretically closed genetic system of twin studies on ALS, although hereditability was strongly established, there were significant numbers of monozygotic twins discordant for ALS.[368]

5. Towards a Synthesis of a Bio-environmental Understanding of ALS

Qusetions of policy and epistemology are mplicit in the task set out for us. Has there really been an increase in ALS rates in the United States? Are there any indicators of causal factors, and therefore potential preventative actions or therapeutic options? What are the policy implications of these findings? Within the epistemological realm, what *is* the nature of ALS? Does the etiological research provide any indication whether or not *any* person might well develop ALS at `random', is risk `pre-determined' by genetic inheritance,

or does the risk of developing ALS fall somewhere along this spectrum of `pre-determined to total randomness'? We suggest that the heuristic paradigm of an interaction of multi-environmental and polymorph genetic endowment is the only one that fits the evidence regarding the development of ALS.

5.1. Diagnosis

This raises the question of the exactitude of the diagnosis of ALS. Is the condition a truly concise, mutually exclusive, unitary diagnosis? Or rather is ALS more of a *syndrome* in which, as in the concept of `fuzzy sets', sufferers of ALS will vary in how closely they fit the classic El Escorial diagnosis? In this model, *where* the person lies along the bioenvironmental spectrum affects *how* ALS is manifested in them. Cases of ALS which start with spinal or bulbar symptoms may be determined by the different pre-determined genetic susceptibility or socio-environmental influences affecting patients. Not surprisingly, Beghi et al found that early on in the diagnostic process, even experts have difficulty in agreeing on a diagnosis of ALS. Although the El Escorial criteria are excellent for research purposes, in clinical and practical terms the criteria exclude people who fall into the possible or probable ALS categories, which possibly leads to underestimating the numbers of people with serious neurodegenerative disorders of an ALS-like type.[369]

5.2. Lessons from Bio-Genetic Research

Diagnostic uncertainty can lead to a plethora of hypothesis and speculation, `for where knowledge fails, wonder grows.' Humanity, and especially medicine, finds an intellectual vacuum intolerable. There is a tendency to over-simplify, and few of the major research papers have looked outside their narrow research focus to account for findings from other relevant fields.

Turning to the genetic and biochemical pathways that underpin ALS, in addition to a multiplicity of intriguing and engaging perspectives, our review revealed a disquieting lack of conclusiveness regarding these pathways. Except for the prominence of investigations into the effects of SOD1 mutations and GluR2 RNA editing on ALS disease progression, the literature remains fragmented. Because of this, we feel that it is appropriate to maintain an inter-disciplinary stance in order to provide a wider understanding upon which to place these different elements in context.

Unusually, Nelson et al's study into macronutrient intake and ALS risk association provide us with a critical exogenous context in which we to situate our etiopathogenic discussion.[370] Nelson and colleagues were first to utilise a questionnaire that accounted for 90% of the nutrients in the American diet, and their study revealed an increased risk of ALS associated with polyunsaturated and saturated fats (a dose-response relationship was observed) and glutamate intake; an inverse risk association was seen with water-soluble and insoluble fibre intake.[371] While Keller and Mattson incriminated increased lipid peroxidation and subsequent oxidative damage associated with elevated fat intake, Nelson et al suggest that fibre may prevent the absorption of a dietary toxin associated with ALS.[372]

AMPA receptors mediate excitatory synaptic signals, and glutamate is one of the premier neurotransmitters in the brain. Glutamate excitotoxicity can be characterised as the chronic over stimulation of AMPA receptors leading to Ca^{2+} intracellular imbalances and eventual neuronal death. Within the subunits of the AMPA receptor the GluR2 subunit controls Ca^{2+} -permeability. Critically, Van Den Bosch and colleagues have shown that neuronal death is

not due to the scarcity/absence of the GluR2; while Kawahara et al have demonstrated that ALS motor neurons present with inefficient RNA editing that consequentially leads to increased Ca^{2+} -permeability and neuronal death.[373]

Mutations in the copper/zinc superoxide dismutase gene (SOD1) account for 20% of all FALS cases. Of the 105 known SOD1 mutations, the A4V and D90A mutations have been investigated in the greatest depth; A4V for its dominant presence in North America, and D90A for its unique pattern of inheritance, penetrance, and European locality.[374] Menzies et al maintain that mutant SOD1 exerts its deleterious effects through a toxic gain of function as opposed to a loss of superoxide dismutase activity.[375] The detrimental effects of mutant SOD1 can be summarised as consisting of: a repression of antioxidant response and detoxification gene transcription, alterations of mitochondrial morphology and subsequent increases in apoptotic vulnerability, and beta amyloid build up upon mutant SOD1 breakdown.[376]

There is also a rare assuredness that mutant SOD1 exerts its toxic function in an apo- (metal free) state, as opposed to the holo state.[377] All of which helps to understand the degenerative process once it has occurred.

Our discussion of peripheral theories included the roles of nitric oxide (as a gaseous and novel neuro-messenger) and vascular endothelial growth factor (VEGF). While nitric oxide has been shown to contribute and increase motoneuronal vulnerability to apoptotic processes, the role of VEGF has only been investigated in the last decade or so and has produced a number of intriguing results.[378] Both Lambrechts et al and Grzenkowicz et al have reported that reduced expression of VEGF caused ALS-like motor neuron degeneration, which prompt us to consider a possible neuroprotective function of VEGF outside of its usual angiogenic occupation. These findings also provoke a temptation to consider VEGF-based therapies. Though we are many years from understanding the true nature of function of VEGF and its role in ALS, it may be the next area for the exploration of an effective treatment.[379]

Choi et al highlight that most of the genetic causal links for ALS are mutations occurring in the copper/zinc superoxide dismutase (Cu.Zn-SOD) but recently they have noted a further three new mutation points. Because these mutation points showed lower SOD1 activity, they might well have treatment implications - yet another illustration that the more we know about the molecular biology of the oxidative and apoptosis process, the greater number of indictable candidates emerge.[380] This reflects Wickland's laconic comment that there are at least 10 genetic foci responsible for FALS.[381]

Migliore et al from the field of neurobiology of ageing, however, highlight the fact that oxidative stress is not exclusive to ALS but is also associated with PD and Alzheimer's disease.[382] In a single case study, Liang et al reports on a woman who died with ALS and other neurodegenerative symptoms.[383] At autopsy she was found to demonstrate three pathological diagnoses: ALS, PD and argyrophilic grain disease. Zhang et al showed some crossover of ALS and Alzheimer's Disease in their proband's having had similar pathological blood pictures, which they interpreted as evidence of systemic immune system alterations.[384]

This new knowledge suggests greater overlap between ALS and the other neurodegenerative

diseases than the earlier diagnostic models suggested. Moreover, the literature on Parkinson's Disease suggests that despite the late age of onset of ALS and PD, both diseases are not simply disorders of ageing, but are to a varying degree influenced by socio-environmental factors.

5.3. Lessons from Public Health & Epidemiology

While acknowledging that the Gompertzian hypothesis has a degree of validity, we have argued in this review that the hypothesis does not adequately fit the evidence. Furthermore, because of its tacit ageism, a Gompertzian approach undermines research that would challenge the inevitability of the effects of the ageing process. By potentially over-emphasizing the genetic factors impacting disease risk, the hypothesis could even undermine health insurance. After all, we buy insurance to protect against the random `slings and arrows of outrageous fortune.' But if we know the odds of not getting certain conditions, why bother insuring ourselves. Worse, if disease risk is pre-determined, insurance companies will not take the business.

The Gompertzian perspective infers a degree of passive inevitability and implies that ALS is simply a disease that certain people are 'programmed' to develop if they reach a certain age. Yet this is an artifact. While the incidence of ALS is linked to age, it is not necessarily age per se that determines onset, but rather the accumulation of `insults/exposures' over the years. Furthermore, by stressing the link between ALS, aging, and genetic predisposition, the Gompertzian approach reassures society, in an inadvertently Panglossian way, that we do not have to change anything in our environment – that this is the best of all possible worlds and therefore nothing needs to be done.

Two interesting public health issues emerge: the link between fatty diet and smoking and ALS, though even here we have the paradoxical results that smoking is negatively associated with PD, while the increasing Westernization of Guamanian and Japanese diets has corresponded with a decline in ALS and neurological deaths in those locations.[385] The reduction of smoking since the 1980's, however, might tangentially lead to a slight reduction in future ALS cases and might account for the flattening of the 55-64 age group found by Noonan et al., which would be broadly co-incident with the decline in lung cancer deaths in the West.[386]

Issues of seasonality, place of birth, clustering, and viral infection and possible latency provide evidence of environmental influences on ALS, while at the same time not excluding possible genetic explanations.

A key finding is that the numbers of people in the U.S. suffering from ALS has risen since both 1969 and 1979, and may be still rising. Crucially, changes in women's morbidity and mortality (especially among those aged <74) strongly indicates a substantial role for socio-environmental factors in the observed increases. However, internal variation among ethnic groups in the USA suggests that genetic predispositions may play a role, although ALS rates in all the three major American ethnic groups have increased.

Studies of the 'ragbag' category of 'Other Nervous Diseases' (OND) are crucial to understanding what is happening with ALS rates in the U.S. Between 1979-98, OND deaths rose by 17% in the 55-64-age band, and 37% in the 65-74 age bracket. All three of these age brackets are *under* the average age of mortality for ALS, with significant increases for women

compared to men.

Furthermore, the latest WHO data (available up to 2000) indicates that the combined rise in neurological deaths in the U.S. is amongst the worst in the Western world. Gender variations in all age bands of this data again point towards socio-environmental influences. Deaths due to neurodegenerative diseases are double those due to HIV, suggesting that it is time for the voice of the neurological patient to be heard.

Moreover, Coffman et al reanalyzed the Horner et al figures of Gulf-War ALS cases, confirming their original findings.[387] This indicates, if anything, that the original numbers were an under-estimate. These studies highlighted what must be seen as essentially external triggers leading to ALS in younger men <55, with genetic susceptibility having little, if any, impact. The policy implication is clear: not only do we need to continue to seek effective treatments, but we should also be thinking in terms of prevention.

Among the many changes clinical medicine has experienced over the past 40 years, one is especially significant - namely the concern about the `interactions' of medicinal drugs. The British National Formulary (2005) states "Two or more drugs given at the same time may exert their effects independently or may interact. The interaction may be potentiation or antagonism of one drug by the other." This wise premise can *also* be interpreted as applying to the changing environment, for as yet we believe there is little research done on the possible interaction of agricultural-domestic-industrial `chemicals' that increasingly impinge upon the modern citizen. Thus we need to understand what, if any, impact the accumulation of minor risks has on the chances of developing either ALS or other neurodegenerative disorders.

5.4. ALS: A bio-genetic-socio-environmental interactive condition?

We conclude, based on the literature, that ALS is a bio-genetic-socio-environmental condition. Paradoxically, while we believe the epidemiological and therefore socio-environmental features have come through most strongly in the literature, genetic susceptibility may help explain some of the observed geographic variation in prevalence. The latter evidence, however, only strengthens the hypothesis of bio-genetic-environmental interface. Indeed, one criticism we have of research in this field is that it is often too uni-dimensional and does not look at the wider context and the possible interfaces between different types of research.

While differences between FALS and SALS remain, distinctions between the two are becoming increasingly blurred as glutamate metabolism dysfunction is found in both (albeit 20% and 2% respectively.) It is quite conceivable that with more advanced technology we will find markers of genetic susceptibility in what are currently described as random SALS, though at present only 10% of ALS cases have been ascribed to having familial origins, with lower than might be expected concordance in twins.[388]

Because of the early optimism that genetic research would lead to effective treatment interventions, we agree with Mitchell that "initiatives aimed at enhancing our understanding of potential environmental factors in the pathogenesis of ALS have been overshadowed by the ascendancy of the study of FALS." This trend has been strengthened by the fact that no single socio-environmental lead has appeared.[389] And of course the search for the `magic bullet', if it existed, potentially could yield immediate treatment results, so desperately needed.

It appears that ALS is more of a syndrome, with possibly no uniform pathological mechanism, but rather a process that develops along a common pathway once the degenerative process is initiated. This process may well start far earlier than is as yet understood, similar to the child-hood chicken pox virus laying dormant until an external trigger re-activates it as adult shingles.

Furthermore, there may be a range of environmental influences impacting the risk of developing ALS - not single factors but rather a combination of many factors, which might include season and place of birth, extreme physical exertion etc, all interacting together, influencing and being influenced by differential genetic predispositions. Consequently "the detoxification mechanisms which result in the degenerative process might be precipitated by different agents," perhaps dependent upon types of genetic susceptibility.[390] Conversely, some people may develop ALS primarily because of multiple environmental stresses, as seen in the increasingly earlier onset of disease (e.g. that observed in the Gulf War veteran cases.)[391]

Professor Edward O. Wilson, who has been described as a new Darwin (Tom Wolfe), highlighted what for us is the key and as yet unresolved question when he said, "different forms of the same gene originate by mutations which are random changes in the long sequence of DNA that compose the gene."[392] The key for us is identifying what causes these random changes. That randomness exists in our biological world is not gainsaid, but we too easily accept it as a matter of fact and do not ask, why. It appears logical that this may well be because of socio-environmental triggers.

The great success of the biological and medical sciences has come about by the reduction of phenomena to their constituent parts, enabling an understanding of `cause', followed by an effective `cure'. Allied to this search are the major strides molecular biology and genetics have made - for example, more than 1,200 physical and psychological disorders can be associated with just one gene.[393] Edward Wilson jocularly suggested that the principle of `One Gene One Disease,' whose mnemonic is OGOD, is so pervasive that the maxim `all disease is genetic,' is almost accepted without question The attraction of the OGOD principle to medicine is that not only does it simplify complex phenomena, but if there is a single or an identifiable multiplicity of genes, they could be used to in diagnosis and treatment. However, as Wilson points out "it does not at all follow that the gene determines the organ or process affected. Typically many genes contribute to the prescription of each complex biological phenomena," the question then becomes how many, what is the interaction, if any and crucially, back to the question of why the mutations occurred in the first place.

It appears that a mutation in a gene or polygenes might well be responsible for the major impact which results in such neurological disorders as Huntington's chorea, the affected gene/s may only be responsible for a small part of what eventually becomes a deviation from the norm. While no modern scientist would seriously adopt the dualism of Descartes, there is a residue of thinking which still perpetuates the Nature or Nurture debate rather than assuming the continued interaction of the bio-genetic and the socio-environmental. For example, when there is incomplete penetrance this is probably due to subtle differences in the organism' environment, distorting the potential of the gene.[394] Hence for example, the pair of monozygotic twins who share the same

inheritance, but among whom only one develops ALS, schizophrenia, or other conditions.

5.5. Is there a `Big Picture' for the Epidemiology and Etiology of ALS?

Are the changes surrounding ALS and the other neurodegenerative disorders part of a bigger picture? After all, the changing pattern of neurological morbidity and mortality is not the only example of major change. For example, almost human sperm counts have also declined in the major Western countries, including the U.S., but not in non-Western countries.[395] This decline has been attributed to be a response to physical, chemical, and other environmental factors - despite DDT being banned more than 20 years ago, the long-term effects are still being seen in South Africa.[396]

This review will also lead to new work which reveals other significant changes in mortality over the same period (namely in the blood disorders) as well as changing patterns in the incidence of cancer.[397]

As long ago as 1968 Rachel Carson's famous or infamous book `The Silent Spring' raised the awareness of ecological issues. Carson predicted that the causes behind the accumulative extinction of simple wildlife species implied threats to human health. Carson also predicted that certain `irritant' diseases of humans would increase, and certainly, despite the major successes in treating malignancies, the rising incidence, especially among younger people cannot be hidden. While the more than doubling of combined neurological deaths amongst the 55-64 and 65-74 in the USA cannot go unremarked. Lord Shackleton said "The science of ecology teaches us that we have to understand the interaction of all livings things in the environment in which we live" and while Rachel Carson's simple statement that in "our modern world, for the first time in history every human being is now subjected to contact with dangerous chemicals from the moment of conception until death" may be considered alarmist, it does pose serious public health questions.[398]

His Royal Highness the Duke of Edinburgh speaking in 1962 said, "Miners used canaries to warn them of deadly gases. It might not be a bad idea if we took the same warning from the dead birds in our countryside."[399] Should we interpret the increases in ALS and other neurodegenerative disorders in the U.S. as a surrogate warning?

It was a Public Health perspective that brought about the greatest improvement in human health; it may be that we have never been in greater need of this tradition.

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APPENDIX A: METHODOLOGY

There are two methodological approaches used in this study, the first is a form of a meta -analysis and review. With the assistance of the medical librarian of the Royal South Hants Hospital, we undertook an international library search using a range of electronic databases, including Medline, Psychinf and Entrez PubMed. The initial trawl commenced for research studies from 1968, but largely focused on studies published in 1980 and onwards. The key search terms were Amyotrophic Lateral Sclerosis, ALS, Motor Neuron Disease, and MND, juxtaposing these terms with epidemiology, etiology, and then a range of terms to seek to elucidate major and recent research papers related to ALS and MND for pesticides, seasonality, clusters, heavy metal, place of birth, injury, trauma, toxic/aema, and a range of biogenetic terms, highlighted from initial searches concerning genetics.

The range of topics and `disciplines' visited was extensive and is illustrated in the attached extensive bibliography. Indeed, such was the extent of the recent bio-genetic material, we were consulting the databases for new research until the 3rd week in June. Consequently, while this review is quite extensive we can not give more than a 90% estimate that it is comprehensive. We tried to identify the key papers, crossing the artificial divide of epidemiology and public health, the bio-genetic and the socio-environmental.

Because of the range of material, few if any professionals could evaluate all the technical details of all the subjects examined, other than examine the general principles of science. However, we only considered papers worthy of inclusion if they were published in notable journals, whose peer-review systems would guarantee at least minimal quality control, as we explored studies from virtually the whole range of medicine, neurology, psychiatry, psychology, neuro-surgery, bio-genetics, micro-biology, toxicology, epidemiology and the social sciences, for the "proper study of man is man", though we could not discover the `conscilience', a unitary theory of humanity that Edward O. Wilson seeks. We also expanded on previous work on the epidemiology of neurological disease, and extended the review from 1997 to 2000, with new analysis that comprehensively covers all major mortality categories for the period 1979 - 1998 for the USA, Canada and England & Wales, as well as the major Western countries. [1]

We utilized the latest standardized mortality data in the WHO format, taking the ICD-9 for 1979 to 1998, uniformly collated at the source by WHO and based upon annual populations by gender within each of the decade age bands. The 1970's baseline are three-year average rates per million population and for the population of each age band for 1979-81 to compare changes in the late 1990's, three-year average rates for 1995-97 by age and gender. To maintain the uniformity necessary in international comparisons we ignored the later ICD-10 data, which is available in some countries, but traditionally are not supposed to be used in comparisons for different ICD editions.[2]

All Cause Deaths (ACD)

All mortality data was drawn from the WHO annual mortality statistics for each country under review, thus ensuring the data was collated in a uniform and standardized way. ACD are the baseline against which to explore any patterns of change in OND and MDD by comparing them against ACD to determine whether these neurological deaths varied from general trends in overall mortality between the endpoints (1970s: 1979-81 vs. 1990s: 1995-97). This was done by comparing OND and MDD (rates per million), against ACD (as a proportion) at each endpoint, and examining the extent of change between the endpoints (ratio of ratios). This demonstrates the degree of any convergence or divergence between OND, MDD and ACD by age and gender over the period, an approach which was found useful in a series of international comparative studies in oncology, public health, child homicide and suicide.[3] It is recognized however that the main drivers that are likely to reduce ACD are unlikely to affect neurological deaths, for example improved treatment for diabetes.[4]

Neurological Deaths

Deaths from meningitis, multiple sclerosis and epilepsy over the period are reviewed as a context in which to compare the two largest neurological mortality categories of OND and MDD. In view of the fact that the vast majority of conditions in the OND category are age related, i.e. often appearing after age 50, we focus mostly upon the WHO age bands of 45-54 years to 65-74 years. Although we include the '75+' year band (for comprehensiveness) for both OND and MDD, the key foci are the 55-64 year and 65-74 year age bands. It is useful to include this older age group to reflect the average age for death in the West (78 for men, 82 for women). However, it is recognized that as life expectancy lengthens, any increases in neurological disease and deaths could be due to simply a reduction in other causes of death (Riggs 1992). This would have some validity in terms of deaths in the 75+ age band (hence the inclusion of that older group) but would not account for any changes in the earlier age bands (45-74 years) for OND or MDD deaths (hence the main focus on changes within these groups). Figure 1 lists the various individual diseases contained in the over-all categories of neurological deaths.

International comparisons

There are inherent problems in international comparisons, due to national variations in data recording. Our chosen approach, utilizing WHO standardized data, resolves these difficulties by essentially comparing a country against itself over time, before comparing national data.[5] To determine the proportional changes in each country, ratios of change are calculated from the baseline and index periods. This is repeated for the average three-year rates for each country, and ratios of change are then used to compare between countries.

The baseline period was taken from the publication of ICD-9 for the 1979 data onwards, and the latest three years for which data was available, mainly 1995-97. In a few countries this is slightly earlier and is noted in the text. The average three-year GPR deaths p.m. was calculated for each age band by gender based upon the populations in the ten-year age bands. Changes over time are determined by comparing baseline and index year rates, from which a ratio of change is calculated. All Western world countries with populations in excess of 16 million were examined.

Gender Differences

To explore gender difference within a country, both rates and the actual number of OND and MDD deaths are shown and a series of cross-tabulation analyses were undertaken to compare between male and female deaths between the two periods.

General data observations: defining substantial change

Only changes in national mortality rates of plus or minus 10% may have clinical significance. To err on the side of caution, as in previous studies, we define "substantial" as being when a ratio lies outside 0.80-1.20; (i.e. is equivalent to a change of 20% or more), which is very marked in terms of standard mortality ratios, to which the ratio of ratios is related.

Statistical analyses

Additionally, we report on those rates that show significant differences ($p < 0.05$), using ANOVA, between the various age groups, and particularly between the study endpoints (1970s vs. 1990s). It is also important to consider whether any apparent changes may be the artefactual result of changes in diagnostic techniques, in complex neuropsychiatric conditions.[6] Age and time effects are included and a stepwise regression analysis examines the contribution of age and year to overall variance. However, time is treated as an independent variable, so it is not reduced to binary, which permits an analysis of the contribution of time to the amount of variance.

Figure 1: Constituent Diseases ICD-9 Classification (1979-98)

CATEGORY	INCLUDES
Mental Disorders	
21 (290-319)	Senile & Pre-senile conditions - Alcoholic psychosis - Drug psychosis - Other organic condition including Huntington's Chorea, hepatolenticular degeneration – psychoses – drug dependence - unspecified mental retardation
Multiple Sclerosis	
223	Disseminated or multiple sclerosis -NOS, brain stem, cord, generalized
Epilepsy	
225	Generalized convulsive and non-convulsive epilepsy – excluded progressive myoclonic epilepsy
Meningitis	
220	Inflammatory Disease of the CNS - Bacterial meningitis- meningitis due to other organism
OND	
23 (360-379)	Disorders of the Globe, ophthalmia nodosa –disseminated choriortinitis, disseminated retinochoroiditis -Disorder of Iris & ciliary body -
24 (380-390)	Disorders of external ear - Eustatian tube disorders
221 (332)	Parkinson's Disease
222 (330, 331, 333-336)	Cerebral degeneration's in childhood- other extra-pyramidal disease [including Hallervorden-Spatz Disease, Olivopontecere, Shy-Drager syndrome, Strionigral degeneration - Spinocerebral disease - anterior horn disease – other diseases of spinal cord -motor neuron disease
224 (343-344)	Infantile cerebral palsy, Little's Disease, hereditary cerebral palsy - Other paralytic syndromes
229 (323-326, 337, 341, 346-359)	Acute disseminated encephalomyelitis [excluding bacterial meningitis], Encephalitis - hereditary degenerative diseases of CNS - disorders of the ANS – Neuromyletis optica, Schilder's Disease, Balo's concentric sclerosis –Migraine - trigeminal nerve disorders- facial nerve disorders- nerve root & plexus disorders- Mononeuritis of limbs – Hereditary peripheral neuropathy – Inflammatory neuropathy- Myoneural disease –muscular dystrophy's & other myopathies

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Clinical Trials in ALS

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A Report to the Department of Public Health, State of Massachusetts on behalf of the ALS Therapy Development Foundation

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Clinical Trials in Amyotrophic Lateral Sclerosis

An Historical Review & Report

Amyotrophic lateral sclerosis (ALS) is an idiopathic neurodegenerative disorder for which there is no known cure and no treatment capable of dramatically slowing or arresting progression. This lack of an effective treatment, and the relatively low prevalence of ALS (an estimated 70,000 patients worldwide) has led to the perception among lay people and non-specialists that clinical investigations in ALS are still in their infancy and occur only rarely.

However, as most ALS researchers are well aware, ALS has a long and at times rich tradition of clinical investigations compared to other diseases that affect similar numbers of people. The first organized clinical trials in ALS were initiated around the time Lou Gehrig's diagnosis with ALS (1939), although this was a coincidence and not the result of Gehrig's diagnosis as has often been assumed.[1] These trials were obviously quite different from contemporary clinical trials - most were unblinded, uncontrolled, followed no definite dosing schedule, and retained many of the features of the classic case study approach to reporting therapeutic efficacy that had been used throughout the 19th and early 20th century. Initial results were reported enthusiastically in the popular press as "cures" for ALS, and it was only upon later reflection in scientific journals that researchers began to realize both the variety of neurological disorders that can mimic ALS and the inter- and intra-individual variation in disease progression.[2]

Excitement over the initiation of the first organized clinical investigations in ALS was quickly dampened by the discovery that certain patients could have transient periods of improvement and that their rate of progression could spontaneously level off – mimicking a possible therapeutic effect if these phenomena coincided with patients' entry into a 'trial.'[3] The failure of mid-century

clinical trial designs to account for this variation in disease progression was so powerful that it would be more than two decades before researchers conducted another prospective, multi-patient clinical investigation in ALS.[4]

The clinical trial landscape in ALS has understandably undergone significant changes since this early period of tentative experimentation and trial design frustration. Although there is still no treatment capable of reversing or arresting the progression of ALS, the clinical trial infrastructure necessary to demonstrate the efficacy of possible treatments has become increasingly more sophisticated in recent years, both as a result of changes in general principles of clinical trial design and conduct, and as a result of an improved understanding of the biology and epidemiology of ALS. Researchers in ALS now have an array of methods for measuring disease progression, structuring efficacy trials to maximize statistical power, balancing the desire to provide treatment to all patients with the need for placebo controls, stratifying patient populations to reduce variability, and calculating and evaluating dosing - all of which have been analyzed and validated through a series of conferences aimed at developing a consensus on clinical trial design in ALS. In addition, the growth in animal and *in vitro* models of ALS and growing attention to laboratory screening of large numbers of therapeutic candidates has provided clinicians with exponentially increasing array of possible treatments for clinical investigation.

These trends have led to considerable increases in the number of clinical trials conducted. Between 1964 and 2004, the results of a total of 134 clinical trials were published in major journals, but the majority of these trials were published in the final 10 years of this period.[5] Between 1964 and 1980 an average of one trial was published each year; by the late 1990s this number had increased to more than 7 trials each year. While these numbers are small, they are significant for the size of the patient population. By comparison, during the same time periods the average number of published clinical trials in Huntington's disease (which has a similar prevalence to

ALS) was 0.5 per year and 2 per year, respectively.[6]

In addition to increases in the number of published trials, there has been one therapeutic success in treating ALS: riluzole, a drug which inhibits pre-synaptic glutamate release. Riluzole slows, rather than stops, progression and has only a very small impact on the course of the disease. By conservative estimates, twelve to eighteen months of treatment with riluzole extends patients' post-diagnosis survival by an average of only 2 to 3 months.[7]

Nevertheless, the success in demonstrating a statistically significant survival advantage after treatment with riluzole has had a number of positive effects on ALS research. Riluzole offered the first clinical evidence that contemporary therapeutic approaches may be able to gain a foothold in slowing or arresting the progression of the disease. Riluzole's modest clinical success, FDA approval for marketing by Aventis under the trade name Rilutek®, and potential for treating a range of related neurodegenerative disorders with larger patient populations have also increased pharmaceutical and biotechnology interest in ALS as a possible therapeutic target for their investigational drug candidates. This has resulted in both new levels of academic-industry collaboration at the pre-clinical level and increased corporate involvement in clinical trials. These collaborations may ultimately reduce the time it takes for promising investigational treatments to reach ALS patients.

Overall, these changes have created a promising climate for clinical research into ALS. Corporate collaboration and expanded pre-clinical investigational techniques will likely continue to increase the range of potential candidates for clinical investigation and will provide a solid experimental basis for investigation. Increasingly sophisticated trial design will reduce the number of patients needed to demonstrate efficacy, meaning that more trials can be conducted simultaneously with the limited patient population available. If treatments are not clinically effective, the chances are higher than ever that researchers will discover this early in the course of the trial and will be able to halt the trial rather than submitting

patients to months or years of futile treatment. Although clinical trials in ALS have unfortunately not yet identified treatments capable of reversing, arresting or dramatically slowing disease progression, these clinical investigations have nevertheless helped refine trial design so that effects observed in the future will be much more likely to be 'real' rather than the result of random (but not therapeutically significant) differences between treatment and control populations.

This report traces the history of clinical trials in ALS over the past forty years, focusing on shifts in trial design and conduct that helped shape contemporary principles of trial design, tracing broad trends in the types of treatments investigated and the rationale for doing so, and assessing the implications of these trends for shaping contemporary research policy on ALS.[8] The review is organized into four separate sections and a series of Appendices. Section 1 summarizes major issues in clinical trial design in ALS, and what – if any – consensus has been reached in the research community on the appropriate resolution of these issues. This section uses these issues to sketch an outline of the ideal clinical trial in ALS as it is understood by contemporary researchers, both in terms of specific prospective design principles and in terms of envisioned outcomes. Sections 2 and 3 summarize major shifts in the study designs, patient populations, and investigational treatments used in clinical trials over the last forty years, focusing on tracing broad trends rather than addressing individual studies. These trends can help illuminate the extent to which actual clinical trial practice has diverged from or approached consensus guidelines on trial design.

Section 4 of this report consists of a series of case studies of investigational therapies on which more than one trial has been conducted and on which at least one major efficacy trial has been conducted.[9] The purpose of these case studies is to provide an overview of the history of clinical investigations of each treatment, with brief comments on the positives or negatives of study design, and to provide references (when available) to any Cochrane or other systematic reviews that have been conducted using the original data

from these trials. Treatments covered in this section include acetylcysteine, brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), creatine, gabapentin, IGF-1, Rilutek®/riluzole, Deprenyl®/selegiline, and thyrotropin-releasing hormone.

The text of this review is followed by two Appendices. Appendix A provides a full citation list of the clinical trials covered in this report. Appendix B includes a summary of each of the 134 clinical trials covered in this report; summaries include the rationale or hypothesis on which each study was based, the intended aim of the study, the primary location of the study, study results, and an overview of major parameters related to study design, patient population, and treatment protocol.

Ultimately, there are two ways of historically interpreting previously published clinical trials in ALS. One arguably misguided interpretation uses contemporary clinical trial design and analytic principles to invalidate the majority of previously published clinical trials. The other interpretation views trial design and therapeutic approaches as necessarily tied to clinical practices and therapeutic paradigms at the time. Under such an interpretation, the ultimate value of a clinical trial lies not in whether it adheres to contemporary design principles, but whether it represented the cutting edge in clinical trial design at the time of its publication, and whether it illuminated issues in design, measurement or analysis that ultimately led to improvements in future clinical investigations.

This report takes the latter of these two approaches, focusing not on the scientific accuracy of each individual trial, but on placing all trials in ALS (and their shortcomings) within a historical narrative in which current clinical trials are part of a process of ever-improving ability to detect or disprove therapeutic effects. Ultimately, this sort of approach will not only help non-specialists in ALS understand the importance of past clinical investigations but will also elucidate the nature and origin of clinical trial design principles that may seem at odds with traditional assumptions about the characteristics of a 'good' clinical trial.

1. Issues in Clinical Trial Design & Conduct in ALS

Before addressing historical trends in clinical trials in ALS, it is important to address the contemporary trial context in which today's clinical researchers assess these earlier efforts at clinical investigation. Both the standards of clinical trials in general and the standards of clinical trials in ALS have changed dramatically over the past forty years. This section will focus on contemporary issues in the design and conduct of clinical investigations aimed at demonstrating the efficacy of a particular treatment in ALS (safety, dose-ranging, and pharmacokinetic studies are a more straightforward endeavor.)

In general clinical practice, the gold standard efficacy study is typically, at the very least, randomized, double-blind, and placebo controlled.[10] Patients are randomly assigned to one of two or more parallel arms of a trial, and then one arm of the study is assigned to receive placebo rather than active treatment. The identity of the medications being taken by each group is kept secret from both doctors and their patients. These design elements are all directed at eliminating different sources of intentional or inadvertent bias in the conduct of these trials.[11]

In the context of this broad standard for clinical trial design, efficacy trials in ALS present their own set of design issues, including the need to account for unusually high variation in rates of progression, the limitations posed by patients' expected post-diagnosis survival time, the difficulty of choosing clinical endpoints in the absence of useful or reliable biomarkers for disease status, concerns over the ability of animal and *in vitro* disease models to predict clinical efficacy, the difficulty of maintaining complete control over patients' treatment regimens, and the challenges of convincing patients to participate in placebo-controlled trials of already FDA-approved or over-the-counter medications.[12]

One of the earliest issues to emerge as a priority for clinical trial design was the wide

variation observed in ALS survival times and rates of progression. (In this context, survival time refers to the time until respiratory failure.)[13] While ALS patients are generally described as surviving an average of 3 years after diagnosis, up to 24% survive for 5 years or more.[14]

On the other hand, between 7% and 10% of patients die in the first year after diagnosis, meaning that a typical efficacy trial that enrolled *only* newly diagnosed patients would already have to plan on losing 7% of the study population during the course of the study. The difficulty lies in figuring out which study participants will have a survival time of 7 months and which will survive 7 years and ensuring that these patients are assigned evenly between the treatment and placebo groups of a trial. Age (≥55 at the time of diagnosis), bulbar onset, rapid time between first symptoms and diagnosis, and early respiratory impairment have all been found to be associated with a shorter survival time.[15] In addition, an array of clinical evaluations can help predict patients' survival time based on their rate of progression.[16] These variables can be used to stratify the randomization process for clinical trials, ensuring the treatment and control groups have an equal distribution of factors that influence prognosis.

However, these clinical evaluations are most accurate at describing time to survival, not the exact rate of progression during each study interval. Despite efforts to develop numerical scales of disease status that decrease linearly over time, the actual course of patients' progression is unlikely to be entirely linear.[17] Thus, while such scales initially promised to reduce the length of clinical trials by providing a surrogate marker of survival, this promise has not necessarily been realized. Clinical investigations still need to collect a range of data points at different intervals in order to develop an accurate linear estimate of disease progression.

Other sources of variation include a sex imbalance in the number of men and women diagnosed with ALS.[18] Although gender does appear to influence one's chances of being diagnosed with ALS (often attributed to hormonal differences between men and women since the difference in diagnostic rates diminishes after menopause), it has not been

reliably demonstrated to play a role in prognosis.

Research has suggested females may have a poorer prognosis than males, but it is unclear whether this is due to differences in the average age of female patients (female patients are more likely to be post-menopausal when diagnosis, and older patients have been shown to have worse prognoses.)[19] Hormonal and genetic factors also appear to cause significant differences in study participants' metabolism of and reaction to therapeutic interventions.[20] In several trials, a distinct portion of the treatment group appeared to respond to treatment while the rest of the treatment group experienced no benefit.[21] In most cases, however, pharmacokinetic studies were unable to identify any variation in drug clearance or bioavailability that could explain this difference.[22]

In certain cases, variation in drug response led researchers to question whether the syndrome currently diagnosed as ALS is actually two or more distinct diseases, although there is no laboratory or epidemiological evidence capable of distinguishing multiple types of ALS, nor are there any relevant etiological or biological differences between familial and sporadic ALS.[23]

Another key issue in clinical trial design has been the selection of appropriate clinical endpoints. In addition to survival, clinical researchers usually take a variety of measurements aimed at assessing the patient's clinical status and, ultimately, his or her rate of decline due to disease. No one measurement has necessarily emerged as the preferred clinical assessment. At least ten different compound scales of clinical progression, each incorporating its own combination of muscle strength, bulbar, respiratory, and activity testing into a final numerical score, have been developed over the past forty years and used in clinical investigations. The most widely used of these scales include the Norris scale,[24] Tufts Quantitative Neuromuscular Exam,[25] Appel ALS Rating Scale,[26] ALS Functional Rating Scale,[27] and Sickness Impact Profile.[28] Although these rating systems have been shown to be highly reproducible from investigator to investigator, the number of rating systems and the failure of clinical trials

in ALS to adhere reliably to one rating system has made it difficult to compare results across trials. Complicating this task is the fact that clinical investigators frequently choose to modify standard scales according to their interpretations of what types of clinical data are most important to track over time.[29] This trend makes it difficult to compare trial results even when the investigators are using the same basic clinical scale.[30]

These efforts at constructing ALS rating scales are in part an attempt to deal with the absence of reliable biological markers of disease. There are no gross anatomical features of ALS that appear in standard clinical imaging techniques (X-ray, MRI, CAT, PET); an absolutely definitive diagnosis still relies on an autopsy, although clinical diagnostic guidelines (known as the El Escorial criteria) have been developed that are reliably predictive of post-mortem findings.[31] Attempts to find a reliable biological or biochemical marker for disease progression have yielded promising results but have so far been unsuccessful.[32]

With no known cause and only one treatment of minimal efficacy, correlating possible biochemical signs of disease progression or regression to clinical observations is difficult. Identifying appropriate biomarkers for ALS would allow pilot efficacy trials to be dramatically shortened and would also permit less labor intensive monitoring of disease progression. Current research into possible biomarkers for ALS will be discussed in the next major section of this report, which deals with current research topics in ALS, but it is important to keep in mind that the absence of appropriate biomarkers for ALS has led to significant issues and inefficiencies in trial design.[33]

A number of other issues in clinical trial design deal with ethical considerations related to patients' expected survival times, placebo-controlled clinical investigation of already FDA-approved or over the counter treatments, and the difficulty of maintaining control over patients' entire treatment regimen during the study. Because patients typically have a limited survival time after diagnosis, this can make them reluctant to participate in placebo-controlled studies in which they may end up receiving placebo for up to 12 or 18 months.[34]

Crossover designs alleviate these fears somewhat by ensuring that all patients receive active treatment for at least part of the study period. Patients are randomized to receive either treatment or placebo to start, and then the groups switch halfway through the study period so that those initially receiving placebo begin active treatment and vice versa. This trial design also has the advantage of requiring fewer patients to demonstrate the same statistical effect as a traditional parallel trial design (in which each group receives either treatment or placebo for the entire study period) but typically must rely on predictors of survival rather than survival rates themselves.[35] Crossover designs were used frequently in the 1980s but have appeared in only a small portion of efficacy trials in recent years.[36]

Most ALS patients are aware that treatments' effects may not always be perceptible, especially for drugs that slow but do not stop progression, and that they may not know whether treatments are effective until the end of the clinical trial. Thus, even in crossover trial designs in which all patients receive treatment at some point, twelve or eighteen months may simply be too long for patients to wait to find out whether the treatment they are taking is futile.

Further complicating this issue is the fact that many investigational treatments in ALS are already FDA-approved for use in other diseases and may be available through off-label prescriptions independent of clinical trials. Certain recent investigational therapies are even available over the counter or from health stores (e.g. creatine, Coenzyme Q10). This means that patients may be reluctant to enter placebo controlled efficacy trials of any sort when they have the option of taking the treatment independently and being assured of not receiving a placebo.

In addition, clinical researchers have few ways of ensuring that patients in one trial do not take additional treatments that may be under investigation in other trials. Depending on the efficacy of these additional drugs and the distribution of their use between treatment and placebo groups, these additional treatments may skew the results of clinical trials or cause unanticipated toxicity. Clinical researchers are thus faced with a range of concerns in addition to basic

statistical and design issues when preparing to conduct clinical trials in ALS.

A number of these issues have been partially resolved through the establishment of consensus guidelines on designing clinical trials in ALS. First developed in 1994, these consensus guidelines were expanded in 1998 and again in 2004 through a conference involving a multinational group of neurologists, statisticians, patient advocates, and representatives from the pharmaceutical industry and an array of regulatory agencies.[37] These guidelines contain a range of recommendations on the appropriate design and conduct of clinical trials in ALS.

However, by their own admission the recommendations describe not the ideal clinical trial, but the absolute minimum required to conduct a valid clinical trial in ALS. Recommendations include specific lengths for safety, pilot efficacy, and full efficacy trials, and specific criteria for enrolling study participants. Particularly noteworthy is the acknowledgement that placebo controls (rather than natural history controls) are still necessary in Phase III efficacy studies. This was an issue of some debate during the late 1990s.[38] A significant portion of the consensus guidelines document is dedicated to a discussion of appropriate statistical planning and analysis. Reviews of earlier clinical trials had highlighted the extent to which 'positive' outcomes were the result

Highlights of Consensus Guidelines

Patient Inclusion
- Between ages of 18 and 85
- Clinical history longer than 5 months
- No more than 5 years post diagnosis
- On no other investigational drugs

Conduct/design
- Phase I minimum 6 months long
- Phase II 6-12 months long
- Phase III needs placebo & sequential design

Information sharing
- Patients should be first to learn about trial results
- Database from study should be available to other researchers after publication

Ethics
- Trials should include an open-label period after controlled study

Statistics
- Careful statistical planning an absolute necessity
- Randomization should be stratified according to covariates highly predictive of outcome
- Distinguish between predictors of progression and effect modifiers
- Post-hoc stratification should be used to generate hypotheses, not confirm them
- Lead-in periods should be used with caution

Endpoints
- Change in muscle strength or survival most useful endpoint
- Surrogate measures of survival, including natural history controls, have yet to be statistically validated
- Quality of life measures should be included in every trial

of faulty or misguided statistical analysis. The guidelines also recommended the use of muscle strength or survival as primary clinical endpoints, and discussed lack of statistical validation of surrogate markers for survival (either biomarkers or clinical rating scales.) Other highlights of the consensus guidelines are outlined in the table on the previous page and should be kept in mind when reading the clinical trial summaries in Appendix B. While it is beyond the scope of this review to evaluate each consensus guideline on a trial by trial basis, the next section traces broad historical trends in ALS clinical trials as they relate to contemporary consensus on clinical trial design in ALS.[39]

2. General trends in clinical trial design in ALS, 1965 - 2004

Although only a limited number of treatments have shown potential benefit in ALS, past failures have also been an occasion for researchers to reevaluate the methods by which they attempted to demonstrate clinical efficacy. The result has been dramatic shifts in the size, design, and scope of clinical trials in ALS. The consensus guidelines for clinical trials in ALS, developed in the late 1990s and early 2000s, are closely tied to these shifts – both to those trends which the consensus guidelines hope will continue and those the guidelines are designed to discourage.

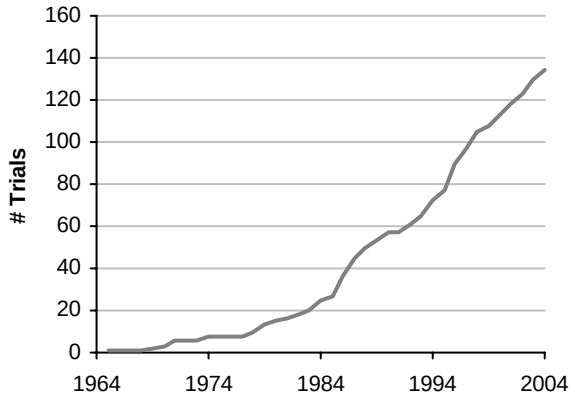
Certainly, trial lengths and the frequency of placebo control in efficacy trials are already well within the range suggested by the consensus guidelines. In a few cases, the current state of clinical trials is at odds with the desires expressed in consensus guidelines – for example, trial population size has increased steadily despite attempts to develop designs that increase the statistical power of small study populations. In some cases, as in the case of sex distribution in trial populations, positive trends in trial design have occurred entirely under the radar of trial guidelines and are not addressed in published recommendations.

This section traces both trends in clinical trial design (as they relate to contemporary issues in trial design) and the types of investigational treatments used in these trials

over the past forty years. It is concerned not with individual treatments or the specific data supporting their use in ALS (these details are covered in later sections), or with assessing the quality of each particular clinical investigation, but instead traces shifting consensus on trial design as expressed through the actual conduct and design of these trials.[40]

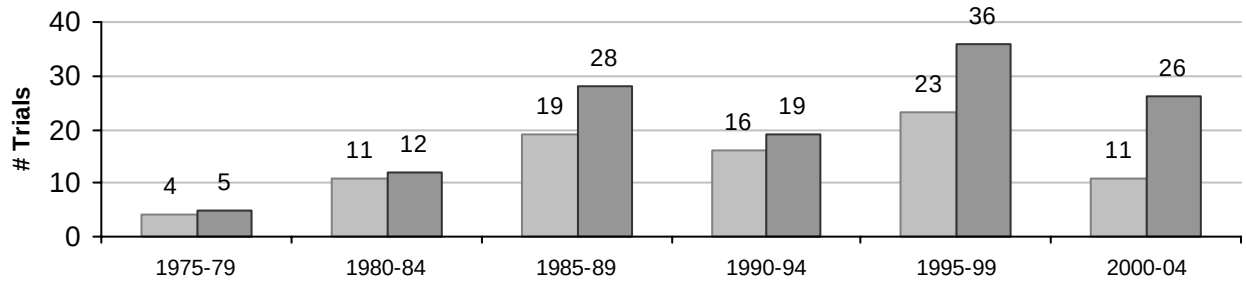
Both the number of published clinical trials and the rate of increase in published clinical trials have risen steadily since 1965. The greatest increases in the number of clinical trials (in terms of the absolute number of new clinical trials published) occurred during the late 1980s and the late 1990s; slightly fewer trials were published between 2000 and 2004 than in the preceding five years. Much of the growth in clinical trials in recent years has been due to an increasing number of safety, dose-ranging, pharmacokinetic, and bio-chemical investigations aimed at developing a better under-standing of drugs' exact biological activity in ALS patients; while in the late 1970s and early 1980s such studies made up less than 20% of all published clinical trials, by the late 1990s they represented a third of these trials, and between 2000 and 2004 this number rose to more than 50% (largely due to ongoing safety and pharmacokinetic studies of Rilutek®.)[41]

Total Clinical Trials in ALS, 1965 - 2004



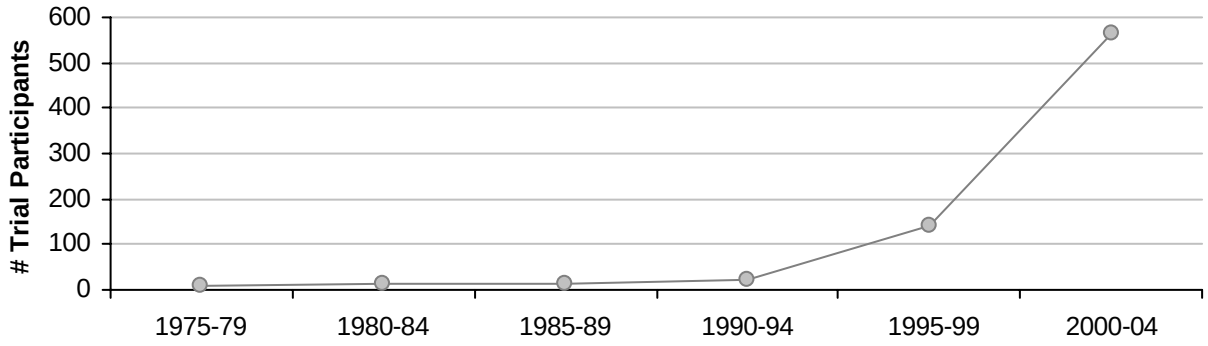
Source: Clinical Trials Database

Total Trials and Trials Directed at Demonstrating Efficacy



Source: Clinical Trials Database

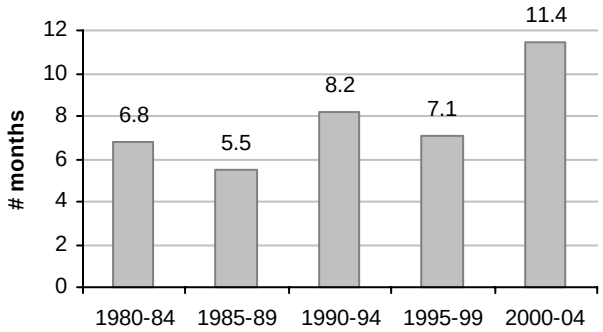
Average Study Size in ALS Clinical Trials



Source: Clinical Trials Database

Despite researchers' interest in designing shorter and smaller trials, the general trend throughout the past forty years has been toward larger and longer trials. From the late 1970s through the early 1990s, the average trial size remained fairly steady, ranging from between 10 and 20 patients on average. In the mid-to-late 1990s, however, average trial size began increasingly rapidly, rising to an average of 140 participants per study between 1995 and 1999 and more than quadrupling to an average of 567 participants between 2000 and 2004.[42] A number of factors contributed to this increase. The 2000 to 2004 period saw the publication of a Phase IIIb safety study of riluzole which enrolled nearly 8,000 participants, which significantly increases the average study size for this period.[43] Even eliminating this outlying data point, clinical trials published between 2000 and 2004 still have an average of 317 participants. The drastic increase in clinical trial size throughout the 1990s and early 2000's can be attributed to three interrelated factors: the organization of multi-center clinical investigation collaboratives on a treatment (e.g. the ALS CNTF Treatment Study Group) and geographic (e.g. the Western ALS Study Group) basis, growing corporate interest in ALS through involvement in clinical trials of Rilutek® (Aventis) and ciliary neurotrophic factor (Regeneron), and the declining popularity of crossover efficacy trials (which require fewer patients.)[44]

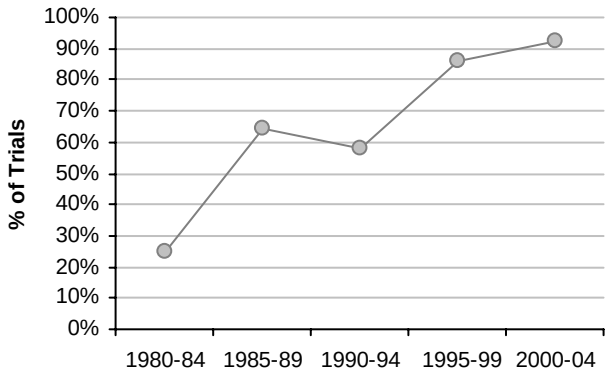
Average Clinical Trial Length in ALS



Source: Clinical Trials Database

In addition to increases in trial size, ALS clinical trials have also increased in length by an average of 4 months since the late 1970s, although for most of that period trial length hovered around 7 months. Although correlating trial length variation to other shifts in trial design is beyond the scope of this review, the increase in trial length between 2000 and 2004 at first appears to be consistent with consensus clinical trial guidelines in ALS which recommended that even safety trials be conducted for a minimum of six months. However, the average safety trial length in the 1980s and 1990s *already* ranged 5 and 6 months; in the 2000 – 2004 period the average safety trial length jumped to 13 months, largely due to a series of Phase IIIb open-label safety trials of Rilutek®.[45] The average length of Phase I safety trials has remained fairly constant and close to the consensus guidelines since at least the early 1980's.[46]

ALS Efficacy Trials with Placebo Controls



Source: Clinical Trials Database

Efficacy trials published between 2000 and 2004 were much more likely to include placebo controls than those conducted in the early 1980s. Although the importance of placebo controls was well documented by the early 1980s, only one out of every four efficacy trials included a placebo arm or time period.[47] In recent years, more than 9 out of every 10 efficacy trials included a placebo group. Placebo controls are particularly important in ALS, since many clinical measures of progression depend on measuring muscle strength (which is highly variable) and patients' subjective assessments of their activities of daily living, both of which can be affected by patients' state of mind. A number of early efficacy trials yielded inconclusive or falsely promising results because the trial design failed to account for the placebo effect.[48]

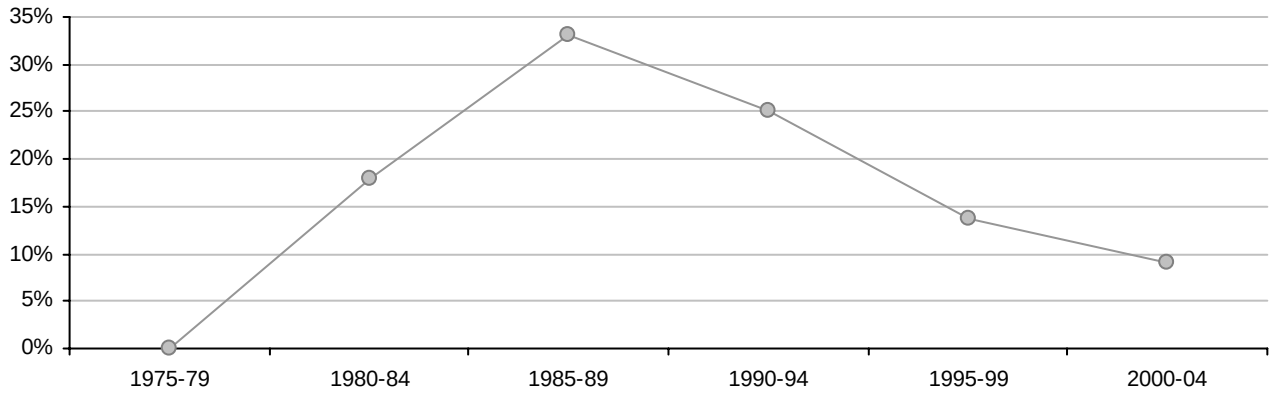
During the 1980s and early 1990s, a number of clinical trials tried to balance the need for placebo controls with the desire to treat all study participants by employing a crossover design. In a crossover trial, patients are

randomized into two or more groups. As discussed earlier, in the typical crossover trial one group starts off receiving the treatment under investigation and the other receives the placebo. At the end of the first treatment period, both groups usually undergo a washout period to allow any residual drug concentrations in the treatment group to be eliminated from the body, and then each group is crossed over to the opposite treatment for a period of time equal to the first.

While this trial design offers significant advantages in terms of study size (and in some cases trial length), it reached its peak in popularity in ALS in the late 1980's and now makes up less than 10% of the efficacy studies published in ALS. The reasons for this decline are not clear, but may have to do with an increasing focus on survival as a more reliably clinical endpoint than muscle strength-based measures of clinical progression. Crossover trials, by design, must rely on these latter endpoints rather than survival, since the decreased study sizes are due to the reductions in variation gained by comparing individual patients' progression during the placebo and treatment periods.[49]

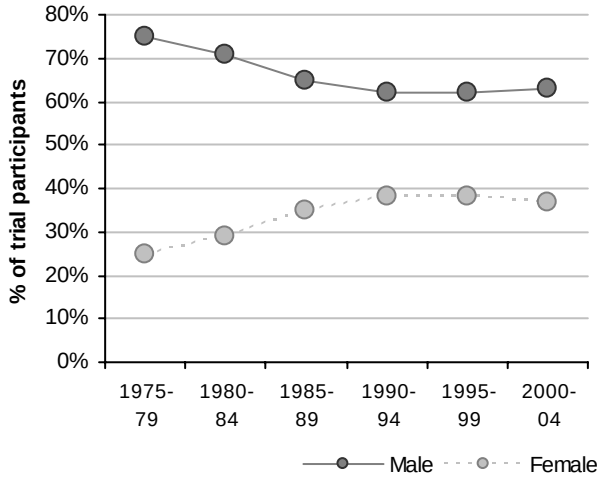
One often overlooked aspect of clinical trials is whether they accurately reflect the sex distribution in the general ALS population. Sex-based differences in drug metabolism are well documented in medical literature, yet until very recently it was still common to conduct clinical trials using only or predominantly male subjects, or to omit analysis of any sex differences in drug

Percent of Efficacy Studies Using Crossover Design



Source: Clinical Trials Database

Gender Balance in ALS Clinical Trials



Source: Clinical Trials Database

response.[50] Including women in disproportionate numbers to the general disease population or excluding women entirely from clinical trials could lead to promising treatments with a sex-specific effect being overlooked *or* being falsely assumed to benefit both men and women despite a predominantly male study population. Complicating the situation is the fact that men are at a higher risk for developing ALS, meaning that the sex distribution in the ALS population differs from the general population. In general, 1.3 to 1.5 men are diagnosed with ALS for every 1 woman, translating to an ALS patient population that is 56% - 60% male and 40% - 43% female.[51] Clinical trials in ALS have approached but not reached this pattern of sex distribution. Between 2000 and 2004, trials enrolled an average of 1.7 men for every 1 woman enrolled, a slightly higher ratio than the highest estimates of sex distribution in ALS. However, this is quite an improvement compared to the late 1970s, when only 25% of study participants were female.[52]

Compared to the earliest clinical investigations, contemporary clinical trials in ALS enroll more participants, last longer, more frequently include placebo controls, and have a more equitable sex distribution among study participants than their predecessors. If the consensus guidelines on ALS become the standard for the design of clinical studies, however, one would hope see a reversal or leveling off of some of these trends. Continued increases in the average trial length or average number of trial participants would certainly be cause for concern given the consensus guidelines' stated desire to reduce trial length and population size through superior statistical planning and design.[53] In addition, if surrogate measures of survival that focus on disease progression rates can be adequately statistically validated, crossover designs may once again rise in popularity. Finally, as researchers refine their

understanding of sex-related or genetic variations in key disease processes, it may be entirely possible that the variation in sex, age, and other characteristics from trial to trial may increase, since study populations may be selected not based on their resemblance to the general disease base, but on their likelihood to respond to the treatment under investigation. These trends, if they occur, should not be interpreted as a 'regression' to outdated modes of clinical trial design, but should be interpreted in the context of the stated goals of clinical trial consensus guidelines, in which trends toward bigger, longer, and more homogeneous studies are not necessarily the ideal direction for clinical trial design in ALS.

3. Etiological Assumptions Underlying Clinical Investigations in ALS

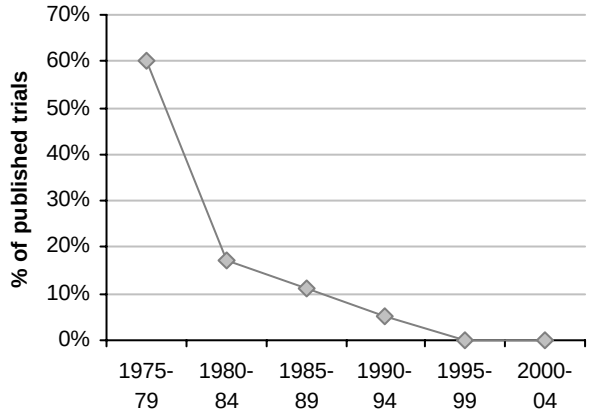
In addition to shifts in clinical trial design, there have been distinct shifts in the reasons investigators initiate clinical trials in ALS. The choice to investigate a particular drug in human subjects must necessarily be based on a strong scientific rationale for believing that drug will be safe and effective in treating the target disease. Usually, the rationale for testing a particular treatment involves not only specific laboratory demonstrations suggesting the possible efficacy of the treatment, but also a statement about the biological causes of the particular disease being studied and the general mechanism of action of the drug under study. In ALS, these statements are necessarily hypotheses, or at best statements about downstream biological effects of the primary disease pathology, since the exact biological cause of the disease is not yet known.

Tracing the rise and fall of these etiological hypotheses, as expressed through the rationale sections of published clinical trials in ALS, provides a window into changes in prevailing scientific thought on ALS over the past forty years. While published pre-clinical investigations over the past forty years have advanced a broad range of etiological hypotheses, not all of these hypotheses were considered equally compelling at the time. Over the past forty years, six major etiological

assumptions about ALS – assumptions that ALS is due to viral infection, autoimmune reaction, neurotrophic deficit, excitotoxic nerve damage, oxidative stress, or metabolic dysfunction – have been used to justify clinical trials, and these assumptions follow distinct patterns of use and disuse over time.

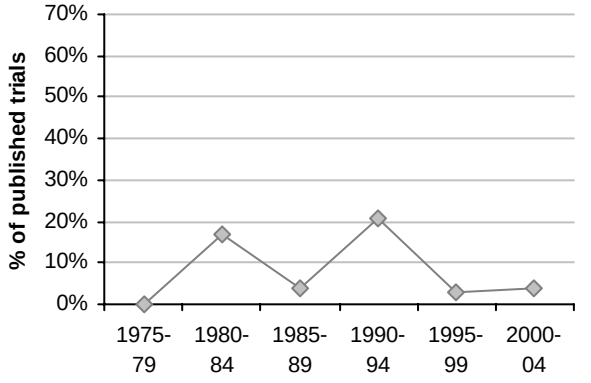
In the late 1970s, the predominant hypothesis behind clinical investigations in ALS was that the disease was caused by a slow acting or chronic viral infection, although trial authors widely acknowledged that there was little laboratory evidence to support this hypothesis. The primary justification for this hypothesis included scattered but irreproducible findings of viruses and virus-like particles in ALS patients pre- and post-mortem, and a growing catalogue of neurological and neurodegenerative ailments which appeared to be caused by chronic viral infections.[54] (A large number of so-called slow virus illnesses that were the focus of intense research interest in the 1970s would later be identified as prion diseases – illnesses caused by infectious protein particles rather than viruses.) Based on this hypothesis, clinicians tested a range of antiviral agents in the hopes of slowing or reversing progression, with little effect. As laboratory evidence in the 1980s began to suggest that earlier findings which had been interpreted as pointing to a viral origin might actually be evidence of an autoimmune component to ALS, the notion of a viral etiology quickly disappeared from ALS trials. While in the late 1970s a viral etiology was used to justify 60% of clinical trials, that number dropped to less than 20% in the early 1980s and

Trials using antiviral agents



Source: Clinical Trials Database

Trials using immune modulators



Source: Clinical Trials Database

continued to decline steadily – by 1993, the notion of a viral etiology had completely disappeared from the rationales of published clinical trials.[55]

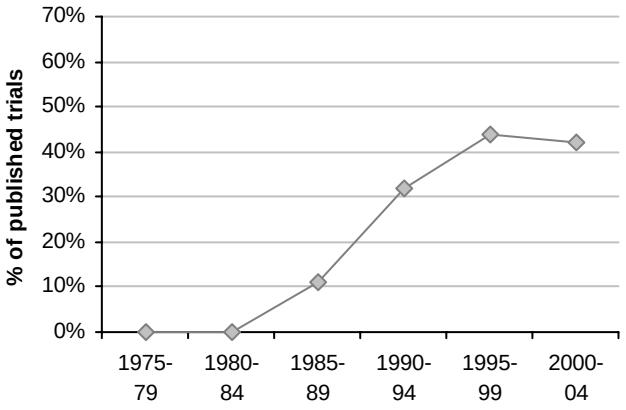
However, declining interest in a possible viral etiology did not mean that immunological findings in ALS were ignored. Throughout the 1980s and 1990s, trials intermittently focused on investigating treatments and procedures which assumed ALS had an autoimmune component. While findings of viral particles in ALS patients were not easily reproducible, the *signs* of apparent infection were reliably detectible using a range of laboratory techniques.[56] This led researchers to hypothesize that the immune reactions observed were directed not against a rare virus, but against patients' own cells and tissues. Studies based on this etiological hypothesis investigated treatments which had been shown to be successful in treating other autoimmune disorders – primarily plasmapheresis (therapeutic blood plasma exchange) and immunosuppressive drugs and procedures. While these interventions experienced brief popularity in the early 1980s and early 1990s, in recent years they have made up only a small proportion of published clinical trials in ALS.

The early 1980s also saw the sudden emergence of a new etiological hypothesis: the idea that ALS was caused by the body's inability to repair the damage done to neurons by the disease (or, more generally, that the most effective treatment for the loss of motor neurons would be to encourage the body to regrow its dead or dying neurons.) Interest in this particular therapeutic avenue coincided with rapid advances in understanding a range of growth hormones and growth factors, and their potential to yield dramatic therapeutic effects.[57] In terms of sheer number of trials, this etiological hypothesis was most prevalent in the 1980s (particularly the late 1980s, when it appeared

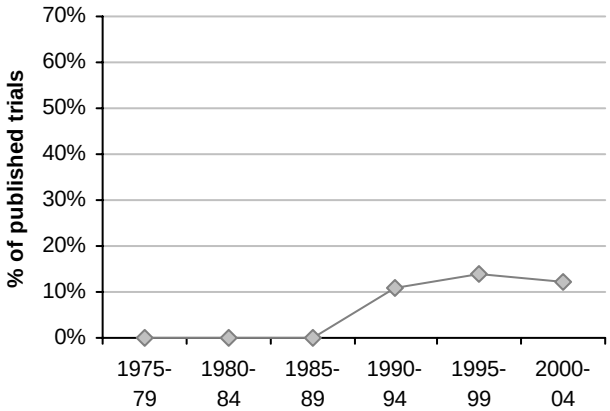
Trials using neurotrophic agents

Source: Clinical Trials Database

Trials using anti-excitotoxic agents



Trials using anti-oxidants



in nearly 60% of published clinical trials.) However, there was a small resurgence of interest in neurotrophic therapies for ALS between 1995 and 1999. Though trials of neurotrophic agents accounted for only 30% of all trials published in ALS during that time period, they were predominantly Phase III efficacy trials. More than 43% of all trial participants between 1995 and 1999 were enrolled in studies testing neurotrophic agents in ALS, even though these studies in absolute numbers represented less than a third of the total clinical published on ALS. The fact that large phase III trials of neurotrophic agents represented only 30% of all clinical trials between 1995 and 1999 was due primarily to the rise of another etiological hypothesis and the accumulation of compelling pre-clinical and clinical data in support thereof. Beginning in the late 1980s, researchers began to conduct clinical investigations which assumed that a key predecessor of motor neuron degeneration in ALS was the flooding of those neurons with massive amounts of synaptic signaling compounds. Usually intended to convey messages from one neuron to the next, when these excitatory compounds accumulate they can be toxic to neurons, a phenomenon called excitotoxicity. Interest in a possible excitotoxic mechanism of action in ALS was based on laboratory findings which suggested animal models of ALS-like syndromes could be induced using excitoxins and data pointing to a possible etiologic role of excitatory amino acids in Huntington's and Alzheimer's Disease.[58] Chief among the possible sources of excitoxicity in ALS was glutamate; studies had shown evidence of altered glutamate metabolism in ALS patients. Strong support

was lent to this hypothesis by investigators' success in demonstrating that Rilutek®, a glutamate antagonist, conferred a small but statistically significant survival benefit to ALS patients treated with the drug. Studies of analogous glutamate antagonists and other anti-excitotoxic compounds have failed to demonstrate similar efficacy, however, and the continued prevalence of excitoxicity as an etiological hypothesis in published clinical trials is due almost entirely to a large number of follow-up safety and efficacy studies on Rilutek®. Even if additional treatments were found to have some efficacy in treating ALS, most researchers assume that excitotoxicity is a downstream event in a much more complicated disease process, and that any therapeutic benefit obtained through anti-excitotoxic treatments will at best have a minimal impact on disease progression.[59]

The 1990s also saw the emergence of oxidative stress as a possible contributing factor to ALS pathology. This interest was due in part to the widespread popularity of research on free radicals, anti-oxidants, and the aging process, as well as a growing interest in anti-oxidants as possible treatments for Alzheimer's and other diseases of aging. A focus on anti-oxidant treatments for ALS was further fueled by researchers' discovery in 1993 that mutations in Cu/Zn Superoxide Dismutase I (SOD1, an antioxidant) were responsible for a significant fraction of familial ALS cases.[60] The mutation has since been identified as a gain-of-function mutation, meaning that familial ALS is caused not by deficiencies in SOD1's ability to mitigate oxidative stress but rather from novel toxic properties possessed by the mutant protein.

However, in the years immediately after the discovery of this gene, a common assumption was that treating patients with either recombinant SOD1 or a range of antioxidants would help slow or reverse the progression of the disease, although this hypothesis has not been borne out in practice. Trials of anti-oxidant treatments have represented between 10 and 15% of clinical trials published in the past fifteen years, but based on disappointing results to date and an evolving understanding of the exact mechanism of the SOD1 mutation, it remains to be seen whether they will continue to appear in such numbers in the future.[61]

In addition to these major trends in etiological assumptions, there have been a range of miscellaneous rationales advanced for trying certain treatments. These rationales have represented between 8% and 40% of clinical trials over time and have included treatments directed primarily at symptomatic management (temporarily improving muscle strength or neural signaling with no expectation of slowing disease progression), studies based on positive therapeutic effects observed in analogous diseases, and studies aimed at confirming anecdotal or case reports of efficacy. In general, no one rationale in this category is prevalent enough to merit a separate discussion at present.

These trends in the rise and fall of certain etiological hypotheses over time help clarify the often confusing array of such hypotheses presented in the introductory paragraphs of many articles on ALS. While the idiopathic nature of ALS means that any number of etiological assumptions might guide clinical research at any given time, there are distinct trends in the prevalence of these hypotheses in the clinical literature over time.

These shifts are not always apparent to readers outside the ALS research community; thus, while a review might offhandedly mention a possible viral etiology among the many theories advanced on the cause of ALS, it is important to place this theory within actual research practice. Though a viral etiology is certainly one of the theories that has been advanced on the cause of ALS, it has long since passed from clinical practice as an

Trends in Etiological Assumptions Behind Clinical Investigations in ALS
(% of total trials conducted in each 5-year period)

	Slow Virus	Autoimmune disorder	Neurotrophic deficit	Excitotoxicity	Oxidative Stress	Misc.
1975-79	60%					40%
1980-84	17%	17%	50%			17%
1985-89	11%	4%	61%	4%		14%
1990-94	5%	21%	21%	32%	11%	11%
1995-99		3%	31%	44%	14%	8%
2000-04		4%	19%	42%	12%	19%

Source: Clinical Trials Database

appropriate target for study. Finally, it is important to keep in mind that the etiological hypotheses advanced in clinical trials usually lag behind pre-clinical and laboratory research by two to four years.[62] As the next chapter (on current research topics in ALS,) will show, a number of promising etiological hypotheses have arisen in the past four years, many of which have yet to appear in published clinical trials but which are being actively investigated both in the laboratory and the clinic.

4. Review of major investigational treatments in ALS, 1965 – 2004

In addition to broad etiological hypotheses, there have been a number of investigational treatments in ALS which have received a great deal of attention. This section reviews some of the major treatments used in published clinical trials on ALS. Treatments included in this portion of the review were selected based on the publication of multiple studies on the same therapeutic compound and on the size of the largest trial conducted (100+ patients for a parallel or sequential trial, 40+ patients for a crossover trial.) These restrictions identified nine investigational treatments which, based on the clinical literature, can be described as receiving the most clinical attention over the past forty years. Each one is reviewed briefly below, and an overview of clinical trials conducted and trials results is included. For a more in-depth description of trial design, rationale, and outcomes, see the appropriate trial summary in Appendix B.

4.1. Thyrotropin-releasing hormone (TRH), a neurotrophic agent

Thyrotropin-releasing hormone (TRH), a tripeptide hormone that stimulates the secretion of thyrotropin from the pituitary gland and also has neurotrophic properties, has almost as lengthy a history of clinical investigation in ALS as Rilutek®, but has been tested only in small scale pilot and crossover efficacy studies. The first such study was published in *Lancet* in 1983 and was based on a hypothesis that the symptoms of amyotrophic lateral sclerosis were primarily due to a metabolic defect.

List of Published Clinical Trials of TRH & TRH analogues

Year	Type	Patients	Length
1983	**Pilot safety & efficacy**	**17**	**<1 month**
1984	Pilot efficacy	6	<1 month
1985	**Pilot efficacy**	**8**	**<1 month**
1986	**Crossover pilot efficacy**	**12**	**<1 month**
1986	Crossover efficacy	41	3 months
1986	Pilot efficacy	30	2 months
1986	Safety/pharmacokinetics	4	6 months
1986	**Pilot efficacy**	**7**	**2-3 months**
1987*	Efficacy	25	<1 month
1987	**Dose-ranging & efficacy**	**19**	**2-3 months**
1987	Pharmacokinetics	15	12 months
1987*	Efficacy	11	2-6 months
1987*	Pilot efficacy	9	<1 month
1988	Efficacy/biomarkers	8	<1 month
1988	Safety	20	5 months
1988	Biomarkers	15	<1 month
1990*	**Pilot safety & efficacy**	**10**	**<1 month**
1990	Biomarkers	6	<1 month
1992	Crossover safety/efficacy	25	6 months

* Indicates study of TRH analogue
Bolding indicates a positive report of efficacy

Only one month long, and conducted in a small patient population with no controls, the promising results of this study would spur eighteen additional studies of TRH and several novel TRH analogues over the next decade, all attempting to confirm or refute these initial findings through a range of study designs, doses, and drug delivery systems. Despite widespread interest in TRH, the largest of these studies (a crossover efficacy study published in 1986) would enroll only 41 patients, and only five out the eighteen studies would report that the treatment was of benefit to study participants. These five reports of efficacy were all based on data from fewer than 20 patients, and consisted of muscle strength or functional improvements in only one or two clinical parameters, such as jaw strength or lower limb strength. In the late 1980s, investigations of analogous compounds and alternative delivery routes (e.g. continuous intrathecal infusion rather than intermittent IV infusion) attempted to reproduce or improve upon these results with little success, and interest in TRH as a possible therapeutic for ALS gradually waned. Later reviews of TRH trials would point out issues in trial design and statistical analysis which called into question the conclusions of these studies.[63]

4.2. Acetylcysteine, an anti-viral agent and anti-oxidant

Acetylcysteine is used as a mucolytic agent to reduce the viscosity of mucous secretions and has also been shown to have anti-viral and anti-oxidant effects. The earliest trials of acetylcysteine were directed primarily toward assessing acetylcysteine's efficacy in alleviating the symptoms of later stages of ALS, where difficulty swallowing and coughing leads to the buildup of excess sputum which in some cases can lead to asphyxiation or respiratory distress. Interest

in acetylcysteine was revived in the mid 1990s in response to a growing interest in anti-oxidant treatment of ALS. In a 12 month, double-blind, placebo controlled efficacy study with 120 participants, treatment with acetylcysteine resulted in a small but not statistically significant survival advantage versus patients treated with placebo. However, this survival advantage was not statistically significant, and the results of this trial have generally been interpreted as failing to demonstrate any benefit of treatment with acetylcysteine.[64]

List of Published Clinical Trials of Acetylcysteine

Year	Type	Patients	Length
1987	Efficacy	40	3-24 months
1987	Efficacy	11	12 months
1995	Efficacy	110	12 months

4.3. Rilutek® / riluzole, an anti-glutamate agent

Rilutek® / riluzole, a glutamate antagonist, is the only FDA-approved treatment for ALS and the only treatment for which clinical trials have repeatedly demonstrated a statistically significant (although small) survival advantage. Originally investigated for a range of neurological conditions, including epilepsy and stroke, by the late 1980s and early 1990s pre-clinical investigations had shown riluzole to be a powerful neuroprotective agent *in vitro*, and had suggested that the source of this neuroprotective effect was the inhibition of both the release and certain post-synaptic effects of glutamate.[65] At the time, research into ALS had begun to suggest that an overproduction or overabundance of glutamate in ALS might play a key role in the biology of the disease.[66]

The first pilot trial, published in 1994, demonstrated a statistically significant survival advantage after 12 months of treatment with riluzole; survival among placebo patients was 58% at the 12-month mark and 74% for patients on active treatment. Among bulbar patients, the difference was more pronounced (35%

List of Published Clinical Trials of Rilutek®

Year	Type	Patients	Length
1994	**Pilot efficacy**	**155**	**12 months**
1996	**Dose-ranging/efficacy**	**959**	**18 months**
1997	**Biomarkers/efficacy**	**5**	**6 months**
1997	Pharmacokinetics	100	1 month
1998	**Reanalysis of trial**	**959**	**18 months**
1998	**Biomarkers/efficacy**	**23**	**<1 month**
1998	Biomarkers	7	<1 month
1999	Biomarkers	17	<1 month
2000	Safety	153	3-36 months
2000	Safety	919	7-8 months
2001	Pharmacokinetics	21	<1 month
2001	Safety	7916	20 months
2002	Safety	168	18 months
2002	Biomarkers	37	18 months
2002	Safety	516	14 months
2002	Safety	2069	3-24 months
2003	Pharmacokinetics	169	<1 month

survival on the placebo and 73% on riluzole.) Although these results did not indicate a dramatic arrest or reversal of progression, they were certainly the largest and most statistically significant demonstration of a survival advantage reported in ALS. Although a larger follow-up efficacy trial was published in 1996, this and subsequent efficacy trials did not demonstrate quite as dramatic a survival advantage. The general consensus upon reviewing all clinical trials of riluzole in ALS is that the drug extends average survival time during the treatment period by 17%, or between 2 and 3 months.[67] Rilutek® was FDA-approved for the treatment of ALS in 1995, but only taken by a fraction of ALS patients due largely to its prohibitive cost and small clinical effect.

However in other countries up to 83% of the ALS population may be treated with Rilutek®.[68] Although the drug's effect may be small, Rilutek offers hope that contemporary therapeutic approaches can gain a foothold in arresting the progression of the disease, and has been credited with eliminating the therapeutically nihilistic attitude which which some physicians previously approached ALS.

4.4. Selegiline hydrochloride / Eldepryl® / Deprenyl®, an MAO-B inhibitor

Selegiline is a selective, irreversible inhibitor of Type B monoamine oxidase with anti-oxidant properties and is used to treat newly diagnosed Parkinson's disease. (Eldepryl® / selegiline and Deprenyl are isomers of the same molecule and have similar biological effects.) Interest in selegiline as a possible treatment for ALS was initially due to its antioxidant properties and its ability to slow Parkinson's disease. Selegiline also appeared to have neuroprotective effects in a rat model of neuronal injury. The treatment has been assessed through three separate efficacy trials, none of which have shown selegiline or Deprenyl® to have any perceptible clinical benefit in ALS.[69]

List of Published Clinical Trials of Selegiline

Year	Type	Patients	Length
1994	Efficacy	111	6 months
1994	Crossover efficacy	10	3 months
1998	Efficacy	104	6 months

4.5. Ciliary neurotrophic factor (CNTF), a neurotrophic agent

Ciliary neurotrophic factor (CNTF) is expressed in glial cells in the central and peripheral nervous systems that appears to be released in response to neuronal injury and

has been shown to have neuroprotective and neurotrophic effects in a range of in vitro and in vivo models of disease.[70] Despite promising results in these in vitro and in vivo studies, two separate efficacy studies of CNTF were unable to demonstrate any clinical benefit of treatment with CNTF. Side effects related to systemic administration of the treatment during early trials may have reduced patients' quality of life; several subsequent trials focused on assessing the safety and tolerability of alternative methods of delivery, including intrathecal pump infusion. While these new delivery methods appeared to be safe and well tolerated, no further efficacy studies of CNTF have been published.[71]

List of Published Clinical Trials of CNTF

Year	Type	Patients	Length
1995	Safety/dose-ranging	57	<1 month
1996	Safety/efficacy	730	9 months
1996	Safety/efficacy	483	6 months
1996	Safety	6	5 months
1996	Safety	72	<1 month
1997	Safety	4	3 months

4.6. Gabapentin, an anti-glutamate agent

Gabapentin, a glutamate antagonist and anti-convulsive agent, was first proposed as a possible treatment for ALS in the mid 1990's due to its neuroprotective effects in an in vitro model of chronic glutamate toxicity and due to early successes in treating ALS with Rilutek®, another glutamate antagonist.[72] The first clinical trial of gabapentin in ALS, published in 1996, showed a modest but not statistically significant reduction in rate of progression (as measured by decline in arm strength.) A subsequent trial appeared to demonstrate a statistically significant reduction in the rate of muscle strength decline, but a confirmatory trial failed to replicate this result. On the basis of these inconsistent results, gabapentin is now presumed to have no relevant clinical effect on ALS progression.[73]

List of Published Clinical Trials of Gabapentin

Year	Type	Patients	Length
1996	Safety/efficacy	140	6 months
1998	**Efficacy**	**231**	**9-12 months**
2001	Efficacy	128	9 months

4.7. Insulin-like growth factor I (IGF-1), a neurotrophic agent

Insulin-like growth factor-I (IGF-I) is a protein growth factor with widely demonstrated neurotrophic and neuroprotective effects, and was proposed for use in amyotrophic lateral sclerosis based on an array of promising results in *in vitro* and *in vivo* models of diseases related to or relevant to ALS.[74] While the first two trials of IGF-I appeared to

demonstrate statistically significant reductions in the rate of FVC decline and increases in survival time, a third trial was unable to replicate these results.[75] A two-year, double-blind, placebo-controlled study is currently underway at the Mayo Medical Center in Rochester, Minnesota to attempt to replicate the findings of the first two trials and definitively determine whether IGF-I slows progression of weakness in ALS.[76]

List of Published Clinical Trials of IGF-I

Year	Type	Patients	Length
1996	**Efficacy**	**141**	**9 months**
1997	**Safety / efficacy**	**266**	**9 months**
1998	Safety / efficacy	96	9 months

4.8. Brain-derived neurotrophic factor (BDNF), a neurotrophic agent

Brain-derived neurotrophic factor (BDNF), like CNTF, is a protein that has neurotrophic and neuroprotective effects on the central nervous system and brain.[77] Its use in ALS was based on positive results in animal models of Alzheimer's and Parkinson's disease, and on positive preliminary clinical results in other diseases. The first study of BDNF failed to demonstrate a statistically significant survival benefit, but post-hoc stratification suggested that both FVC scores of 91% or lower and adverse reactions to

List of Published Clinical Trials of BDNF

Year	Type	Patients	Length
1999	**Efficacy**	**1135**	**9 months**
2000	Safety / dose-ranging	25	3 months
2003	Biomarkers / efficacy	11	<1 month

BDNF within the first two weeks of treatment were predictive of statistically significant survival advantages on treatment with BDNF. A smaller follow-up study published in 2003 was unable to replicate this survival advantage. A 300- patient study testing the effectiveness of intrathecal and subcutaneous BDNF was terminated in early 2001 after preliminary results showed the trial was highly unlikely to demonstrate an improvement in survival.[78]

4Creatine, a dietary supplement and muscle strength enhancer

Creatine, an amino acid available as an over-the-counter dietary supplement, is currently of interest in ALS primarily for its role in mitochondrial energy production. Studies have shown that mitochondrial dysfunction occurs relatively early in the course of the disease, and the positive effect of creatine on a murine model of ALS suggested to researchers that mitochondria might be an important target for treatment.

List of Published Clinical Trials of Creatine

Year	Type	Patients	Length
2001	Pilot efficacy	28	6 months
2002	Pilot efficacy	27	4 months
2003	Efficacy	175	16 months
2004	Efficacy	104	6 months

The first two published trials of creatine in ALS, however, were primary interested in creatine's effect on muscle strength (creatine is frequently used as a nutritional supplement to help improve muscle strength.) The results of these trials were inconclusive - one suggested that creatine temporarily increases muscle strength in ALS patients, while the other could demonstrate no beneficial effect of creatine on respiratory function. The two most recently published trials focused on creatine's involvement in mitochondrial energy production, and attempted – without success - to demonstrate a beneficial effect on survival. A third efficacy trial, a 9-month, multi-site, sequential placebo-controlled study focusing on survival rates and short-term and long-term effects on muscle strength, is currently being coordinated by the Carolinas Neuromuscular/ ALS Center.[79]

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[5] This number refers only to those clinical trials for which the original full text would be widely accessible to American ALS researchers. An initial list of trials was created using the National Library of Medicine's Pubmed database. "Trials" which were actually case studies of one or two patients, studies which were retrospective rather than prospective, or studies which were clearly not part of a research effort whose end goal was an assessment of efficacy in ALS were obviously excluded. Clinical trials published in languages other than English, German or French were thus excluded from this study. Studies published in scientific journals available in fewer than 10 libraries nationwide were also excluded as unlikely to be read by all but a very small fraction of the ALS research community (roughly 7 studies were excluded using this criteria.)

[6] In absolute numbers, the results 13 ALS and 7 Huntington's disease clinical trials were published between 1964 and 1979. Between 1995 and 1999, the results of 36 ALS and 11 Huntington's disease clinical trials were published. The rate of publication in ALS appeared to decrease slightly between 2000 and 2004 but this may be due to delays in indexing of certain periodicals for 2004.

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[8] The purpose of this report is obviously not to review past trials with a scientific eye or with the intent to discover some new kernel of knowledge on the mechanism of action of riluzole, or whether thyrotropin-releasing hormone is or is not effective in ALS. Scientific reviews of past trials that aim to make definitive statements about efficacy or therapeutic potential are widespread in the literature and will be cited throughout this report as appropriate.

[9] For the purposes of this study, a major efficacy trial is defined as either a traditional parallel 2+ arm placebo-controlled trial with 100+ participants or a crossover trial with 40+ participants.

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[33] Another topic that will be addressed in the report on current research topics is the connection between pre-clinical research (particularly research using animal models of ALS) and clinical efficacy. Although animal models of neurodegenerative disease are general assumed to be predictive of clinical efficacy, the historical record in ALS shows that this has rarely been the case, even for contemporary (and presumably more advanced) animal models of disease. Clinicians must reconcile this evidence when articulating the rationale for investigating a particular treatment. For an overview of this topic, see J.D. Rothstein, "Of mice and men: reconciling preclinical ALS mouse studies and human clinical trials," *Ann Neurol,* Apr 2003, 53(4):423-6.

[34] G.J. Groeneveld, I. van der Tweel, J.H. Wokke, L.H. van den Berg, "Sequential designs for clinical trials in amyotrophic lateral sclerosis," *Amyotroph Lateral Scler Other Motor Neuron Disord,* 2004, 5(4):202-7.

[35] For an outline of the advantages of crossover designs (albeit in a disease other than ALS), see: A. Richens, "Proof of efficacy trials: cross-over versus parallel-group," *Epilepsy Res,* 2001, 45(1-3):43-7.

[36] See Section II for more detail.

[37] World Federation of Neurology (WFN) Research Group on Neuromuscular Diseases Subcommittee on Motor Neuron Disease, "Therapeutic trials in amyotrophic lateral sclerosis," *J Neurol Sci,* 1995, 129:1-10; R.G. Miller, T.L. Munsat, M. Swash, B.R Brooks, "Consensus guidelines for the design and implementation of clinical trials in ALS. World Federation of Neurology Committee on Research," *J Neurol Sci,* Oct 1999, 169(1-2):2-12; P.N. Leigh, M. Swash, Y. Iwasaki, et al., "Amyotrophic lateral sclerosis: a consensus viewpoint on designing and implementing a clinical trial," *Amyotroph Lateral Scler Other Motor Neuron Disord,* Jun 2004, 5(2):84-98.

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[40] For additional reviews of past clinical trials, see: M. Dib, "Amyotrophic lateral sclerosis: progress and prospects for treatment," *Drugs,* 2003, 63(3):289-310; M. Jackson, J. Llado, J.D Rothstein, "Therapeutic developments in the treatment of amyotrophic lateral sclerosis," *Expert Opin Investig Drugs,* Oct 2002, 11(10):1343-64; M.R. Turner, M.J. Parton, P.N. Leigh, "Clinical trials in ALS: an overview," *Semin Neurol,* Jun 2001, 21(2):167-75.

[41] Statistics on trial design cited in this and subsequent paragraphs in this section was calculated using a database of all previously published clinical trials in ALS, which was compiled by the author in the process of preparing this report. The contents of this database are reproduced in full in Appendix B. Any graphs and trendlines that accompany these statistics are included for illustrative purposes only and should not be interpreted as reflecting detailed statistical analysis. Statistics and data which were generated using this database will be footnoted to that effect, along with any additional comments on calculations or graphing.

[42] Trend lines in the graphs illustrating this and other changes in clinical trial design are included only to better illustrate changes over time and should not be interpreted to represent any statistical modeling or significance assessment of shifts in clinical trial design over time. Standard deviation calculations for all averages have been completed but hold little significance to the content of this report and have not been included or indicated on the graphs.

[43] C. Debove, et al., "The Rilutek (riluzole) Global Early Access Programme: an open-label safety evaluation in the treatment of amyotrophic lateral sclerosis," *Amyotroph Lateral Scler Other Motor Neuron Disord,* 2001, 2(3): 153-8.

[44] The Syntex-Synergen ALS/CNTF Study Group, "A brief quality-of-life measure for ALS clinical trials based on a subset of items from the sickness impact profile," *J Neurol Sci,* 1997, 5(Suppl 1):18-22; R.G. Miller, D.H. Moore, C.E. Jackson, WALS Study Group, "Western ALS Study Group," *Amyotroph Lateral Scler Other Motor Neuron Disord,* 2004, 5(Suppl 1):121-4; M. Dib, "Amyotrophic lateral sclerosis: progress and prospects for treatment," *Drugs,* 2003, 63(3):289-310; ALS CNTF Study Group, "Prognostic indicators of survival in ALS," *Neurology,* 1998, 50(1): 66-72. The decline in popularity of crossover trials is discussed in greater detail later in this chapter.

[45] C.Debove, et al., "The Rilutek (riluzole) Global Early Access Programme…"; L. Lacomblez, et al., "Long-term safety of riluzole in amyotrophic lateral sclerosis," *Amyotroph Lateral Scler Other Motor Neuron Disord,* 2002, 3(1):23-9

[46] Because the original study data was not available for most published trials, in cases where the duration of a study varied on a patient-by-patient basis and in which the authors did not indicate an average study duration across the entire trial population, trial length was calculated by averaging the shortest and longest trial durations.

[47] G. Haegerstam, "Placebo in clinical drug trials – a multidisciplinary review," *Methods Find Exp Clin Pharmacol,* 1982, 4(4): 261-78.

[48] W.W. Bryan, "Can we eliminate placebo in ALS clinical trials?" *Amyotroph Lateral Scler Other Motor Neuron Disord,* 2003, 4(1): 11-5; G.D. Borasia, "Amyotrophic lateral sclerosis: lessons in trial design from recent trials," *J Neurol Sci,* 1997, 152(Suppl 1):23-8.

[49] P.H. Gordon, "Advances in clinical trials for amyotrophic lateral sclerosis," *Curr Neurol Neurosci Rep,* 2005, 5(1): 48-54.

[50] See, for example: I. Beierle, M. Meibohm, H. Derendorf, "Gender differences in pharmacokinetics and pharmacodynamics," *Int J Clin Pharmacol Ther,* Nov 1999, 37(11):529-47; C.C. Gotay, P.H. Phillips, B.D. Cheson, "Male-female differences in the impact of cancer therapy," *Oncology,* Feb 1993, 7(2):67-74; S.K. Keitt, C.R. Wagner, C. Tong, S.A. Marts, "Understanding the biology of sex and gender differences: using subgroup analysis and statistical design to detect sex differences in clinical trials," *MedGenMed,* Jun 2003, 5(2):39.

[51] J.T. Caroscio, M.N. Mulvihill, R. Sterling, B. Abrams, "Amyotrophic lateral sclerosis: its natural history," *Neurol Clin,* Feb 1987, 5(1):1-8; J.F. Kurtzke, "Risk factors in amyotrophic lateral sclerosis," *Advances in Neurology,* 1991, 56:245-70; L.M. Nelson, "Epidemiology of ALS," *Clin Neurosci,* 1995-96, 3(6):327-51.

[52] These statistics are based on somewhat incomplete data - not all published clinical trials

provide details on sex distribution between the treatment and control groups.

[53] These measures are important not only for ethical reasons, but also for practical ones – in a disease with a limited patient population and an even more limited population of eligible, willing trial participants, larger trials tie up unnecessary clinical research resources in testing treatments that may eventually prove futile.

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[55] The proportion of clinical trials assuming a viral etiology in the late 1970's may be overexaggerated due to the relatively small number of trials published in that time period (five trials, out of which three tested anti-viral agents.)

[56] D.B. Drachman, R.W. Kuncl, "Amyotrophic lateral sclerosis: an unconventional autoimmune disease?" *Ann Neurol,* Aug 1989, 26(2): 269-74.

[57] W.A. Bijlsma, F.G. Jennekens, P. Schotman, W.H. Gispen, "Neurotrophic factors and regeneration in the peripheral nervous system," *Psychoneuroendocrinology,* 1984, 9(3):199-215.

[58] A. Doble, "The role of excitoxicity in neurodegenerative disease: implications for therapy," *Pharmacol Ther,* Mar 1999, 81(3):163-221.

[59] L. Brighina, G. Sala, C. Ceresa, L. Tremolizzo, C. Ferrarese, "Recent advances in the therapy of amyotrophic lateral sclerosis: focus on excitotoxicity," *Funct Neurol,* 2001, 16(Suppl 4):189-202.

[60] D.R. Rosen, T. Siddique, D. Patterson, et al., "Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis," *Nature,* Mar 1993, 362(6415):59-62.

[61] N. Delanty, M.A. Dichter, "Oxidative injury in the nervous system," *Acta Neurol Scand,* Sep 1998, 98(3):145-153.

[62] That is, it takes at least 2 to 4 years before promising pre-clinical studies can be translated into a completed, published clinical investigation. Although no formal analysis has been conducted, this estimate is upheld by the timelines described in most of the clinical trials cited in this report.

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[66] A. Plaitakis, J.T. Caroscio, "Abnormal glutamate metabolism in amyotrophic lateral sclerosis," *Ann Neurol,* Nov 1987, 22(5):575-9; A. Plaitakis, E. Constantakakis, J. Smith, "The neuroexcitotoxic amino acids glutamate and aspartate are altered in the spinal cord and brain in amyotrophic lateral sclerosis," *Ann Neurol,* Sep 1988, 24(3):446-9; T.L. Munsat, D. Hollander, "Excitotoxins and amyotrophic lateral sclerosis," *Therapie,* May-Jun 1990, 45(3): 277-9.

[67] R.G. Miller, J.D. Mitchell, D.H. Moore, "Riluzole for amyotrophic lateral sclerosis (ALS) / motor neuron disease (MND)", *Cochrane Database Syst Rev,* 2001(4):CD001447.

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[69] S.S. Jordan, "Double blind cross over trial with deprenyl in amyotrophic lateral sclerosis," *J Neural Transm Suppl*, 1994, 41: 237-41; L. Mazzini, et al., "An open-randomized clinical trial of selegiline in amyotrophic lateral sclerosis," *J Neurol*, 1994, 241(4):223-7; D.J. Lange, et al., "Selegiline is ineffective in a collaborative double-blind, placebo-controlled trial for treatment of amyotrophic lateral sclerosis," *Arch Neurol*, 1998, 55(1):93-6.

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APPENDIX B: CLINICAL TRIAL SUMMARIES, 1965-2005

Jörg Baumann.

Results of treatment of certain diseases of the central nervous system with ACTH and corticosteroids.

Acta Neurologica Scandinavia, 1965, 13(Suppl 2): 453 - 461.

Hypothesis/Rationale: The authors were testing the ability of the "antiphlogistic, antiallergic and antitoxic components of the corticosteroids" to reverse the symptoms of diseases characterized by inflammation of the central nervous system. This study followed a series of late 1950s and early 1960s reports of varying (and often anecdotal) success in using ACTH and/or corticosteroids to treat multiple sclerosis, myasthenia gravis, and other similar disorders. The study included primarily multiple sclerosis patients, but did include three ALS patients along with a handful of patients diagnosed with other neurological diseases as controls in a study. Amyotrophic lateral sclerosis patients were thus included in the study for variety, not as a primary target of study.

Location of Study: Finland

Study Results: **No benefit**

The treatment regimen had no apparent effect on the disease.

Study Design	
Length	2 – 6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	3
Treatment	3
Control	0
% Male	66%

Dosing	
Treatment(s)	ACTH, prednisolone
Dose(s)/Schedule	100 - 120 unit intramuscular loading dose, followed by either 20 units intramuscularly twice a week or 10-15 mg prednisolone daily

Dorman JD, Engel WK, Fried DM.

Therapeutic trial in amyotrophic lateral sclerosis

JAMA, 1969, 209(2): 257 - 8.

Hypothesis/Rationale: The authors attempted to confirm earlier case reports that Vitamin and pancreatic extract were effective in treating ALS.

Location of Study: Bethesda, MD

Study Results: **No benefit.**
All patients continued to decline, and the authors could not perceive any reductions in the rate of decline in these patients.

Study Design	
Length	7 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	✓
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	12
Treatment	12
Control	0

Dosing	
Treatment(s)	Pancreatic extract (Viokase) and DL-alpha tocopherol (Aquasol E)
Dose(s)/Schedule	6.3 gm Viokase/day and 1500 IU Aquasol E/day oral

Liversedge LA, Swinburn WR, Yuill GM.

Idoxuridine and motor neurone disease

Br Med J, 1970, 1(698): 755 - 6.

Hypothesis/Rationale: The authors tested the efficacy of idoxuridine, a then-novel anti-viral agent, in an attempt to probe the possible viral etiology of ALS. The authors acknowledged that despite widespread hypothesizing, there was little factual rationale at the time for assuming a viral etiology of ALS.

Location of Study: U.K.

Study Results: **No benefit.**
The treatment had no influence on the progression of disease.

Study Design	
Length	2 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	10
Treatment	10
Control	0
% Male	20%

Dosing	
Treatment(s)	Idoxuridine
Dose(s)/Schedule	1.5 g/day IV over three hours for 5 days

Mendell JR, Chase TN, Engel WK.

Amyotrophic lateral sclerosis: a study of central monoamine metabolism and therapeutic trial of levodopa.

Archives of Neurology, 1971, 25(4): 320 - 5.

Hypothesis/Rationale: The authors attempted to use a pilot therapeutic trial of levodopa in ALS to confirm their earlier molecular findings that suggested central dopamine synthesis could be diminished in ALS. The trial was based on both dramatic results in the use of levodopa to treat Parkinson's disease, and on the authors' own molecular studies of ALS patients, which showed dimished levels of homovanilic acid (a dopamine catabolite) in the CSF of ALS patients.

Study Results: **No benefit.**

The authors observed no clinical effect of dopamine treatment on the ten treated ALS patients.

Study Design	
Length	2 – 9 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	10
Treatment	10
Control	0

Dosing	
Treatment(s)	Levodopa
Dose(s)/Schedule	0.25 to 0.5 gm/day to start, increased to a maximum of 6 to 7 gm/day over the course of several weeks

Percy AK, Davis LE, Johnston DM, Drachman DB.

Failure of isoprinosine in amyotropic lateral sclerosis.

New England Journal of Medicine, 1971, 285(12): 689.

Hypothesis/Rationale: The authors attempted to confirm a previously reported case study in which isoprinosine had a positive therapeutic effect in a single patient. Isoprinosine was considered as a potential therapeutic agent for its anti-viral properties.

Location of Study: Baltimore, MD

Study Results: **No benefit**.
Isoprinosine had no measurable effect on clinical parameters.

Study Design	
Length	4 – 6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	14
Treatment	9
Control	5
Data collected	Muscle strength evaluation, electromyography

Dosing	
Treatment(s)	Isoprinosine
Dose(s)/Schedule	4 g/day, increased to 6 g/day after 6 weeks

Fareed GC, Tyler HR.

The use of isoprinosine in patients with amyotrophic lateral sclerosis.

Neurology, 1971, 21(9): 937 - 40.

Hypothesis/Rationale: The authors attempted to confirm single case study reports (published in both medical journals and the lay press) that isoprinosine was effective in treating two ALS patients. Isoprinosine was considered as a potential therapeutic agent for its anti-viral properties.

Location of Study: Boston, MA

Study Results: **No benefit.**

Isoprinosine had no measurable clinical effect of the progression of ALS.

Study Design	
Length	3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	25
Treatment	25
Control	25
Data collected	Muscle testing, inspiratory and expiratory chest films, recordings of clinical examinations, chemical analyses of patients' blood and urine..

Dosing	
Treatment(s)	Isoprinosine
Dose(s)/Schedule	3 - 6 gm/day

Brody JA, Chen KM, Yase Y, Holden EM, Morris CE.

Inosiplex and amyotrophic lateral sclerosis. Therapeutic trial in patients on Guam.

Archives of Neurology, 1974, 30(4): 322 - 3.

Hypothesis/Rationale: The authors attempted to either confirm or refute an earlier trial which showed inosiplex to have no effect on ALS.Inosiplex had at the time been shown to be effective as an antiviral agent for both DNA and RNA viruses. The possible viral etiology of ALS was a popular hypothesis at the time.

Location of Study: Bethesda, MD

Study Results: **No benefit.**

Inosiplex had no statistically significant effect on the progression of ALS in the patients studied.

Study Design	
Length	24 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	21
Treatment	13
Control	8

Dosing	
Treatment(s)	Inosiplex
Dose(s)/Schedule	3 gm/day for the first week and 4gm/day

Norris FH Jr, Calanchini PR, Fallat RJ, Panchari S, Jewett B.

The administration of guanidine in amyotrophic lateral sclerosis.

Neurology, 1974, 24(8): 721 - 8.

Hypothesis/Rationale: The authors attempted to verify their own earlier anecdotal observations regarding possible dramatic effects of guanidine in slowing or stabilizing the progression of ALS.The exact mechanism of action of guanidine was unclear at the time of the study, despite several decades of inquiry into the molecule.

Location of Study: San Francisco, CA

Study Results: **Possible benefit.**

Patients in the controlled study receiving the higher dosage of guanidine appeared to have a better clinical status at six months versus the low-dose group, and also appeared to have lower mortality rates at the nine to ten month mark. However, this data and a smattering of apparent cases of arrested progression proved difficult to interpret in light of diagnostic uncertainty and emerging knowledge on inter- and intra-individual variation in disease progression.

Study Design	
Length	6 – 24 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III
Add'l comments	The authors conducted one uncontrolled study with 84 patients and one controlled study with 24 patients in which low doses of guanidine served as the placebo.

Study Participant Demographics	
Total Participants	108
Treatment	95
Control	13
Control selection	Selected from practice based on age and sex matched patients who did not receive guanidine or physical therapy
% Male	58.3%

Dosing	
Treatment(s)	Guanidine
Dose(s)/Schedule	5 - 40 mg/kg/day

Comments/Analysis:

Olson WH, Simons JA, Halaas GW.

Therapeutic trial of tilorone in ALS: lack of benefit in a double-blind, placebo-controlled study.

Neurology, 1978, 28(12): 1293 - 5.

Hypothesis/Rationale: The authors attempted to test the therapeutic efficacy of tilorone in light of recent theories on a possible viral etiology of ALS.Tilorone had been shown at the time to be active against a range of DNA and RNA viruses in animals, and had minimal toxicity in humans, making it an attractive treatment with which to probe possible therapies for ALS and verify a possible viral etiology.

Location of Study: Fargo, ND

Study Results: **No benefit.**

There was no difference in outcome between the two groups. If anything, the placebo group appeared, at least on a supericial level, to fare better during the course of the study. However, the authors attributed this to differences in average duration of disease between the two groups.

Study Design	
Length	24 – 48 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	16
Treatment	8
Control	8
% Male	75%

Dosing	
Treatment(s)	Tilorone
Dose(s)/Schedule	1 gm/wk

Maida E, Gerstenbran F, Grundig E, Binder H.

Über die Anwendung von Guanidinhydrochlorid in der Behandlung degenerativer Nerven- un Muskelerkrankungen.

[On the application of guanidine hydrochloride to the treatment of degenerative nervous and muscular diseases.]

C, *Wien Klin Wochenschr*, 1978, 90(2): 43 - 56.

Location of Study: Germany

Study Design	
Length	0 - 0
Purpose:	
	0 Safety Dose-ranging Pharmacokinetic/molecular Efficacy
Controlled?	
Crossover?	0
Placebo?	
Randomized?	
Double-blind?	0
Blind?	
Phase?	
	0 Pilot Phase I Phase II Phase III
Add'l comments	

Study Participant Demographics	
Total Participants	
Treatment	0
Control	0
Control selection	
% Male	0
Data collected	

Dosing	
Treatment(s)	Guanidine hydrochloride
Dose(s)/Schedule	20 - 40 mg/kg/day

Study Results:

Comments/Analysis:

Monstad I, Dale I, Petlund CF, Sjaastad O.

Plasma exchange in motor neuron disease - a controlled study

Journal of Neurology, 1979, 221(1): 59 - 66.

Hypothesis/Rationale: Assuming that an unidentified factor in the sera of ALS patients played a role in perpetuating the disease, the authors attempted to test whether the removal of this factor could influence to course of the disease. Earlier studies had shown that the serum of ALS patients had a toxic effect on myelinated CNS fibers and on anterior horn cells in vitro.

Location of Study: Norway

Study Results: **No benefit.**

The rate of deterioration was approximately the same in treatment and control groups.

Study Design	
Length	6 – 15 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	14
Treatment	7
Control	7
% Male	85.7%
Data collected	Muscular power, tests for motor speed, coordination, pulmonary function

Dosing	
Treatment(s)	Plasma exchange
Dose(s)/Schedule	Plasma exchange was performed weekly

Olarte MR, Gersten JC, Zabriskie J, Rowland LP.

Transfer factor is ineffective in amyotrophic lateral sclerosis.

Annals of Neurology, 1979, 5(4): 385 - 8.

Hypothesis/Rationale: The use of transfer factor in ALS was based on hypotheses that ALS might be due to a persistent or slow-acting viral infection.At the time, transfer factor was a substance of unknown nature in white blood cells that had been shown to confer lasting immunity when transferred from immune to nonimmune subjects. (Today, transfer factor is primarily used in alternative medicine.)

Location of Study: New York, NY

Study Results: **No benefit.**

No significant difference between the two groups. No evidence of therapeutic value for transfer factor.

Study Design	
Length	2 – 26 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	28
Treatment	16
Control	12
% Male	65.2%
Data collected	Norris score

Dosing	
Treatment(s)	Transfer factor

Norris FH Jr., Sachais B, Carey M.

Trial of baclofen in amyotrophic lateral sclerosis

Archives of Neurology, 1979, 36(11): 715 - 6.

Hypothesis/Rationale: The authors tested the ability of baclofen to reduce short-term spasticity and/or alter the long-term progression of ALS.Baclofen had been shown to have anti-spastic effects in a number of other diseases, including multiple sclerosis.

Location of Study: San Francisco, CA

Study Results: **Possible benefit.**

There were no statistically significant differences between treatment and placebo groups, although a small subset of patients appeared to benefit from long-term treatment with baclofen, with baclofen showing a pronounced anti-spastic effect on two of these patients.

Study Design	
Length	2 – 6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	20
Treatment	9
Control	11
Data collected	Norris score

Dosing	
Treatment(s)	Baclofen
Dose(s)/Schedule	80 mg/day

Rivera VM, Grabois M, Deaton W, Breitbach W, Hines M.

Modified snake venom in amyotrophic lateral sclerosis - lack of clinical effectiveness.

Archives of Neurology, 1980, 37(4): 201 - 3.

Hypothesis/Rationale: The authors tested Sanders' and Fellows' 1975 hypothesis that detoxified snake venom might have a therapeutuc effect on ALS through inducing the synthesis of an interferon-like substance.Modified neurotoxin from cobra and krait venom had shown positive therapeutic effects on poliomyelitis (in monkeys), ocular herpes simplex, and pseudorabies (in rabbits.)

Location of Study: Houston, TX

Study Results: **No benefit.**

The authors observed no benefit in the patients who received treatment. Transient periods of improvement were more common in patients who received placebo.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	31
Treatment	14
Control	17
Data collected	Self assessment, neurological score (105 pt scale), manual testing of 13 muscle grous, quantitative EMG, videotape esophagrams, FVC, FEV.

Dosing	
Treatment(s)	Snake venom
Dose(s)/Schedule	1.3 ml IM every other day

Olarte MR, Schoenfeldt RS, McKiernan G, Rowland LP.

Plasmapheresis in amyotrophic lateral sclerosis

Annals of Neurology, 1980, 8(6): 644 - 5.

Hypothesis/Rationale: The authors hoped to establish whether or not circulating factors play a role in amyotrophic lateral sclerosis by testing the effect of plasmapheresis on disease progression.The study was based on earlier studies that showed plasmapheresis was effective in treating myasthenia gravis. The rationale for extrapolating a possible therapeutic effect for amyotrophic lateral sclerosis was based on studies that suggested a possible immune component in ALS, including studies that showed deposits of immune complexes in renal glomeruli, excessive serum complement consumption, and toxic effects of ALS patients' sera on cultured neurons.

Location of Study: New York, NY

Study Results: **No benefit.**

The authors observed no long-range benefit from treatment, nor were any clinical changes - positive or negative - observed immediately after individual treatments.

Study Design	
Length	1 – 6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	✓ Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	10
Treatment	10
Control	0
Data collected	Norris score

Dosing	
Treatment(s)	Plasmapheresis
Dose(s)/Schedule	2 liters plasma exchange with frequency ranging from 4 treatments in 7 days to 15 treatments over 180 days.

Munsat TL, Easterday CS, Levy S, Wolff SM, Hiatt R.

Amantadine and guanidine are ineffective in ALS.

Neurology, 1981, 31(8): 1054 - 5.

Hypothesis/Rationale: The authors were testing the effectiveness of amantadine and guanidine.Amantidine had shown effectiveness in Creutzfeld-Jacob disease, a neurological disorder thought at the time to have a viral etiology. The choice of guanidine as a treatment was based on anecdotal reports of efficacy.

Location of Study: San Francisco, CA

Study Results: **No benefit.**

Neither drug had any measurable effect on the clinical parameters measured.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	20
Treatment	20
Control	20
Data collected	68-item clinical evaluation of respiratory, oropharyngeal, gait, arm, and leg function

Dosing	
Treatment(s)	Amantadine, guanidine
Dose(s)/Schedule	25 mg/kg/day guanidine OR 100 mg 3x/day amantidine

Kelemen J, Hedlund W, Murray-Douglas P, Munsat TL.

Lecithin is not effective in amyotrophic lateral sclerosis

Neurology, 1982, 32(3): 315 - 6.

Hypothesis/Rationale: The authors tested the efficacy of lecithin in treating ALS.Lecithin had been used successfully to treat a variety of neurological disorders. The authors believed lecithin to be of interest in ALS for its role both as a precursor of acetylcholine and as an essential component in membrane synthesis.

Location of Study: Boston, MA

Study Results: **No benefit.**

There was no percetible difference in outcome between the treatment and control groups.

Study Design	
Length:	2 – 13 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	21
Treatment	10
Control	11
Data collected	Maximal isometric strength, grip strength, FVC, maximal voluntary ventilation

Dosing	
Treatment(s)	Lecithin
Dose(s)/Schedule	20 gm/day in 3 doses

Conradi S, Ronnevi LO, Nise G, Versterberg O.

Long-time penicillamine-treatment in amyotrophic lateral sclerosis with parallel determination of lead in blood, plasma, and urine

Acta Neurologica Scandinavia, 1982, 65(3): 203 - 11.

Hypothesis/Rationale: The authors tested whether penicillamine, a chelating agent used in the treatment of lead poisoning, had an affect on either the clinical course of ALS or on measurable levels of lead in patients' blood, plasma, and urine.Previous studies had suggested that overexposure to lead and other heavy metals might play a role in ALS. The authors hypothesized that ALS might involve an abnormal updake of lead in motor neurons.

Location of Study: Sweden

Study Results: **No benefit.**

There was no measurable effect on the disease. In one case, the onset of treatment coincided with an increase in the rate of progression and, ultimately, death.

Study Design	
Length	6 – 12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	6
Treatment	6
Control	0
Data collected	Subjective clinical status, levels of lead in blood, plasma, urine

Dosing	
Treatment(s)	Penicillamine
Dose(s)/Schedule	0.15 g 2x/day for first week, then 0.30 g 2x/wk

Engel WK, Siddique T, Nicoloff JT.

Effect on weakness and spasticity in amyotrophic lateral sclerosis of thyrotropin-releasing hormone.

Lancet, 1983, 2(8341): 73 - 5.

Hypothesis/Rationale: The authors tested the short-term effects of thyrotropin-releasing hormone on muscle strength and to test the side effects of IV infusions of TRH at a range of doses.The authors had long held the hypothesis that ALS was caused by a metabolic defect. Thyrotropin-releasing hormone, with its known trophic effects in animals, was a promising treatment with which to test this hypothesis.

Location of Study: Los Angeles, CA

Study Results:

Short-term benefit.

The authors observed moderate but marked improvement of both weakness and spasticity, sustained through infusion and for 1 hr afterward. Slight improvement was sometimes observed up to 20 hours after infusion. Both the benefits and side-effects appeared to be more evident in men than women.

Study Design	
Length	< 1 month
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	17
Treatment	17
Control	0
% Male	70.5%

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	2-19 mg/min

Kelemen J, Hedlund W, Orlin JB, Berkman EM, Munsat TL.

Plasmapheresis with immunosuppression in amyotrophic lateral sclerosis

Archives of Neurology, 1983, 40(12): 752 - 3.

Hypothesis/Rationale: Attempted to confirm Norris et al.'s 1979 report that plasma removal was effective in ALS, hypothesizing that follow-up trials had failed to confirm this effect because they did not include concomitant immunosuppression.

Location of Study: Boston, MA

Study Results: **No benefit.**

The authors observed no therapeutic benefit of plasmapheresis on the progression of ALS.

Study Design	
Length	6 – 13 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	8
Treatment	4
Control	4

Dosing	
Treatment(s)	Plasmapheresis, azathroprine
Dose(s)/Schedule	2 mg/kg azathioprine starting one week prior to apheresis procedures. Apheresis (removal of at least 2 liters of plasma during each procedure) occurred three times per week for two weeks, then once per week for three months.

Bradley WG, Hedlund W, Cooper C, Desousa GJ, Gabbai A, Mora JS, Munsat TL, Scheife R..

A double-blind controlled trial of bovine brain gangliosides in amyotrophic lateral sclerosis.

Neurology, 1984, 34(8): 1079 - 82.

Hypothesis/Rationale: The authors investigated whether exogenous gangliosides could induce neuronal regeneration in ALS.Interest in gangliosides as a potential treatment for ALS was predicated mainly on their observed impact on various neuronal cellular processes (including membrane excitability, enzyme activity, and receptor function) and its observed trophic effects in vitro.

Location of Study: Boston, MA

Study Results: **No benefit.**

The authors observed no significant improvements and no significant differences between the treatment and placebo groups.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	40
Treatment	19
Control	21
Data collected	120 clinical and electrophysiologic parameters of neuromuscular function

Dosing	
Treatment(s)	Gangliosides
Dose(s)/Schedule	40 mg/d IM

Imoto K, Saida K, Iwamura K, Saida T, Nishitani H..

Amyotrophic lateral sclerosis: a double-blind crossover trial of thyrotropin-releasing hormone.

Journal of Neurology, Neurosurgery, & Psychiatry, 1984, 47(12): 1332 - 4.

Hypothesis/Rationale: The authors attempted to confirm Engel et al. (1983) and Yamane et al.'s (1984) clinical studies which suggested that thyrotropin-releasing hormone had a positive therapeutic impact on ALS. Thyrotropin-releasing hormone had been shown to have trophic effects on motor neurons.

Location of Study: Japan

Study Results: **No benefit.**

Half of the patients reported subjective improvement, but objective evaluation failed to demonstrate the therapeutic effectiveness of TRH at this dosage.

Study Design	
Length	< 1 month
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	✓
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	6
Treatment	6
Control	6

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	5 mg IM

Bradley WG.

Double-blind controlled trial of purified brain gangliosides in amyotrophic lateral sclerosis and experience with peripheral neuropathies.

Advances in Experimental Medicine & Biology, 1984, 174: 565 - 73.

Hypothesis/Rationale: The authors were interested in gangliosides (a series of complex glycolipids found in neuronal cell membranes) because of their purported ability to enhance axonal regeneration and reinnervation of denervated muscles after injury.

Location of Study: Burlington, VT

Study Results: **Possible benefit.**

Although there were no consistent statistically significant differences between the treatment and control groups, three patients receiving treatment improved over the course of the study while no patients receiving placebo appeared to improve.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	40
Treatment	19
Control	21
Data collected	70 tests of neurological function

Dosing	
Treatment(s)	Gangliosides
Dose(s)/Schedule	40 mg/day IM

Harrington H, Hallett M, Tyler HR.

Ganglioside therapy for amyotrophic lateral sclerosis: a double-blind controlled trial.

Neurology, 1984, 34(8): 1083 - 5.

Hypothesis/Rationale: The study investigated whether or not ganglioside therapy had the potential to impact the progression of ALS. Recent studies had shown that exogenous gangliosides could be incorporated into nerve cell membranes and might promote nerve regeneration. Based on these studies and positive results in treating human diabetic and alcoholic neuropathies, the authors and others hypothesized that gangliosides might help slow or reverse the symptoms of ALS by acting as neurotrophic factors.

Location of Study: Boston, MA

Study Results: **No benefit.**

The study failed to demonstrate any significant difference between the two groups in the progression of muscle weakness.over the course of the disease.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	32
Treatment	18
Control	14
Data collected	10 different objective tests of muscle strength

Dosing	
Treatment(s)	Gangliosides
Dose(s)/Schedule	40 mg/d IM

Hallett M, Harrington H, Tyler HR, Flood T, Slater N.

Trials of ganglioside therapy for amyotrophic lateral sclerosis and diabetic neuropathy.

Advances in Experimental Medicine and Biology, 1984, 174: 575 - 9.

Hypothesis/Rationale: Gangliosides were selected as a treatment because of studies that had suggested they increased axonal sprouting in reaction to a range of neuronal injuries. The authors hypothesized that this neuroprotective effect would help slow or reverse the effects of ALS.

Location of Study: Boston, MA

Study Results:

No benefit.

No significant improvements were observed in either the treatment or control groups. There was no perceptible difference in the rates of decline in either group.

Study Design	
Length	6 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	32
Treatment	18
Control	14

Dosing	
Treatment(s)	Gangliosides
Dose(s)/Schedule	40 mg/day daily muscular injection

Serratrice G, Desnuelle C, Guelton C, Meyer-Dutour A, Richard P, Braguer D, Crevat A.

Essai du facteur de libération de l'hormone thyréotrope dans la sclérose latérale amyotrophique.

[Trial of thyrotropin-releasing factor in amyotrophic lateral sclerosis.]

Presse Med, 1985, 14(8): 487 - 8.

Hypothesis/Rationale: The authors were attempting to confirm Engel (1983) and Munsat's (1984) earlier findings on the efficacy of TRH in ALS.Thyrotropin-releasing hormone had been shown to have neuromodulatory effects on motor neurons. This study was primarily an attempt to confirm earlier findings by American researchers.

Location of Study: France

Study Results: **Short-term benefit.**

The authors observed an objective improvement in muscle strength in four cases and an improvement in speech and swallowing in 2 (these measurements were taken immediately after individual injections of TRH.) These improvement were transitory, lasting for between 1 and 24 hours, and became less and less pronounced with repeat injections.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	8
Treatment	8
Control	0
Data collected	Muscle strength, other clinical observations (not discussed in detail)

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	0.5 to 2 ml TRH every 2 to 4 days for a total of between 3 and 6 injections

Olarte MR, Shafer SQ.

Levamisole is ineffective in the treatment of amyotrophic lateral sclerosis.

Neurology, 1985, 35(7): 1063 - 6.

Hypothesis/Rationale: The authors investigated whether levamisole, a drug with immunostimulatory properties, could affect the clinical course of ALS. A popular but unproven hypothesis at the time was that ALS was of viral origin. Levamisole had been successfully used as a T-cell stimulator in a range of other diseases or viral or immune origin (including viral hepatitis, rheumatoid arthritis, spondyloarthropathies, aphthous stomatitis, recurrent herpes, candidiasis, and multiple sclerosis.)

Location of Study: New York, NY

Study Results: **No benefit.**

Levamisole had no effect on the rate of score decline in the 20 patients who completed the trial.

Study Design	
Length	6 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	20
Treatment	20
Control	20
Data collected	ALS score, blood chemistries

Dosing	
Treatment(s)	Levamisole
Dose(s)/Schedule	150 mg oral

Caroscio JT, Cohen JA, Zawodniak J, Takai V, Shapiro A, Blaustein S, Mulvihill MN, Loucas SP, Gudesblatt M, Rube D, et al.

A double-blind, placebo-controlled trial of TRH in amyotrophic lateral sclerosis.

Neurology, 1986, 36(2): 141 - 5.

Hypothesis/Rationale: The authors conducted a placebo controlled trial of TRH in which patients' status (treatment or placebo) varied by dose; effects were measured on a per-treatment basis.Earlier reports of transient improvements in clinical parameters following treatment with TRH had no placebo-controlled element to them. By varying patients' treatment randomly from dose to dose, the authors hoped to be better able to probe the actual transient effects, if any, of TRH.

Location of Study: New York, NY

Study Results: **Possible benefit.**

Improvement was seen only in dynametric strength 1 hour after subcutaneous injection and one patient had an improvement in subjective speech testing during IV infusion of TRH. Otherwise, there was no significant difference between TRH and placebo doses.

Study Design	
Length	< 1 month
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	12
Treatment	12
Control	12
Data collected	Bulbar score, spinal score, upper motor neuron score, voice recordings, vital capacity, dynamometer score.

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	150 mg subcutaneous AND IV infusions of 500 mg at 72- to 96-hour intervals

Dalakas MC, Aksamit AK, Madden DL, Sever JL.

Administration of recombinant human leukocyte alpha 2-interferon in patients with amyotrophic lateral sclerosis

Archives of Neurology, 1986, 43(9): 933 - 5.

Hypothesis/Rationale: The authors attempted to demonstrate a possible efficacious effect of IFN-a on the clinical course of ALS, altering their treatment protocol from earlier unsuccessful studies to include pure (rather than partially purified) recombinant human interferon, and administering the interferon continuously (rather than intermittently) for four months. In the context of a possible viral etiology of ALS, interferon was of interest because of its potent antiviral effects.

Location of Study: Bethesda, MD

Study Results: **No benefit.**

The treatment was ineffective in improving, arresting, or slowing the progression of the disease.

Study Design	
Length	4 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	6
Treatment	6
Control	0
Data collected	Modified Norris ALS score, blood chemistries

Dosing	
Treatment(s)	rhIFN-a
Dose(s)/Schedule	2 million units 3x/wk

Mitsumoto H, Salgado ED, Negroski D, Hanson MR, Salanga VD, Wilber JF, Wilbourn AJ, Breuer AC, Leatherman J..

Amyotrophic lateral sclerosis: effects of acute intravenous and chronic subcutaneous administration of thyrotropin-releasing hormone in controlled trials.

Neurology, 1986, 36(2): 152 - 9.

Hypothesis/Rationale: The authors attempted to resolve debate over the clinical potential of TRH by conducting a double-blind, placebo-controlled crossover study of TRH in ALS. The choice of TRH was based on earlier reports of its effectiveness in improving muscle strength and reducing spasticity in ALS.

Location of Study: Cleveland, OH

Study Results: **No benefit.**

Ten patients receiving daily TRH noted subjective improvement without objective evidence. However, statistical analysis showed no beneficial effects from acute or chronic TRH administration versus the placebo period.

Study Design	
Length	3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	✓
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	41
Treatment	41
Control	41
% Male	51.2%
Data collected	Vital signs, respiratory function, neurologic function, muscle strength (manual & dynamometer), EMG

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	500 mg given intravenously at 1 week intervals (16) OR 25 mg/day subcutaneously (25)

Aquilonius SM, Askmark H, Eckernas SA, Gillberg PG, Hilton-Brown P, Rydin E, Stalberg E..

Cholinesterase inhibitors lack therapeutic effect in amyotrophic lateral sclerosis. A controlled study of physostigmine versus neostigmine.

Acta Neurologica Scandinavia, 1986, 73(6): 628 - 32.

Hypothesis/Rationale: The authors' choice of the cholinesterase inhibitors physostigmine and neostigmine was based on their hypothesis that cholinergic hypofunction played a key etiological role in ALS.A range of studies between 1977 and 1985 had suggested a disturbance of cholinergic function in the spinal cord was implicated in the disease processes of ALS.

Location of Study: Sweden

Study Results: **No benefit.**

The treatments appeared to have no effect on muscle strength or other physiological parameters.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	✓
Placebo?	
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	5
Treatment	5
Control	0
Data collected	Subjective patient assessments, maximal isometric strength, decremental response, reflexes, somatosensory evoked potentials

Dosing	
Treatment(s)	Physostigmine, neostigmine
Dose(s)/Schedule	10 mg/day oral physotigmine 45 mg/day neostigmine

Brooke MH, Florence JM, Heller SL, Kaiser KK, Phillips D, Gruber A, Babcock D, Miller JP..

Controlled trial of thyrotropin releasing hormone in amyotrophic lateral sclerosis.

Neurology, 1986, 36(2): 146 - 51.

Hypothesis/Rationale: The authors attempted to expand on earlier studies which suggested subcutaneous and IV TRH had beneficial effects in ALS. In this study, the drug was administered directly into patients' muscles. While earlier studies had attempted intramuscular delivery of TRH, these and other studies lacked placebo controls and in many cases delivered low (and possibly non-therapeutic) doses of the hormone.

Location of Study: St. Louis, MO

Study Results: **No benefit.**

The authors observed a termporary increase in the strength of some muscles following the administration of TRH, but there was no change in functional performance. The conclusion was that the course of the illness was not altered by treatment.

Study Design	
Length	2 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	30
Treatment	15
Control	15
% Male	66%
Data collected	Strength evaluation, functional ability, respiratory function

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	150 mg/day IM

Mora JS, Munsat TL, Kao KP, Finison LJ, Hedlund W, Bradley GA, Scheife R, Georgiades JA..

Intrathecal administration of natural human interferon alpha in amyotrophic lateral sclerosis.

Neurology, 1986, 36(8): 1137 - 40.

Hypothesis/Rationale: The authors investigated whether intrathecal administration of interferon could impact the progression of ALS.The choice of natural human interferon alpha was based on its observed anti-viral properties and assumed an as-yet-uncharacterizd viral etiology for ALS.

Location of Study: Boston, MA

Study Results: **Well tolerated. No benefit.**

There were no significant alterations in neuromuscular functional decline observed for the control (pre-study) vs treatment periods. The treatment was well-tolerated, and indomethacin and ibuprofen were able to block interferon side effects.

Study Design	
Length	2 – 6 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	6
Treatment	6
Control	0
% Male	33.3%
Data collected	TQNE, blood chemistry

Dosing	
Treatment(s)	Interferon Alpha
Dose(s)/Schedule	1 million units/wk

Kaplan MM, Taft JA, Reichlin S, Munsat TL.

Sustained rises in serum thyrotropin, thyroxine, and triiodothyronine during long term, continuous thyrotropin-releasing hormone treatment in patients with amyotrophic lateral sclerosis

Journal of Clinical Endocrinology & Metabolism, 1986, 63(4): 808 - 14.

Hypothesis/Rationale: The authors investigated the effect of long-term, high-dose TRH on both clinical progression andpituitary and thyroid function in ALS patients. This article reports only the authors' data on changes in the hormones regulated by TRH, not the outcomes of the neuromuscular evaluations.Previous studies had been inconclusive on whether TRH was of benefit in ALS. The authors believed the difficulty in assessing TRH arose from the fact that it did not freely cross the blood brain barrier (earlier studies had delivered TRH intravenously) and believed a study with continuous infusion of TRH directly into the CSF would be better designed to detect any positive effect of TRH.

Location of Study: Boston, MA

Study Results: **Inconclusive.**

The authors observed that TRH caused modest, sustained rises in serum TSH and thyroid hormones, but could not draw any conclusions regarding any possible adverse consequences these changes might cause in thyroid function.

Study Design	
Length	6 months
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	4
Treatment	4
Control	0
Data collected	Blood chemistry

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	3000 mcg/day via infusion pump, plus monthly 500 mcg IV bolus stimulation tests

Serratrice G, Desnuelle C, Crevat A, Guelton C, Meyer-Dutour A.

Traitement de la sclérose latérale amyotrophique par le facteur de libération de l'hormone thyréotrope (TRH)

[Treatment of amyotrophic lateral sclerosis with thyrotropin-releasing hormone.]

Rev Neurol, 1986, 142(2): 133 - 139.

Hypothesis/Rationale: The authors tested the effects of sequential IV administration of TRH in ALS.The authors' interest in TRH was based not only on its purportedly neurotrophic properties, but also on the debate in the scientific literature over the proper dosing route. While most debates focused on IV vs IT delivery, the authors believed that alterations in the dosing schedule and rate of infusion for IV TRH could alleviate issues with achieving therapeutic efficacy.

Location of Study: France

Study Results: **Possible benefit.**

The authors observerd a statistically significant improvement in muscle strength in nine patients by five infusions over a 4-week period, and 5 patients at 8 infusions over a 10 week period.

Study Design	
Length	2 – 3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	7
Treatment	7
Control	0
% Male	42.9%
Data collected	Muscle strength before and 24 post infusion, objective/subjective assessment of spastificty

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	240 mg TRH intravenously over 1 hr every 2 weeks

Norris FH, Denys EH, Fallat RJ.

Trial of octacosanol in amyotrophic lateral sclerosis.

Neurology, 1986, 36(9): 1263 - 4.

Hypothesis/Rationale: The authors attempted to verify anecdotal reports of the efficacy of octacosanol in ALS.The choice of octacosanol was based on promising results of a trial of the drug in Parkinson's disease, and on anecdotal reports of effectiveness in treating ALS.

Location of Study: San Francisco, CA

Study Results: **No benefit.**

There were no measurable differences in status between the treatment and placebo periods in any of the neurophysiological parameters measured.

Study Design	
Length	3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	11
Treatment	11
Control	11
% Male	90.9%
Data collected	ALS score, MVV

Dosing	
Treatment(s)	Octacosanol
Dose(s)/Schedule	40 mg/day

Pinelli P, Mazzini L, Mora G, Pisano F, Villani A.

A follow-up electromyographic investigation of ALS patients treated with high dosage gangliosides.

Advances in Experimental Medicine & Biology, 1987, 209: 285-91.

Hypothesis/Rationale: The choice of brain gangliosides was based on earlier studies which suggested a possible benefit in ALS. The authors hoped that studying electromyographic parameters might help them better elucidate what effects, if any, gangliosides have on ALS.

Location of Study: Italy

Study Results: **Possible benefit.**
Gangliosides appeared to improve the body's own compensatory mechnanisms during the early stages of the disease and temporarily reduce the frequency of fasciculations.

Study Design	
Length	3 – 12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	0 Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	33
Treatment	26
Control	7
% Male	60%
Data collected	Kinetic and electromyographic data, including fasciculations, variability in action potential, and EMG of maximal voluntary effort.

Dosing	
Treatment(s)	Gangliosides
Dose(s)/Schedule	100 mg/day IM

Study Results:

Provinciali L, Giovagnoli AR, Di Bella P, Baroni M, Dallantonio R.

A therapeutic trial of thymic factor in amyotrophic lateral sclerosis

Advances in Experimental Medicine & Biology, 1987, 209: 293 - 296.

Hypothesis/Rationale: The authors were interested in thymic factor for its role in replacing deficits in cell-mediated immunity in a range of immunodeficiency and viral disease.

Location of Study: Italy

Study Results: **No benefit.**

There was no perceptible benefit of treatment with thymic factor over the course of the study compared to placebo.

Study Design	
Length	4 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	17
Treatment	9
Control	8
% Male	71%
Data collected	Modified Norris score

Dosing	
Treatment(s)	Thymic factor
Dose(s)/Schedule	100 mg 1x/day for first 20 days, followed by 50 mg 1x/day next 20 days, and 50 mg 1x/wk for last 20 days

de Jong JM, den Hartog Jager WA, Vyth A, Timmer JG.

Attempted treatment of motor neuron disease with N-acetylcysteine and dithiothreitol

Advances in Experimental Medicine & Biology, 1987, 209: 277 - 280.

Hypothesis/Rationale: Both N-acetylcysteine and dithiothreitol had been shown to prevent the development of amyotrophic lateral sclerosis in a Vitamin C-deficient guinea pig model of the disease.

Location of Study: Netherlands

Study Results: **Possible benefit.**

The authors reported a stabilization of more than 60% of all cases at the 6 month mark, but this data was not analyzed statistically with regard to patients' clinical history, the natural history of ALS, or control data.

Study Design	
Length	3 – 24 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	40
Treatment	40
Control	0

Dosing	
Treatment(s)	N-acetylcysteine (NAC), dithiothreitol (DTT)
Dose(s)/Schedule	50-100 ml 5% NAC/day subcutaneously, plus 26 patients received 250 mg DTT/day orally

Brooks BR, Sufit RL, Montgomery GK, Beaulieu DA, Erickson LM..

Intravenous thyrotropin-releasing hormone in patients with amyotrophic lateral sclerosis. Dose-response and randomized concurrent placebo-controlled pilot studies.

Neurological Clinics, 1987, 5(1): 143 - 58.

Hypothesis/Rationale: The authors extended on earlier investigations of TRH, which were conducted for short periods of time and had inconclusive and contradictory results. Thyrotropin-releasing hormone was of significant interest in ALS for its neurotrophic properties. TRH increased motor neruon function in animals and increased the likelihood of recovery in an animal model of spinal cord trauma.

Location of Study: Madison, WI

Study Results: **Clear benefit.**

The authors recorded a statistically significant effect of TRH on jaw muscle strength, and a positive effect on wrist and thumb flexor strength that did not reach statistical significance. Patients receiving placebo treatment had a statistically significant decline in forced vital capacity versus TRH treated patients. There was also a statistically significant difference in TRH-treated patients' continued ability to complete the "arising from chair" test of lower extremity function, versus a loss of this ability in placebo-treated patients. All patients receiving TRH treatment experience improvement in strength of more than two standard deviations in one to five muscles, while only half of the patients in the placebo group improved by a similar amount in no more than 2 muscles. This difference approached statistical significance.

Study Design	
Length:	2 – 3 months
Purpose:	
✓	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	19
Treatment	12
Control	7
Data collected	MVC, FVC, MVV, Neuromuscular Evaluation Grading Scale, oxygen consumption

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	10 mg/kg IV every other day

Munsat TL, Taft J, Jackson IM.

Pharmacokinetics of intrathecal thyrotropin-releasing hormone

Neurology, 1987, 37(4): 597 - 601.

Hypothesis/Rationale: The authors investigated the pharmacokinetics of intrathecally-administered thyrotropin-releasing hormoneEarlier studies had revealed difficulties in achieving effective CSF concentrations of TRH through peripheral administration of the peptide. The authors investigated an intrathecal route of administration and the attendant pharmacodynamics of the TRH administered.

Location of Study: Boston, MA

Study Results:

Pharmacokinetics.

Elimination half-life was 54 minutes. During a 2-hour infusion 2.75% crossed CSF/blood-brain barrier and entered the bloodstream. Mean CSF steady state concentrations ranged from 2.42 to 2.88 micrograms/ml. There was little change in the pharmacokinetics of TRH over time in patients treated chronically for up to 12 months. The observed half-life suggested to the authors that single bolus IT administration of TRH might not be an effective route of delivery.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
✓	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
✓	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	15
Treatment	15
Control	0
Data collected	Blood and CSF chemistry

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	3 mg/d intrathecal infusion

Thielen T, Stober T, Schimrigk K.

Therapeutic trial of intrathecal thyrotropin-releasing hormone (TRH) and a TRH-analogue in amyotrophic lateral sclerosis

Advances in Experimental Medicine & Biology, 1987, 209: 304 - 8.

Hypothesis/Rationale: Earlier studies had observed both neurotrophic effects of TRH and decreased levels of TRH in the anterior horn regions and cerebrospinal fluid of ALS patients.

Location of Study: West Germany

Study Results: **Possible benefit.**

Although none of the patients receiving TRH showed any improvement, the authors reported both transient and long-term improvement in a subset of patients receiving the TRH analogue.

Study Design	
Length	2 – 6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	11
Treatment	11
% Male	36%
Data collected	Muscle strength, vital capacity, atrophy

Dosing	
Treatment(s)	TRH, TRH analogue
Dose(s)/Schedule	1000 – 2500 mcg 1x-2x/wk , 6 – 24 mg/day (for four days)

Kuther G, Struppler A.

Therapeutic trial with N-acetylcysteine in amyotrophic lateral sclerosis

Advances in Experimental Medicine & Biology, 1987, 209: 281 - 284.

Hypothesis/Rationale: The authors were attempting to confirm earlier results which suggested N-acetylcysteine was beneficial in ALS patients.

Location of Study: West Germany

Study Results: **No benefit.**

Treatment with N-acetylcysteine did not appear to benefit patients or slow progression. The authors attributed this to differences in the drug delivery mechanism between their study and the study it attempted to replicate.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	11
Treatment	11
Control	0
% Male	55%
Data collected	

Dosing	
Treatment(s)	N-acetylcysteine
Dose(s)/Schedule	50 ml 5% N-acetylcysteine/day subcutaneously

Hawley RJ, Kratz R, Goodman RR, McCutchen CB, Sirdofsky M, Hanson PA.

Treatment of amyotrophic lateral sclerosis with the TRH analog DN-1417.

Neurology, 1987, 37(4): 597 - 601.

Hypothesis/Rationale: The authors tested the efficacy of DN-1417, a TRH analogue, in a pilot, open-label trial.The authors hypothesized that DN-1417 might have a greater impact on strength parameters in ALS than previously reported experiences with TRH, since DN-1417 appeared to be more targeted to impact the affected areas in ALS (e.g. anterior horn cells) and had fewer endocrine effects than TRH.

Location of Study: Washington, DC

Study Results: **No benefit.**

No objective improvement in strength was observed during the treatment period.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	9
Treatment	9
Control	0
Data collected	Muscle strength

Dosing	
Treatment(s)	TRH analogue
Dose(s)/Schedule	2 mg 2x/day

Appel SH, Stewart SS, Appel V, Harati Y, Mietlowski W, Weiss W, Belendiuk GW..

A double-blind study of the effectiveness of cyclosporine in amyotrophic lateral sclerosis.

Archives of Neurology, 1988, 45(4): 381 - 6.

Hypothesis/Rationale: The authors tested the efficacy of cyclosporine, an immunosuppressant, in slowing or reversing the clinical progression of ALS.Although the authors acknowledged that little laboratory data existed to support an autoimmune component in ALS, they believed the higher incidence of autoimmune diseases in families of ALS patients vs. the general population merited attempting to treat ALS with immunosuppressive therapies. Cyclosporine had been shown to be successful in a variety of transplant situation, and had also been used with some success in juvenile diabetes.

Location of Study: Houston, TX

Study Results: **Possible benefit.**

Cyclosporine appeared to benefit men with recent onset of disease at the time they entered the study (reducing their monthly rate of progression by almost a third), but appeared to be of little benefit to women or to patients with a diagnosis dating more than 18 months before study entry.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	74
Treatment	36
Control	38
Data collected	Survival, Appel ALS rating scale (composed of scores for muscle strength, in addition to bulbar, respiratory, lower extremity, and upper extremity function

Dosing	
Treatment(s)	Cyclosporine
Dose(s)/Schedule	10 mg/kg to start, adjusted downward as necessary to maintain whole blood concentrations of 400 to 600 ng/ml

Gueguen B, Puymirat J, Grouselle D, Piketty ML, Bleton JP, Bourdel MC, Rondot P.

Effets cliniques, électrophysiologiques et endocriniens de la TRH en perfusion à doses élevées dans la sclérose latérale amyotrophique.

[Clinical, electrophysiologic and endocrine effects of the perfusion of high doses of TRH in amyotrophic lateral sclerosis.]

Revue Neurologique, 1988, 144(11): 704 - 9.

Hypothesis/Rationale: The authors were attempted to help resolve debates over the clinical effect of TRH by observing quantitative clinical, electrophysiological, and hormonal changes induced by TRH.

Location of Study: France

Study Results: **No benefit.**

The authors reported that three patients noted subjective improvement in strength following the treatment period. However, clinical muscular testing and H response tests showed no change. The authors observed changes in prolactin, growth hormone, TSH and T3 serum levels of patients, and expressed concern over the repercussions of long-term treatment with TRH.

Study Design	
Length	< 1 month
Purpose:	
✓	Safety
	Dose-ranging
✓	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	8
Treatment	8
Control	0
% Male	0.5
Data collected	Clinical mscle testing, blood chemistry

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	500 mg TRH intravenously over 3 hours

Plaitakis A, Smith J, Mandeli J, Yahr MD.

Pilot trial of branched-chain aminoacids in amyotrophic lateral sclerosis.

Lancet, 1988, 1(8593): 1015 - 8.

Hypothesis/Rationale: The authors tested the efficacy of branched-chain amino acids based on the hypothesis that abnormal glutamate metabolism had a significant etiological role in ALS.The rationale for assuming abnormal glutamate metabolism in ALS was at the time based on studies which had shown deficiencies of glutamate dehydrogenase and other disturbances of glutamate metabolism in multi-system atrophic disorders which often mimicked ALS. The branched chain amino acids L-leucine and L-isoleucine were used to treat these diseases, since they had been shown to activate GDH.

Location of Study: New York, NY

Study Results: **Clear benefit.**

Patients in placebo group showed a linear decline in functional status consistent with the natural history of the disease, while treated patients showed a statistically significant maintenance of extremity muscle strength and continued ability to walk.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	11
Treatment	11
Control	11
% Male	86.4%
Data collected	Plaitakis spinal and bulbar scores

Dosing	
Treatment(s)	Branched-chain amino acids
Dose(s)/Schedule	12 g L-leucine oral 8 g L-isoleucine oral 6.4 g L-valine oral

Munsat TL, Taft J, Kasdon D, Jackson IM.

Prolonged intrathecal infusion of thyrotropin releasing hormone in amyotrophic lateral sclerosis.

Annals of the New York Academy of Sciences, 1988, 531: 187 - 93.

Hypothesis/Rationale: Since IV infusion of TRH had been shown to induce temporary improvements in muscle strength and other clinical parameters in ALS, the authors investigated a novel means of ensuring continuous delivery of TRH to the CSF (and, presumably, continuous therapeutic benefits) via an intrathecal infusion pump.

Location of Study: Boston, MA

Study Results:

Mild to moderate toxicity.

All patients experienced some degree of toxicity lasting 8-16 hours and beginning within 6-8 hours of starting the infusion. These symptoms included shivering, tachycardia, and sweating. Because of the small number of patients, no conclusions could be made regarding the possible efficacy of long-term TRH administration.

Study Design	
Length	5 – 32 months
Purpose:	
	✓ Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	✓ Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	20
Treatment	20
Control	0
Data collected	Adverse events

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	3 mg/24 hrs via intrathecal pump

Brooks BR, Kalin N, Beaulieu DA, Barksdale C, Sufit RL, Dills DG.

Thyrotropin-releasing hormone uptake into serum and cerebrospinal fluid following intravenous or subcutaneous administration

Neurological Research, 1988, 10(4): 236 - 8.

Hypothesis/Rationale: The authors conducted a study of the pharmacokinetics of intravenously and subcutaneously administered TRH.Although TRH had been advocated as a promising treatment for ALS, there was little data at the time on whether peripherally administered TRH could reach adequate serum of CSF concentrations to be therapeutically effective.

Location of Study: Madison, WI

Study Results:

Pharmacokinetics.

The authors observed that the rate of entry of TRH into the CSF is roughly equivalent between subcutaneous and intravenous administration. Although many intravenously administered neuropeptides were observed to be efficiently excluded from the central nervous system, this did not appear to be completely the case with TRH. Although higher doses of TRH failed to increase CSF concentrations of TRH, peripheral treatment with TRH did increase CSF levels of TRH to targeted therapeutic levels.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	✓ Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	15
Treatment	15
Control	0
% Male	86.7%
Data collected	Blood and CSF chemistry

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	10 mg/kg IV (6), 2.5 mg/kg subcutaneous (4), or 5.0 mg/kg subcutaneous (5)

Lacomblez L, Bouche P, Bensimon G, Meininger V.

A double-blind, placebo-controlled trial of high doses of gangliosides in amyotrophic lateral sclerosis.

Neurology, 1989, 39(12): 1635 - 7.

Hypothesis/Rationale: The authors attempted to demonstrate a beneficial effect of gangliosides on ALS by using higher doses than in previous studies.

Location of Study: France

Study Results: **No benefit.**
Gangliosides appeared to have no measurable effect on any clinical parameter measured.

Study Design	
Length	3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	34
Treatment	17
Control	17
% Male	75%
Data collected	Norris score

Dosing	
Treatment(s)	Gangliosides
Dose(s)/Schedule	300 mg Cronassial

Testa D, Caraceni T, Fetoni V.

Branched-chain amino acids in the treatment of amyotrophic lateral sclerosis

Journal of Neurology, 1989, 236(8): 445 - 7.

Hypothesis/Rationale: The authors conducted a pilot investigation of the effectiveness of branched chain amino acids in ALS.Branched chain amino acids had been suggested as a possible treatment primarily because of their potential to modulate glutamate metabolism (and thus mitigate glutamate-induced excitotoxicity.)

Location of Study: Italy

Study Results: **No benefit.**

The authors observed no statistically significant differences between treatment and control patients. They hypothesized that the failure of BCAA might be due to a range of different diseases being included under the diagnosis of ALS.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	32
Treatment	16
Control	16
% Male	68.8%
Data collected	Norris score

Dosing	
Treatment(s)	Branched-chain amino acids
Dose(s)/Schedule	3 g L-leucine, 2 g L-isoleucine, 1.6 g L-valine (oral, powder) 4x/day

Comments/Analysis:

Blin O, Serratrice G, Pouget J, Aubrespy G, Guelton C, Crevat A.

Essai en double aveugle contre placebo à court terme de la L-thréonine dans la sclérose latérale amyotrophique.

[Short-term double-blind vs. placebo trial of L-threonine in amyotrophic lateral sclerosis.]

Presse Medécine, 1989, 18(30): 1469 - 70.

Hypothesis/Rationale: The authors tested the ability of L-threonine to slow or reverse the progression of ALSL-threonine was attractive as a potential treatment for ALS because of its role as a glycine precursor and it's possible ability to mitigate glutamate toxicity.

Location of Study: France

Study Results: **Clear benefit.**

Grip strength improved during the treatment period and deteriorated during the placebo period - the authors assessed this difference to be statistically significant. Manual testing and subjective clinical observation of the patients also suggested a statistically significant difference in outcome between the treatment and control periods.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	12
Treatment	12
Control	12
Data collected	Grip strength, manual muscle testing, subjective clinical observation

Dosing	
Treatment(s)	L-threonine
Dose(s)/Schedule	2 g/day

Modarres-Sadeghi H, Guiloff RJ..

Comparative efficacy and safety of intravenous and oral administration of a TRH analogue (RX77368) in motor neuron disease.

Journal of Neurology, Neurosurgery & Psychiatry, 1990, 53(11): 944 - 7.

Hypothesis/Rationale: The authors were conducting a more extensive follow-up to a 1987 study on the effects and pharmacokinetics of the same TRH analogue. The purpose of the study was to establish an oral dose range that produced a similar profile of effects as the earlier tested IV doses.The authors do not explicitly discuss their rationale for investigating the effectiveness of a TRH analogue, since at the time this study was published, the rationale for investigating TRH in amyotrophic lateral sclerosis was well known and had recently been clarified through a rather extensive review article.

Location of Study: U.K.

Study Results: **Clear benefit.**

Improvements in speech, swallowing, and in tongue and jaw movements seen after IV and Oral admin in (respectively) 9, 5, and 8 patients. The improvement correlated to plasma levels of drug, but the clinical effects persisted when levels of the drug decreased and became undetectable after 24 hours.

Study Design	
Length	< 1 month
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	10
Treatment	10
Control	0
% Male	80%

Dosing	
Treatment(s)	RC77368 (TRH analogue)
Dose(s)/Schedule	0.15 - 0.25 mg/kg IV over two hours, followed within 3 to 7 days by increasing oral doses (0.7 - 3 mg/kg) given every 2 - 4 days

Askmark H, Aquilonius SM, Gillberg PG, Hartvig P, Hilton-Brown P, Lindstrom B, Nilsson D, Stalberg E, Winkler T..

Functional and pharmacokinetic studies of tetrahydroaminoacridine in patients with amyotrophic lateral sclerosis.

Acta Neurologica Scandinavia, 1990, 82(4): 253 - 8.

Hypothesis/Rationale: The authors revisited the question of possible cholinergic hypofunction in ALS with 9-amin-1,2,3,4-tetrahydroacridine (THA), a cholinesterase inhibitor.Despite earlier failures using cholinesterase inhibitors to treat ALS, the authors believed THA to be a cholinesterase of special significance and possibly a different level of efficacy. THA had at the time been recently reported to be effective in treating Alzheimer's disease, a disease which, like ALS, is classed as neurodegenerative in origin. Other studies at the time suggested THA might also be an NMDA-receptor agonist, which was of interest because the connection between ALS and neuroexcitotoxic amino acids was beginning to emerge as an event of possible etiologic significance.

Location of Study: Sweden

Study Results: **No benefit.**

The authors observed no conclusive changes in neurophysiological parameters during drug administration. Two patients showed an increase in muscle strength, but there were no pharmacokinetic observations that could explain this effect. Side effects were common.

Study Design	
Length	2 months
Purpose:	
	Safety
	Dose-ranging
✓	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	8
Treatment	8
Control	0
Data collected	Muscle strength, neurophysiological parameters

Dosing	
Treatment(s)	Tetrahydroaminoacridine (THA)
Dose(s)/Schedule	Pharmacokinetic study using 30 mg THA via intravenous injection followed by pilot efficacy study using 100-200 mg/day oral THA plus 11 g/day lecithin

Werdelin K, Boysen G, Jensen TS, Mogensen P.

Immunosuppressive treatment of patients with amyotrophic lateral sclerosis

Acta Neurologica Scandinavia, 1990, 82(2): 132 - 4.

Hypothesis/Rationale: The authors' study revisited the question of immunosuppression and ALS, which was the subject of a series of clinical trials with conflicting conclusions in the mid 1980s.The study was based on earlier studies that suggested an immunological etiology for ALS and a possible positive effect of immunosuppressants. The authors chose to ignore two studies which seemed to provide fairly definitive evidence that immunosuppressive treatment was of no benefit in ALS.

Location of Study: Denmark

Study Results: **No benefit.**

There was no definite difference between survival in treated patients and their historical case-matched controls, nor was there any perceptible difference in clinical progression between the treated patients and historical controls.

Study Design	
Length	12 months
Purpose:	
	✓ Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	✓ Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	21
Treatment	21
Control	0
Control selection	Historical case-control matching
% Male	81%
Data collected	Survival, scoring system assessing upper motor neuron function, manual strength testing

Dosing	
Treatment(s)	Prednisolone, azathioprine
Dose(s)/Schedule	2 mg/kg/day azathioprine, plus initial loading dose of 1 mg methylprednisolone IV over 3 days followed by 50-100 mg prednisolone every 2 days thereafter

Chiodini PG, Attanasio R, Liuzzi A, Cozzi R, Orlandi P, De Palo C, Dallabonzana D, Girotti F, Testa D.

Prolactin response to growth hormone-releasing hormone during chronic thyrotropin-releasing homone infusion in the treatment of amyotrophic lateral sclerosis.

Journal of Endocrinological Investigations, 1990, 13(8): 631 - 636.

Hypothesis/Rationale: The authors were attempting to confirm an earlier study (Kaplan et al, 1986) which suggested that TRH caused endocrine alterations (increased TSH and thyroid hormone levels.)In contrast to that earlier study, which delivered TRH directly to the CSF, this study delivered TRH chronically via IV pump. The authors do not discuss their rationale for this change in treatment protocol.

Location of Study: Italy

Study Results: As in Kaplan's 1986 study, the authors observed a significant rise of thyroid hormone levels, and an upward trend of basal TSH levels. They observed no change in basal prolactin (PRL) and growth hormone (GH) levels. However, TSH and PRL response to TRH stimulation appeared to be blunted during therapy. Treatment with TRH also caused PRL to be responsive to growth hormone-releasing hormone, a phenomenon which had not been observed before treatment. At the time, conflicting reports on PRL response to GHRH in healthy patients made it difficult to interpret the significance of this latter finding.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	✓ Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	6
Treatment	6
Control	0
% Male	33.3%
Data collected	Blood chemistry, clinical observation

Dosing	
Treatment(s)	TRH
Dose(s)/Schedule	2 mg/day TRH-tartrate continuous IV pump infusion

Blin O, Pouget J, Aubrespy G, Guelton C, Crevat A, Serratrice G..

A double-blind placebo-controlled trial of L-threonine in amyotrophic lateral sclerosis.

Journal of Neurology, 1992, 239(2): 79 - 81.

Hypothesis/Rationale: The authors were extending their earlier short-term crossover study of L-threonine in ALS to investigate the long-term therapeutic effects of the treatment.L-threonine was attractive as a potential treatment for ALS because of its role as a glycine precursor and it's ability to increase levels of glycine in the CNS. Increasing levels of glycine was at the time thought to be an ideal strategy for mitigating glutamate-induced exctotoxicity.

Location of Study: France

Study Results: **No benefit.**

Patients in the placebo group showed an expected rate of decline in function consistent with the natural history of ALS, and this decline was not statistically different from the outcomes of patients treated with L-threonine.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	23
Treatment	12
Control	11
% Male	56.5%
Data collected	Muscle function, grip strength, self/evaluation

Dosing	
Treatment(s)	L-threonine
Dose(s)/Schedule	2 g/day

Testa D, Caraceni T, Fetoni V, Girotti F.

Chronic treatment with L-threonine in amyotrophic lateral sclerosis: a pilot study.

Clinical Neurology and Neurosurgery, 1992, 94(1): 7 - 9.

Hypothesis/Rationale: The authors tested the effectiveness of L-threonine in chronic treatment of ALS patients.Threonine is a precursor of glycine. At the time of this trial, there were debates over whether glycine would enhance or prevent glutamate toxicity to neurons. The authors sided with a series of reports that suggested glycine might help neutralize excess excitotoxic amino acids. Another study reported that treatment with threonine improved muscle strength in ALS patients.

Location of Study: Italy

Study Results: **No benefit.**

No statistical differences observed between treated group and control patients; Thr-treated patients complained less frequently of respiratory failure than the control group despite more frequent bulbar involvement in the L-threonine group.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	13
Treatment	9
Control	4
Data collected	Norris scale

Dosing	
Treatment(s)	L-threonine
Dose(s)/Schedule	1g 4x/day (oral, powder)

Westarp ME, Westphal KP, Kolde G, Wollinsky KH, Westarp MP, Dickob M, Kornhuber HH..

Dermal, serological and CSF changes in amyotrophic lateral sclerosis with and without intrathecal interferon beta treatment.

International Journal of Clinical Pharmacology, Therapy & Toxicology, 1992, 30(3): 81 - 93.

Hypothesis/Rationale: This study assumed a viral etiology of ALS, and the purpose of the trial was primarily to contribute to an understanding of the biological and immunological changes induced by treatment with IFN-b. The authors hoped in the process to identify possible biological markers for therapeutic efficacy.The authors were interested in IFN-b primarily because of its anti-viral properties, especially in neurological diseases of viral origin. At the time, it was being investigated as a treatment for subacute sclerosing panencephalitis and visna virus (a retrovirus causing CNS pathology.)

Location of Study: Germany

Study Results: In both treatment and control, there were significantly elevated serum levels of IgG immune complexes and creatine kinase (especially in non-bulbar disease). The erythrocyte sedimentation rate rose during intratheal IFN therapy in 9/10 patients. Both treated and untreated patients had similar dermal alterations known to be associated with ALS. Treatment with IFN did not appear to have any effect on the appearance of dermal tissue.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	✓ Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	✓ Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	21
Treatment	12
Control	9
Data collected	Various blood chemistries, erythrocyte sedimentation rate, CSF total protein, light and electron miscroscopy of dermal tissues.

Dosing	
Treatment(s)	Interferon Beta
Dose(s)/Schedule	10,000,000 IU human natural fibroblast IFN-b 2x/wk for 1 month, followed by 1x/2 wks for the next five months

Munsat TL, Taft J, Jackson IM, Andres PL, Hollander D, Skerry L, Ordman M, Kasdon D, Finison L..

Intrathecal thyrotropin-releasing hormone does not alter the progressive course of ALS: experience with an intrathecal drug delivery system.

Neurology, 1992, 42(5): 1049 - 53.

Hypothesis/Rationale: The authors hypothesized that previous negative therapeutic trials of TRH in ALS were due to difficulties of getting TRH to cross the blood-brain-barrier. To test this, they initiated a therapeutic trial using intrathecally administered TRH.

Location of Study: Boston, MA

Study Results: **No benefit.**

The authors observed no change in progressive course of disease. However, the implanted pump delivery system was safe, reliable, and well tolerated.

Study Design	
Length	6 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	
Double-blind?	
Blind?	✓
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	25
Treatment	25
Control	25
% Male	80%
Data collected	TQNE

Dosing	
Treatment(s)	TRH

Askmark H, Aquilonius SM, Gillberg PG, Liedholm LJ, Stalberg E, Wuopio R.

A pilot trial of dextromethorphan in amyotrophic lateral sclerosis.

Journal of Neurology, Neurosurgery & Psychiatry, 1993, 56(2): 197 - 200.

Hypothesis/Rationale: The authors tested the efficacy of dextromethorphan, an NDMA-receptor antagonist, in light of recent experimental findings and hypotheses surroundin the role of glutamate toxicity in ALS.Dextromethorphan is an NMDA receptor antagonist; the NMDA receptor was thought to play a crucial role in glutamate-induced neuro-toxicity.

Location of Study: Sweden

Study Results: **No benefit.**

No positive effects on clinical or neurophysiological parameters were observed. Patients treated with dextromethorphan appeared to have a faster rate of decline in their bulbar scores, but this difference was not statistically significant.

Study Design	
Length	3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	✓
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	14
Treatment	14
Control	0
% Male	35.7%
Data collected	Norris score, Plaitakis bulbar and spinal score, compound muscle action potentials

Dosing	
Treatment(s)	Dextromethorphan
Dose(s)/Schedule	150 mg daily for 3 months, then 300 mg daily for 6 months after crossover portion of trial

Eisen A, Stewart H, Schulzer M, Cameron D.

Anti-glutamate therapy in amyotrophic lateral sclerosis: a trial using lamotrigine.

Canadian Journal of the Neurological Sciences, 1993, 20(4): 297 - 301.

Hypothesis/Rationale: The authors tested the effect of lamotrigine, a drug used at the time to treat refractory epilepsy. Lamotrigine was of interested primarily because it inhibited the release of glutamate and blocks voltage-sensitive sodium channels.Lamotrigine was tested in response to a growing interest in the possible etiological role of glutamate toxicity in ALS.

Location of Study: Canada

Study Results: **No benefit.**

Lamotrigine at the doses administered did not alter the course of ALS.

Study Design	
Length	18 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	40
Treatment	19
Control	21
Data collected	Routine electrophysiological tests, motor evoked potentials, and various clinical scores of bulbar function, ambulatory function, and overall disability

Dosing	
Treatment(s)	Lamotrigine
Dose(s)/Schedule	100 mg/d oral

Smith RA, Melmed S, Sherman B, Frane J, Munsat TL, Festoff BW.

Recombinant growth hormone treatment of amyotrophic lateral sclerosis.

Muscle Nerve, 1993, 16(6): 624 - 33.

Hypothesis/Rationale: Based on the hypothesis that ALS was due to an abnormality or deficiency in an as-yet-discovered motor neuron growth factor, the authors tested the effects of growth hormone on ALS.Growth hormone (GH) had been showed to have trophic effects on nerve and muscle, and GH deficient rats and mice showed myelin deficits which were able to be reversed through administration of exogenous GH.

Location of Study: Los Angeles, CA

Study Results: **No benefit.**

Survival analysis (performed 12 months after cessation of treatment) showed no difference between treatment and placebo groups. There was also no difference in the rate of decline of TQNE scores.

Study Design	
Length	18 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	75
Treatment	38
Control	37
Data collected	Survival, Quantitative neuromuscular (TQNE) and manual (MRC) exam results, and laboratory chemistries

Dosing	
Treatment(s)	rGH
Dose(s)/Schedule	0.1 mg/kg human recombinant methionyl growth hormone IM 3x/wk

Norris FH, Tan Y, Fallat RJ, Elias L.

Trial of oral physostigmine in amyotrophic lateral sclerosis.

Clinical Pharmacology & Therapeutics, 1993, 54(6): 680 - 2.

Hypothesis/Rationale: The authors tested the efficacy of oral physostigmine salicylate in slowing the progression of ALS. For earlier studies, only an injectable form of physostigmine was available - both the short half life and narrow safety margin made this earlier form of the drug an unfeasible treatment. The choice of physostigmine was based on an earlier clinical trial using an injectable form of the drug, and on studies which suggested that certain anticholinesterases temporarily improved strength in ALS patients.

Location of Study: San Francisco, CA

Study Results: **Possible benefit.**

The authors observed a slight benefit in reduced loss of grip strength, but the rates of decline for body weight, ALS score, forced vital capacity, maximum voluntary ventilation, and megascore were similar for both the treatment and placebo periods of the crossover trial.

Study Design	
Length	4 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	13
Treatment	13
Control	13
Data collected	Body weight, ALS score, Jamar grip strength, forced vital capacity, maximum voluntary ventilation

Dosing	
Treatment(s)	Physostigmine
Dose(s)/Schedule	16 mg/day (4mg 4x/day) stepped up over a two week period from an initial dose of 1 mg/day

Bensimon G, Lacomblez L, Meininger V.

A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group.

N Engl J Med, 1994, 330(9): 585 - 91.

Hypothesis/Rationale: The authors conducted a placebo-controlled trial or riluzole to determine whether the anti-glutamate agent was beneficial to patients with amyotrophic lateral sclerosis.Riluzole was found in preclnical studies to modulate glutamatergic transmission, making it an attractive drug candidate to mitigate the glutamate-induced excitotoxicity hypothesized to play a major role in ALS causation.

Location of Study: France

Study Results:

Clear benefit.

Riluzole conferred a statistically significant survival advantage at the twelve month mark and at the end of the study. After 12 months, the survival rate for placebo patients was 58%, while for riluzole-treated patients it was 74%. Among bulbar patients, the difference was even more pronounced - 35% survival with placebo and 73% with riluzole.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	155
Treatment	77
Control	78
% Male	58.7%
Data collected	Survival, changes in functional status (using modified Norris score), muscle testing scores, respiratory function, Clinical Global Impression of Change, patients' subjective evaluations.

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	100 mg/day

Mazzini L, Testa D, Balzarini C, Mora G.

An open-randomized clinical trial of selegiline in amyotrophic lateral sclerosis.

Journal of Neurology, 1994, 241(4): 223 - 7.

Hypothesis/Rationale: Based on the assumption that free radicals play a role in motor neurons, the authors tested the ability of selegiline to alter the progressive course of ALS.Selegiline is monoamine oxidase B inhibitor. Selegiline was found to slow the progression of of Parkinson's disease at the same dosage used in this study.

Location of Study: Italy

Study Results: **No benefit.**

There were no differences in clinical parameters between treated and untreated patients.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	✓
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	111
Treatment	53
Control	58
Data collected	Mortality, MRC and Norris disability scores, FVC

Dosing	
Treatment(s)	Selegiline
Dose(s)/Schedule	10 mg/d oral

Jossan SS, Ekblom J, Gudjonsson O, Hagbarth KE, Aquilonius SM..

Double blind cross over trial with deprenyl in amyotrophic lateral sclerosis.

Journal of Neural Transmission, 1994, 41(Suppl): 237 - 41.

Hypothesis/Rationale: The authors tested the efficacy of deprenyl in ALS in a placebo-controlled, crossover pilot efficacy study.Deprenyl was a monoamine oxidase-B inhibitor that had been successfully used in the treatment of Parkinson's disease. Low concentrations of deprenyl also appeared to have a neuroprotective effect in a rat model of neuronal injury.

Location of Study: Sweden

Study Results: **No benefit.**

MAO-B activity in blood platelets was completely inhibited during treatment with deprenyl. However, the authors could not detect that the treatment had any effect on the progression of the disease. The patients treated with deprenyl appeared to have more rapid decline in Norris scores, but this difference was not statistically significant.

Study Design	
Length	3 months
Purpose:	
	Safety
	Dose-ranging
✓	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	10
Treatment	10
Control	10
% Male	80%
Data collected	Norris score

Dosing	
Treatment(s)	Deprenyl
Dose(s)/Schedule	10 mg/day oral

Dalakas MC, Stein DP, Otero C, Sekul E, Cupler EJ, McCrosky S.

Effect of high-dose intravenous immunoglobulin on amyotrophic lateral sclerosis and multifocal motor neuropathy

Archives of Neurology, 1994, 51(9): 861 - 4.

Hypothesis/Rationale: The authors primarily assessed whether intravenous immunoglobulin impacted muscule strength or clinical progression in ALS.The specific choice of intravenous immunoglobulin was based on both immunoglobulin's success in treating various autoimmune neuromuscular diseases and on specific findings suggesting an autoimmune component in ALS that might respond positively to immunoglobulin therapy.

Location of Study: Bethesda, MD

Study Results: **No benefit.**

All patients continued to decline. The authors concluded that IVIG has no apparent therapeutic role in improving the symptoms or arresting the pace of progression in ALS patients.

Study Design	
Length	3 months
Purpose:	
	✓ Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	✓ Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	9
Treatment	9
Control	0
Data collected	Muscle strength (MVIC) and self-assessment scores

Dosing	
Treatment(s)	Immunoglobulin
Dose(s)/Schedule	1 g/kg IV 2x/day once a month for three consecutive months

Hollander D, Pradas J, Kaplan R, McLeod HL, Evans WE, Munsat TL.

High-dose dextromethorphan in amyotrophic lateral sclerosis: phase I safety and pharmacokinetic studies.

Annals of Neurology, 1994, 36(6): 920 - 4.

Hypothesis/Rationale: The authors believed dextromethorphan to be the most appropriate therapy for use in human trials testing excitotoxic theories of ALS etiology, based on dextromethorphan's selective NMDA-receptor antagonism and its long history of use in humans (as a cough suppressant).

Location of Study: Boston, MA

Study Results: **Well tolerated.**
Contrary to the authors' fears, long-term high dose treatment with dextromethorphan failed to have any measurable negative impact on patients' memory. (This was a major concern, since previous studies had suggested NMDA receptor agonists might block crucial memory processes if the drugs reached high enough concentrations in the brain.) There were no unexpected adverse events or major side effects of high-dose dextromethorphan; side effects included light-headedness, slurred speech, fatigue, and decreased coordination.

Study Design	
Length	1 – 7 months
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	✓ Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	13
Treatment	13
Control	0
% Male	53.8%
Data collected	Blood chemistry, adverse events, standard pharmacokinetic studies

Dosing	
Treatment(s)	Dextromethorphan
Dose(s)/Schedule	Pharmacokinetic study using 2.5 mg/kg (oral, syrup) followed by a starting dose of 2-3 mg/kg/day increasing by 0.25 mg/kg every two days up to the maximum tolerated dose or the target dose of 10 mg/kg/day

Smith SA, Miller RG, Murphy JR, Ringel SP.

Treatment of ALS with high dose pulse cyclophosphamide.

Journal of the Neurological Sciences, 1994, 124(Suppl): 84 - 7.

Hypothesis/Rationale: Based on the assumption of an autoimmune component in ALS, the authors tested the effects of high-dose cyclophosphamide in slowing or reversing the progression of ALS.Cyclophosphamide, an immunosuppressant, had shown promise in treating multifocal motor neuropathy, a condition which can mimic ALS.

Location of Study: Minneapolis, MN

Study Results: **No benefit.**

The course of the disease was not measurably altered by cyclophosphamide treatment.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	36
Treatment	18
Control	18
Control selection	Natural history (from WALS database)
Data collected	FVC, bilateral isometric muscle strength testing, bulbar function, fine motor coordination

Dosing	
Treatment(s)	Cyclophosphamide
Dose(s)/Schedule	Loading dose of 3 g/m2 plus montly injections of 750-1000 mg/m2

Drachman DB, Chaudhry V, Cornblath D, Kuncl RW, Pestronk A, Clawson L, Mellits ED, Quaskey S, Quinn T, Calkins A, et al.

Trial of immunosuppression in amyotrophic lateral sclerosis using total lymphoid irradiation.

Annals of Neurology, 1994, 35(2): 142 - 50.

Hypothesis/Rationale: The study explored the possibility that previous attempts to treat ALS failed because suppression was neither powerful enough nor continued long enough to be effective. The authors were attempting both to find an effective treatment for ALS and to probe the specific role, if any, of the immune system in ALS pathology.The study was based on a range of studies which suggested an autoimmune component to ALS, including studies which found circulating immune complexes, increased incidence of a specific histocompatibility type among patients, association with other autoimmune diseases, and calcium-channel-specific antibodies. Although previous trials of immunosuppressive treatments had failed to demonstrate any benefit, the authors interpreted these studies in certain cases to have yielded intriguing and suggestive results inconsistent with a total denial of efficacy.

Location of Study: Baltimore, MD

Study Results: **No benefit.**

The authors found no statistically significant differences in motor function or overall survival. However, total lymphoid irradiation did successfully suppress cellular and humoral immune function throughout the 2-year follow-up period.

Study Design	
Length	23 – 24 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	61
Treatment	30
Control	31
Data collected	Muscle strength, functional motor activity, humoral/cellular immune status

Dosing	
Treatment(s)	Immunosuppression
Dose(s)/Schedule	Total lymphoid irradiation

M.L. Aisen, D. Sevilla, G. Gibson, H. Kutt, A. Blau, L. Edelstein, J. Hatch, J. Blass.

3,4-diaminopyridine as a treatment for amyotrophic lateral sclerosis

Journal of the Neurological Sciences, 1995, 129(1): 21 - 4.

Hypothesis/Rationale: The authors attempted to develop a rational dosing schedule of DAP in ALS patients and also measure the effect of DAP on ALS-related motor weakness.3,4-diaminopyridine had been shown to improve neuronal function (through increasing neurally evoked acetylcholine release) and had been used with success in treating motor impairment in multiple sclerosis and myasthenic syndrome.

Location of Study: White Plains, NY U.S.

Study Design	
Length	4 months
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	7
Treatment	7
Control	0
% Male	57.1%

Dosing	
Treatment(s)	3,4-diaminopyridine (DAP)
Dose(s)/Schedule	10 - 100 mg/day

Study Results: DAP was tolerated in all patients, and was associated with a small but statistically significant increase in motor score.

[No authors listed].

A phase I study of recombinant human ciliary neurotrophic factor (rHCNTF) in patients with amyotrophic lateral sclerosis.

Clinical Neuropharmacology, 1995, 18(6): 515 - 32.

Hypothesis/Rationale: The authors conducted a Phase I trial primarily aimed at testing the safety of rhCNTF at range of different doses.This study was the first step in preparing for more extensive therapeutic trials of rhCNTF in ALS. The authors had conducted previous studies which suggested rhCNTF supported the survival of motor neurons in vitro and slowed the progression of three naturally occurring rodent models of motor neuron disease.

Location of Study: Tarrytown, NY

Study Results:

Well-tolerated at lower doses.

The authors observed dose-limiting toxicity (febrile reactions, fatigue, nonproductive cough) at 30 mcg/kg. There were no adverse neurological consequences observed. Pharmacokinetically, peak plasma concentrations occurred 180 to 260 minutes after dosing.

Study Design	
Length	< 1 month
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	✓ Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	57
Treatment	57
Control	0

Dosing	
Treatment(s)	CNTF
Dose(s)/Schedule	0.5 - 30 mcg/kg subcutaneously 3x/wk for 2 weeks

E.S. Louwerse, G.J. Weverling, P.M. Bossuyt, F.E. Meyjes, J.M. de Jong, B. Vianney.

Randomized, double-blind, controlled trial of acetylcysteine in amyotrophic lateral sclerosis.

Archives of Neurology, 1995, 52(6): 559 - 64.

Hypothesis/Rationale: The authors attempted to investigate the effectiveness of acetylcysteine, a free radical scavenging agent, in patients with amyotrophic lateral sclerosis.Based on the hypothesis that glutamate and, in particular, free radicals play a role in ALS. This study came out shortly after the discovery of the first gene for ALS, which involved mutations of the gene encoding copper-zinc-binding superoxide dismutase

Location of Study: Netherlands

Study Results: **Possible benefit.**

Patients treated with acetylcysteine had an 11% different in 12-month survival vs. the placebo group, which translated to a motrality risk reduction of 26% relative to the placebo group. This difference, however, was not found to be statistically signifi

Study Design	
Length	12 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	110
Treatment	55
Control	55
Data collected	Non-event survival (avoidance of death, tracheostomy, or persistent assisted ventilation)

Dosing	
Treatment(s)	Acetylcysteine
Dose(s)/Schedule	50 mg/kg subcutaneous

Pinto AC, Evangelista T, Carvalho M, Alves MA, Sales Luis ML.

Respiratory assistance with a non-invasive ventilator (Bipap) in MND/ALS patients: survival rates in a controlled trial.

Journal of the Neurological Sciences, 1995, 129(Suppl): 19 - 26.

Hypothesis/Rationale: The authors investigated whether non-invasive positive pressure ventilation (frequently referred to bipap) could be effective in improving survival in ALS.

Location of Study: Portugal

Study Results: **Clear benefit.**

Treatment with bipap yielded a statistically significant improvement in total survival time. Although NINV increases survival time, it does not arrest or slow the progression of the disease.

Study Design	
Length	12 – 36 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	20
Treatment	10
Control	10
% Male	55%

Dosing	
Treatment(s)	Bipap
Dose(s)/Schedule	Continuous

Bastone A, Micheli A, Beghi E, Salmona M.

The imbalance of brain large-chain aminoacid availability in amyotrophic lateral sclerosis patients treated with high doses of branched-chain aminoacids.

Neurochemistry International, 1995, 27(6): 467 - 72.

Hypothesis/Rationale: The authors investigated plasma concentrations of large amino acids, glutamic acid, and large neutral aminoacid brain flux in an attempt to explain evidence of increased mortality among ALS patients treated with high daily doses of branched-chain amino acids.

Location of Study: Italy

Study Results: The branched chain amino acid brain influx of the treated group was 110-140% of that in patients receiving placebo and that of healthy controls, while the aromatic amino acid brain influx was lower in the treated group than in the placebo group or healthy controls. The authors interpreted this data to indicate a possible impairment of brain large neutral aminoacid availability during treatment with BCAA, which could contribute to enhancing symptom progression.

Study Design	
Length	< 1 month
Purpose:	
✓	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	54
Treatment	12
Control	42
Data collected	Blood chemistry, amino acid brain influx

Dosing	
Treatment(s)	Branched-chain amino acids
Dose(s)/Schedule	12 g L-leucine, 6 g L-leucine, 6 g L-valine daily

Miller RG, Smith SA, Murphy JR, Brinkmann JR, Graves J, Mendoza M, Sands ML, Ringel SP.

A clinical trial of verapamil in amyotrophic lateral sclerosis.

Muscle & Nerve, 1996, 19(4): 511 - 5.

Hypothesis/Rationale: Interest in verapamil was based on both its possible ability to stop the calcium-mediated activation of excitatory amino acid receptors (and thus reduce excitoxicity), and for its positive side effect and tolerability profile versus other calcium channel blockers.

Location of Study: San Francisco, CA

Study Results: **No benefit.**

The rate of decline in pulmonary function and limb megascores was not significantly different during periods of drug treatment vs. non-treatment. Verapamil appeared to have no measurable effect on the clinical progression of ALS.

Study Design	
Length	6 months
Purpose:	
	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	72
Treatment	72
Control	72
Control selection	
% Male	62.1%
Data collected	Pulmonary function, limb megascores

Dosing	
Treatment(s)	Verapamil
Dose(s)/Schedule	40 mg 2x/day oral for first two weeks, increased by 40 mg increments every 2-3 days to maximum tolerated dose up to 240 mg/day

Lacomblez L, Bensimon G, Leigh PN, Guillet P, Powe L, Durrleman S, Delumeau JC, Meininger V..

A confirmatory dose-ranging study of riluzole in ALS. ALS/Riluzole Study Group-II.

Neurology, 1996, 47(6 Suppl 4): 0 - 50.

Hypothesis/Rationale: The authors were attempting to confirm earlier studies which suggested that riluzole decreased mortality and muscular degeneration.

Location of Study: France

Study Results: **Clear benefit.**

Patients treated with riluzole had a dose-related decrease in risk of death or tracheostomy, which equated to a statistically significant 35% reduced risk of death at the 100-mg dose at 18 months versus placebo. However, the study could not confirm an earlier study's observation that riluzole decreased the rate of muscle strength deterioration by up to 35%.

Study Design		
Length		15 – 18 months
Purpose:		
		Safety
	✓	Dose-ranging
		Pharmacokinetic/molecular
	✓	Efficacy
Controlled?		✓
Crossover?		
Placebo?		✓
Randomized?		✓
Double-blind?		✓
Blind?		
Phase?		
		Pilot
		Phase I
		Phase II
		Phase III

Study Participant Demographics	
Total Participants	959
Treatment	717
Control	242
% Male	60%
Data collected	FVC, muscle tests, bulbar scale, limb scale

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50, 100, and 200 mg/day

Blin O, Azulay JP, Desnuelle C, Bille-Turc F, Braguer D, Besse D, Branger E, Crevat A, Serratrice G, Pouget JY.

A controlled one-year trial of dextromethorphan in amyotrophic lateral sclerosis.

Clinical Neuropharmacology, 1996, 19(2): 189 - 92.

Hypothesis/Rationale: The authors were attempting to either confirm or refute the results of pilot and short-term studies which suggested dextromethorphan had no measurable impact on the progression of ALS.Interest in dextromethorphan was primarily due to its action as an NMDA receptor antagonist, meaning it was likely to help prevent or alleviate excitoxic damage to neurons.

Location of Study: France

Study Results: **No benefit.**

The authors observed no significant difference in the rate of progression between treated patients and controls (placebo).

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	49
Treatment	24
Control	25
% Male	59.6%
Data collected	Norris scale

Dosing	
Treatment(s)	Dextromethorphan
Dose(s)/Schedule	1.5 mg/kg/day

Tandan R, Bromberg MB, Forshew D, Fries TJ, Badger GJ, Carpenter J, Krusinski PB, Betts EF, Arciero K, Nau K..

A controlled trial of amino acid therapy in amyotrophic lateral sclerosis: I. Clinical, functional, and maximum isometric torque data.

Neurology, 1996, 47(5): 1220 - 6.

Hypothesis/Rationale: The authors attempted to confirm or refute earlier studies of branched-chain amino acids in ALS.Earlier studies of amino acid therapy in ALS were mostly open-label; only one of the few controlled trials conducted was able to demonstrate possible effectiveness along the lines of that demonstrated in the open-label trials. In addition, the authors criticized these earlier trials for basing their assessments of clinical progression on subjective criteria rather than objective measurements of muscle strength and other neurophysiological parameters.

Location of Study: Burlington, VT

Study Results: **No benefit.**

Patients in the BCAA group gained weight while those in the other two treatment groups lost weight, but this difference did not reach statistical significance. The BCAA and L-threonine groups had a 2.5x greater decline in FVC versus the placebo group.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	77
Treatment	52
Control	25
% Male	55.8%
Data collected	Clinical muscle strength, maximum isometric muscle torque, FVC, activities of daily living, timed tasks, weight

Dosing	
Treatment(s)	Amino acids
Dose(s)/Schedule	12 g L-leucine 8 g L-isoleucine 6.4 g L-valine OR 4 g L-threonine with 160 mg pyridoxal phosphate

ALS CNTF Treatment Study Group.

A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. ALS CNTF Treatment Study Group.

Neurology, 1996, 46(5): 1244 - 9.

Hypothesis/Rationale: The authors investigated the safety, tolerability, and efficacy of subcutaneous administration of recombinantly produced human CNTF in slowing the progression of ALS.Earlier Phase I-II studies had showed rhCNTF to be safe and relatively well tolerated when administered subcutaneously, and had also suggested rhCNTF administration resulted in the expected biological and neurotrophic effects.

Location of Study: Tarrytown, NY

Study Results: **No benefit.**

There were no statistically significant differences between rhCNTF and placebo-treated patients' muscle strength or any of the secondary measures of progression. Early in the study, rhCNTF-treated patients showed a statistically significant decrease in strength.

Study Design	
Length	9 months
Purpose:	
✓	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
✓	Phase II
✓	Phase III

Study Participant Demographics	
Total Participants	730
Treatment	489
Control	241
Data collected	Slope of decline of isometric muscle strength, ALSFRS, Schwab & England Scale, Global Clinical Impression of Change (GCIC)

Dosing	
Treatment(s)	CNTF
Dose(s)/Schedule	30 mcg/kg or 15 mcg/kg

Aisen ML, Sevilla D, Edelstein L, Blass J.

A double-blind placebo-controlled study of 3,4-diaminopyridine in amyotrophic lateral sclerosis patients on a rehabilitation unit.

Journal of the Neurological Sciences, 1996, 138(1-2): 93 - 6.

Hypothesis/Rationale: The authors followed up on their earlier study of DAP with a double-blind crossover study assessing whether single dose oral therapy could improve motor strength, functional status, or nerve conduction.The authors' rationale for choosing DAP was based on its successful use in improving peripheral synaptic efficiency, its success in treating other neurological disorders, and reports that another drug that enhanced acetylcholine release had yielded short-term benefits in ALS.

Location of Study: White Plains, NY

Study Results: **No benefit.**

Subjects experienced an improvement in functional status, but this improvement was the same for both the drug and placebo treatment periods. Motor strength scores improved, but this improvement was not statistically significant. The authors concluded that DAP did not have a useful role in the treatment of advanced ALS.

Study Design	
Length	< 1 month
Purpose:	
	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
✓	Phase II
	Phase III

Study Participant Demographics	
Total Participants	9
Treatment	9
Control	9
% Male	55.5%
Data collected	Functional independence measurement (FIM), Ashworth, grip strength, limb strength measurements, nerve conduction studies, and speech assessments

Dosing	
Treatment(s)	3,4 - diaminopyridine
Dose(s)/Schedule	10 mg, increasing daily to maximum tolerated dose or 80 mg/day

Miller RG, Petajan JH, Bryan WW, Armon C, Barohn RJ, Goodpasture JC, Hoagland RJ, Parry GJ, Ross MA, Stromatt SC..

A placebo-controlled trial of recombinant human ciliary neurotrophic (rhCNTF) factor in amyotrophic lateral sclerosis.

Annals Neurol, 1996, 39(2): 256 - 60.

Hypothesis/Rationale: The authors tested the safety and efficacy of rhCNTF at three different doses.Earlier Phase I trials had demonstrated rhCNTF was safe and well-tolerated at doses up to 5 micrograms/kg/day. Interest in rhCNTF as a possible treatment for ALS was based on studies that suggested the neuro-protective protein (typically expressed upon injury to the nervous system in order to limit the extent of injury-induced neuronal damage) promoted the survival of motor neurons in an animal model of ALS.

Location of Study: San Francisco, CA

Study Results: **No benefit. Well tolerated at lower doses.**

There were no significant clinical differences observed among the four groups. There were, however, an increased number of deaths and adverse events associated with the highest rhCNTF dose level.

Study Design	
Length	6 months
Purpose:	
✓	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
✓	Phase II
	Phase III

Study Participant Demographics	
Total Participants	483
Treatment	360
Control	123
Data collected	Limb strength & pulmonary megascore, individual arm & leg megascores, pulmonary function tests, activities-of-daily-living outcome measure, survival

Dosing	
Treatment(s)	rhCNTF
Dose(s)/Schedule	0.5, 2, or 5 mcg/kg/day

Miller RG, Shepherd R, Dao H, Khramstov A, Mendoza M, Graves J, Smith S.

Controlled trial of nimodipine in amyotrophic lateral sclerosis.

Neuromuscular Disorders, 1996, 6(2): 101 - 4.

Hypothesis/Rationale: The study tested whether nimodipine, a Calcium channel blocker, could slow or arrest the progression of ALS.The choice of nimodipine was based on the assumption that glutamate excitotoxicity played a major etiologic role in ALS, and that the cause of this toxicity was high levels of intracellular calcium.

Location of Study: San Francisco, CA

Study Results: **No benefit.**

There was no difference in the rate of decline of pulmonary function or limb strength during treatment with nimodipine.

Study Design	
Length	3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	87
Treatment	87
Control	87
% Male	63.2%
Data collected	TQNE

Dosing	
Treatment(s)	Nimodipine
Dose(s)/Schedule	30 mg 3x/day

Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V.

Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II.

Lancet, 1996, 347(9013): 1425 - 31.

Hypothesis/Rationale: The authors assessed the efficacy of riluzole at three different dose levels.An earlier trial suggested riluzole conferred significant survival benefits, but these benefits appeared to decline as the trial went on. This study was an attempt to replicate that earlier experience in a larger population and clarify its' findings.

Location of Study: France

Study Results: **Clear benefit.**

At the end of the study, there was a statistically significant higher rate of survival among treated vs. placebo patients in the 100 mg group. These differences also existed at the 50 mg and 200 mg levels. The authors concluded that Riluzole at 100 mg provided the best benefit/risk profile.

Study Design	
Length	18 months
Purpose:	
✓	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	959
Treatment	717
Control	242
% Male	60%
Data collected	Survival, muscle strength, limb score, bulbar score, vital capacity

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg, 100 mg, or 200 mg daily

Aebischer P, Schluep M, Deglon N, Joseph JM, Hirt L, Heyd B, Goddard M, Hammang JP, Zurn AD, Kato AC, Regli F, Baetge EE..

Intrathecal delivery of CNTF using encapsulated genetically modified xenogeneic cells in amyotrophic lateral sclerosis patients.

Nature Medicine, 1996, 2(6): 696 - 699.

Hypothesis/Rationale: The authors investigated an alternative method of delivery of CNTF capable of mitigating the serious side effects observed with systemically administered CNTF, which had caused clinical trials of the treatment to be discontinued.The authors hypothesized that placing encapsulated genetically engineered cells (designed to release 0.5 micrograms of human CNTF per day) in the lumbar intrathecal space of ALS patients would be an effective, safe, long-term means of continuously administering CNTF to ALS patients. CNTF demonstrated a number of neuroprotective effects in animal models of motor neuron degeneration, but trials of the drug had been plagued by troubling side effects, the short half-life of the molecule, and issues with ensuring delivery of the drug to the CNS.

Location of Study: Switzerland

Study Results:

No major side effects.

CNTF was detectable in patients' cerebrospinal fluid for at least 17 weeks after transplantation (it had been undetectable before transplantation.) This method of drug delivery was no associated with the limiting side effects observed with systemic delivery of CNTF.

Study Design	
Length	5 months
Purpose:	
	✓ Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	✓ Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	6
Treatment	6
Control	0
Data collected	FVC, TQNE, Norris score, blood chemistry

Dosing	
Treatment(s)	CNTF
Dose(s)/Schedule	0.5 mcg/d via ex vivo gene therapy

Miller RG, Moore D, Young LA, Armon C, Barohn RJ, Bromberg MB, Bryan WW, Gelinas DF, Mendoza MC, Neville HE, Parry GJ, Petajan JH, Ravits JM, Ringel SP, Ross MA..

Placebo-controlled trial of gabapentin in patients with amyotrophic lateral sclerosis. WALS Study Group. Western Amyotrophic Lateral Sclerosis Study Group.

Neurology, 1996, 47(6): 1383 - 8.

Hypothesis/Rationale: The authors were investigating the efficacy, safety, and tolerability of gabapentin, and also attempting to confirm or refute anecdotal reports of efficacy.Gabapentin had been shown to have a neuroprotective effect in tissue culture models of excitotoxicity, and also prolonged survival in the mSOD1 mouse model of the disease.

Location of Study: San Francisco, CA

Study Results: **Possible benefit.**

The authors observed a trend toward slower decline of arm strength in patients taking gabapentin, but this difference was not statistically significant. The treatment had no observable effect on forced vital capacity, but was well tolerated. The authors interpreted these results to mean that gabapentin was a promising treatment for future study.

Study Design	
Length	6 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
✓	Phase II
	Phase III

Study Participant Demographics	
Total Participants	140
Treatment	70
Control	70
% Male	67.1%
Data collected	Slope of arm megascore (average maximum voluntary isometric strength from eight arm muscles standardized against reference ALS population), FVC

Dosing	
Treatment(s)	Gabapentin
Dose(s)/Schedule	800 mg tid

Lange DJ, Felice KJ, Festoff BW, Gawel MJ, Gelinas DF, Kratz R, Lai EC, Murphy MF, Natter HM, Norris FH, Rudnicki S..

Recombinant human insulin-like growth factor-I in ALS: description of a double-blind, placebo-controlled study.

Neurology, 1996, 47(4 Suppl 2): S93 - 4.

Hypothesis/Rationale: The study assessed the effects of rhIGF-I in patients with mild or moderate ALS.The choice of rhIGF-I was based on the hypothesis that the treatment would exert neurotrophic effects which would slow the rate of decline in treated patients vs. those receiving placebo.

Location of Study: Houston, TX

Study Results: **Clear benefit.**

The authors observed statistically significant, dose-related differences in the rate of progression between patients receiving treatment and the placebo. Patients treated with IGF appeared to have a statistically significant slower rate of FVC decline, and a statistically significant difference in survival time between the two groups. There were no clinically significant adverse experiences with the treatment.

Study Design	
Length	9 months
Purpose:	
	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	141
Treatment	99
Control	42
Data collected	Appel ALS score, Sickness Impact Profile (SIP)

Dosing	
Treatment(s)	IGF-1
Dose(s)/Schedule	0.05 mg/kg/day or 0.10 mg/kg/day

Miller RG, Bryan WW, Dietz MA, Munsat TL, Petajan JH, Smith SA, Goodpasture JC.

Toxicity and tolerability of recombinant human ciliary neurotrophic factor in patients with amyotrophic lateral sclerosis.

Neurology, 1996, 47(5): 1329 - 31.

Hypothesis/Rationale: The authors tested the tolerability and toxicity of subcutaneous rhCNTF at a variety of doses.

Location of Study: San Francisco, CA

Study Results:

Well tolerated at lower doses.

The tolerability of rhCNTF was equivalent to placebo at <= 5 mcg/day. Higher doses caused anorexia, weight loss, reactivation of herpes simplex virus, labialis/stomatitis, cough, and increased oral secretions.

Study Design	
Length	< 1 month
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	72
Treatment	50
Control	22
Data collected	Adverse events

Dosing	
Treatment(s)	CNTF
Dose(s)/Schedule	2 to 100 mcg/day

Gredal O, Werdelin L, Bak S, Christensen PB, Boysen G, Kristensen MO, Jespersen JH, Regeur L, Hinge HH, Jensen TS..

A clinical trial of dextromethorphan in amyotrophic lateral sclerosis.

Acta Neurologica Scandinavia, 1997, 96(1): 8 - 13.

Hypothesis/Rationale: The authors were following up on two pilot trials of dextromethorphan, an NMDA receptor agonist, conducted in the early 1990s. The approval of Rilutek, an anti-excitotoxic drug, in the mid 1990s encourage researchers to research or revisit a wide variety of possible anti-excitotoxic compounds.Dextromethorphan is a non-competitive NMDA-glutamate recepter antagonist, and had been shown to reduce both NMDA- and glutamate-induced excitotoxicity in vitro.

Location of Study: Denmark

Study Results: **Possible benefit.**

The authors observed no difference in survival between the two groups, and no difference in rates of disease progression except for a significantly less pronounced rate of decline in the lower extremities for the dextromethorphan group.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	45
Treatment	22
Control	23
% Male	48.9%
Data collected	Survival, FVC, functional disability

Dosing	
Treatment(s)	Dextromethorphan
Dose(s)/Schedule	150 mg/day

Lai EC, Felice KJ, Festoff BW, Gawel MJ, Gelinas DF, Kratz R, Murphy MF, Natter HM, Norris FH, Rudnicki SA..

Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study.

Neurology, 1997, 49(6): 1621 - 30.

Hypothesis/Rationale: The authors were investigating the safety and efficacy of rhIGF-1.Earlier trials of rhIGF-I had been smaller, and had focused primarily on assessing the safety, tolerability, and appropriate dose range for the treatment. IGF-I was of interest primarily for its purported neurotrophic effects.

Location of Study: Houston, TX

Study Results: **Clear benefit.**

Patients receiving high-dose rhIGF-1 showed a statistically significant slowing of disease symptom progression and were less likely to terminate the study because of advanced progression. Incidence of adverse events was comparable among all three treatments.

Study Design	
Length	9 months
Purpose:	
	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
✓	Phase II
✓	Phase III

Study Participant Demographics	
Total Participants	266
Treatment	176
Control	90
% Male	62.3%
Data collected	Disease symptom progression (Appel ALS rating scale, Sickness Impact Profile)

Dosing	
Treatment(s)	IGF-1
Dose(s)/Schedule	0.05 or 0.10 mg/kg/day

Sojka P, Andersen PM, Forsgren L.

Effects of riluzole on symptom progression in amyotrophic lateral sclerosis.

Lancet, 1997, 349(9046): 176 - 7.

Hypothesis/Rationale: The authors attempted to determine whether riluzole affected the progression of symptoms, or merely improved survival rates in ALS.Earlier studies demonstrating the efficacy of ALS had focused almost exclusive on survival, and were unable to show any notable effect on the clinical symptoms of ALS. The authors hoped that by closely monitoring a wide range of clinical parameters in a small number of patients, they would be able to characterize what, if any, impact riluzole has on the outward clinical symptoms of ALS.

Location of Study: Sweden

Study Results: **Clear benefit.**

The authors observed significant alterations in the natural course of ALS for four out of the five riluzole-treated patients. This alteration, however, varied greatly among the four patients, suggesting to the authors that ALS patients did not constitute a homogeneous group with regard to the efficacy or biological activity of riluzole.

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	5
Treatment	5
Control	0
% Male	80%
Data collected	TQNE

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg 2x/day (oral)

Cudkowicz ME, Warren L, Francis JW, Lloyd KJ, Friedlander RM, Borges LF, Kassem N, Munsat TL, Brown RH Jr..

Intrathecal administration of recombinant human superoxide dismutase 1 in amyotrophic lateral sclerosis: a preliminary safety and pharmacokinetic study.

Neurology, 1997, 49(1): 213 - 22.

Hypothesis/Rationale: The authors were conducting a preliminary study on the pharmacokinetic and safety profile of intrathecally administered recombinant human superoxide dismutase.At the time, mutations in the SOD1 gene had recently been identified as a cause of one form of familial ALS. The exact mechanism of action of this mutation had not yet been fully investigated. The pursuit of rhSOD1 as a treatment for ALS was based on the assumption that the mutation was a loss-of-function mutation resulting in increased oxidative stress.

Location of Study: Boston, MA

Study Results: **No benefit.**

The authors detected no benefit of the treatment in the two patients with familial ALS. Chronic infusion of rhSOD1 yielded a forty-fold increase in cerebrospinal SOD1 levels; intrathecal injection of 20 micrograms of rhSOD1 showed a half-life of 2.2 hours

Study Design	
Length	3 – 6 months
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	16
Treatment	16
Control	0
% Male	81.3%
Data collected	TQNE, blood and CSF chemistry

Dosing	
Treatment(s)	rSOD-1
Dose(s)/Schedule	2 familial ALS patients received Intrathecal infusion of 5-10 mg/day for 3 - 6 months 14 non-familial patients received a single, 20 mcg intrathecal injection

Penn RD, Kroin JS, York MM, Cedarbaum JM.

Intrathecal ciliary neurotrophic factor delivery for treatment of amyotrophic lateral sclerosis (phase I trial).

Neurosurgery, 1997, 40(1): 94 - 99.

Hypothesis/Rationale: The purpose of the study was to test the safety and tolerability of delivering rhCNTF directly and continuously into CSF via an implanted infusion pump.Systemic administration of CNTF had resulted in unacceptable side effects; this study was the first to investigate a new means of delivering CNTF directly into the CSF and thereby avoiding the consequences of the high systemic doses of CNTF required to ensure therapeutic concentrations in the CSF.

Location of Study: Chicago, IL

Study Results: **Well tolerated.**

The authors observed that the distribution and clearance of rhCNTF was similar to that of many small, water-soluble agents. The steady-state concentration of rHCNTF at cervical level was 18 to 36% that at the lumbar level. No asthenia, fever, chills, nausea, weight loss, or other major side effects were observed.

Study Design	
Length	1 – 3 months
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	✓ Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	4
Treatment	4
Control	0
Data collected	Adverse events, CSF levels of CNTF, muscle/respiratroy tests

Dosing	
Treatment(s)	CNTF
Dose(s)/Schedule	0.4 - 8 micrograms/h over 48 hours using drug pump implanted in lumbar subarachnoid space

Bruno R, Vivier N, Montay G, Le Liboux A, Powe LK, Delumeau JC, Rhodes GR.

Population pharmacokinetics of riluzole in patients with amyotrophic lateral sclerosis

Clinical Pharmacology & Therapeutics, 1997, 62(5): 518 - 526.

Hypothesis/Rationale: The purpose of the study was to test whether the pharmacokinetics of Riluzole differed based on age, gender, treatment duration, and a range of other factors.Population pharmacokinetics (assessing differences in drug metabolism between individuals) was a crucial step in developing dosage guidelines for rilute, since earlier pharmacokinetic studies had been done in only a limited number of patients, most of whom were healthy, non-ALS volunteers.

Location of Study: France

Study Results: Interindividual variation in plasma clearance was greater than intraindividual variation. Clearance was independent of dosage, treatment duration, age, and renal function. Gender and smoking were the most important factors influencing clearance variation.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	✓ Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	100
Treatment	100
Control	0

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	25 to 100 mg bid

Gourie-Devi M, Nalini A, Subbakrishna DK.

Temporary amelioration of symptoms with intravenous cyclophosphamide in amyotrophic lateral sclerosis.

Journal of the Neurological Sciences, 1997, 150(2): 167 - 72.

Hypothesis/Rationale: The authors tested the effectiveness of cyclophosphamide in treating ALS.Cyclophosphamide was chosen for its immunosuppressant properties; this choice was based on the assumption that autoimmunity played a role in the pathogenesis of ALS.

Location of Study: India

Study Results: **Clear benefit.**

A large subset of treated patients (23 out of 44) showed a statistically significant improvement in composite and individual neurological scores. The improvements lasted for 2 to 3 months after the cessation of cyclophosphamide treatment. The authors were unable to find any differences between non-responders and responders that might explain the difference between the two groups.

Study Design	
Length	1 – 3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	44
Treatment	44
Control	0
% Male	79.5
Data collected	Neurological score electromyography, nerve conduction, haematological & biochemical tests, and chest X-rays

Dosing	
Treatment(s)	Cyclophosphamide
Dose(s)/Schedule	1.5 g/m2 IV over 8 to 10 days

Borasio GD, Robberecht W, Leigh PN, Emile J, Guiloff RJ, Jerusalem F, Silani V, Vos PE, Wokke JH, Dobbins T.

A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. European ALS/IGF-I Study Group.

Neurology, 1998, 51(2): 583 - 6.

Hypothesis/Rationale: The authors tested the safety and efficacy of rhIGF-1 in ALS and attempted to replicate the findings of an earlier study, which suggested treatment with IGF-I conferred a 26% survival benefit.IGF-I was of interested for it putative neurotrophic and neuroprotective effects.

Location of Study: Germany

Study Results: **No benefit. Well-tolerated.**

There was a slight, but not statistically significant, slowing of progression observed in the treated vs. placebo groups. Although the authors could demonstrate no statistically significant difference in primary efficacy outcome measure, rhIGF-1 appeared to be safe and well-tolerated.

Study Design	
Length	9 months
Purpose:	
	✓ Safety
	Dose-ranging
	Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	✓ Phase II
	Phase III

Study Participant Demographics	
Total Participants	96
Treatment	65
Control	31
Data collected	Change in disease progression as measured by Appel ALS rating scale, Sickness Impact Profile (SIP)

Dosing	
Treatment(s)	IGF-1
Dose(s)/Schedule	0.1 mg/kg/day subcutaneously

M. Riviere, V. Meininger, P. Zeisser, T. Munsat.

An analysis of extended survival in patients with amyotrophic lateral sclerosis treated with riluzole.

Archives of Neurology, 1998, 55(4): 526 - 8.

Hypothesis/Rationale: This study reanalyzed the data from an earlier study in order to better assess whether riluzole slows the progression of ALS or simply confers an end-of-life survival benefit.

Location of Study: Boston, MA

Study Results: **Clear benefit.**

Among patients who entered the study in the milder states of ALS, the data suggested that significant prolongation of the time before progression to the next health state for treated patients vs placebo.

Study Design	
Length	18 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	959
Treatment	717
Control	242
% Male	60%
Data collected	ALS Health State Scale based on a modified Norris scale

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50, 100, and 200 mg/day

Kaji R, Kodama M, Imamura A, Hashida T, Kohara N, Ishizu M, Inui K, Kimura J.

Effect of ultrahigh-dose methylcobalamin on compound muscle action potentials in amyotrophic lateral sclerosis: a double-blind controlled study.

Muscle & Nerve, 1998, 21(12): 1775 - 8.

Hypothesis/Rationale: The study investigated whether methylcobalamin could slow or reverse the loss of motor neurons, using CMAP amplitudes as a surrogate marker for neuronal loss.Methylcobalamin is a vitamin B12 analog and had been shown to protect neurons against glutamate-induced toxicity in vitro.

Location of Study: Japan

Study Results: **Possible benefit.**

Patients treated with the low dose methylcobalamin had not significant changes in CMAP amplitude either 2 or 4 weeks after treatment. The twelve patients who received ultra-high-dose methylcobalamin demonstrated a significant increase in CMAP amplitude at 4 weeks. The authors concluded that a larger, extended trial of methylcobalamin was warranted.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	✓ Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	24
Treatment	24
% Male	45.8%
Data collected	Compound muscle action potential amplitudes

Dosing	
Treatment(s)	Methylcobalamin
Dose(s)/Schedule	25 mg/day or 0.5 mg/day IM

Apostolski S, Marinkovic Z, Nikolic A, Blagojevic D, Spasic MB, Michelson AM..

Glutathione peroxidase in amyotrophic lateral sclerosis: the effects of selenium supplementation.

Journal of Environmental Pathology, Toxicology and Oncology, 1998, 17(3-4): 325 - 9.

Hypothesis/Rationale: The authors tested the efficacy of a variety of antioxidants in slowing or reversing the clinical progression of ALS.The authors selected antioxidant therapy based on the discovery that a mutation of CuZN SOD (an antioxidant protein) caused a form of familial ALS, and on other studies which suggested reactive oxygen species played a key etiological role in sporadic ALS.

Location of Study: Yugoslavia

Study Results:

Clear benefit.

ALS patients had significantly decreased activity of both GSH-Px and CuZn SOD compared to controls - suggesting that a disturbed oxidative/antioxidative balance exists not only in motor neurons but also in the blood. Alsamin (a combination of selenium, antioxidants, amino acids, and nimodipine) appeared to enhance the activity of GSH-Px and increased levels of Vitamin E. The authors reported that Alsamin halted the course of the disease in the 7 patients who received the treatment, while patients in the other treatment groups and those receiving placebo continued to decline. (However, the study does not include calculations of statistical significance, nor does it indicate that patients were assigned randomly to the various treatment groups or that the study was double-blind. Given the wide range of literature on 'benign' courses of ALS which occur independently of treatment, these omissions are obviously problematic.)

Study Design	
Length	3 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	
Blind?	
Phase?	
✓	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	35
Treatment	28
Control	7
Data collected	Norris score, blood chemistry

Dosing	
Treatment(s)	Alsamin (selenium, Ca2+ blocker, amino acids, antioxidants)
Dose(s)/Schedule	N/A

Kalra S, Cashman NR, Genge A, Arnold DL.

Recovery of N-acetylaspartate in corticomotor neurons of patients with ALS after riluzole therapy.

Neuroreport, 1998, 9(8): 1757 - 61.

Hypothesis/Rationale: The authors investigated a possible mechanism of action of Riluzole, specifically whether Riluzozle reduced the rate of glutamate-toxicity-induced motor neuronal loss.The authors used N-acetylaspartate (NAA) as surrogate marker for neuronal loss - decreases in relative intensity of NAA as measured by MRI would indicate a loss of motor neurons. If levels of NAA increased or stabilized after treatment with Riluzole, that would indicate a reduction in the rate of neuronal loss. A secondary task would be to correlate this reduction, if observed, with the outward clinical signs of progression.

Location of Study: Canada

Study Results: **Clear benefit.**

The authors observed an increase in NAA/Cr relative intensity in the treatment group and a decrease in the untreated group. Though each of these trends was on its own not statistically significant, the difference between the two groups did reach statistical significance. The authors concluded that Riluzole leads to a reversal in sublethal neuronal loss within weeks of intiating treatment.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	✓ Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Add'l comments	Relative intensity Levels of NAA/Creatine before and 3 weeks after treatment with riluzole measured via proton magnetic resonance spectroscopy. (Increases in relative intensity would indicate a reduction in neuronal loss.)

Study Participant Demographics	
Total Participants	23
Treatment	11
Control	12
% Male	56.5%

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg bid

Desai J, Sharief M, Swash M.

Riluzole has no acute effect on motor unit parameters in ALS.

Journal of the Neurological Sciences, 1998, 160(Suppl 1): 0 - 72.

Hypothesis/Rationale: The study explored potential biological markers for therapeutic efficacy by testing the acute impact of riluzole on measurable neurophysiological parameters in ALS.By taking a drug which had already been shown to have efficacy in ALS, and tracking its impact on measurable neurophysiological parameters of ALS, the authors hoped to come up with a biological marker capable of bypassing the long trial timelines typically required in ALS.

Location of Study: U.K.

Study Results: The study suggested that riluzole has no acute effect on the motor unit, and that the therapeutic effect of the drug was not due to any acute biological effect. The authors concluded that EMG could not be used as an acute marker of progression or predictor of drug effect in the context studied.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
✓	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	✓
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	7
Treatment	7
Control	7
Data collected	EMG

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	100 mg/day

Lange DJ, Murphy PL, Diamond B, Appel V, Lai EC, Younger DS, Appel SH..

Selegiline is ineffective in a collaborative double-blind, placebo-controlled trial for treatment of amyotrophic lateral sclerosis.

Archives of Neurology, 1998, 55(1): 93 - 6.

Hypothesis/Rationale: The study assessed whether selegiline, an MAO-B inhibitor, could slow or reverse the progression of ALS.Selegiline had been found to slow the progression of Parkinson's; combined with emerging theories on the role of oxidative stress in ALS, this made it an attractive drug for clinical investigation.

Location of Study: New York, NY

Study Results: **No benefit.**

There was no difference in the rate of progression between the treatment and placebo groups.

Study Design	
Length	6 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	104
Treatment	53
Control	51
% Male	61.6%
Data collected	Appel ALS score

Dosing	
Treatment(s)	Selegiline
Dose(s)/Schedule	5 mg bid

Mazzini L, Mora G, Balzarini C, Brigatti M, Pirali I, Comazzi F, Pastore E..

The natural history and the effects of gabapentin in amyotrophic lateral sclerosis.

Journal of the Neurological Sciences, 1998, 160(Suppl 1): 57 - 63.

Hypothesis/Rationale: The study expanded on earlier studies of gabapentin and assessed the effect of different dosages and treatment durations on the clinical progression and survival of ALS patients.Gabapentin had been shown to prolong survival in a mouse model of ALS and to slow the decline of arm strength in human ALS.

Location of Study: Italy

Study Results: **Clear benefit.**

Patients treated with gabapentin had a statistically significant reduction in the rate of muscle strength deterioration versus controls. This difference was also observed (and reached statistical significance) using natural history controls. Patients receiving gabapentin at the high dose or for the longer time period had a significantly longer survival than controls.

Study Design	
Length	6 – 12 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	231
Treatment	110
Control	121
Data collected	ALSFRS, maximum isometric muscle strength, FVC, urinalysis, routine hematological tests, death or tracheostomy

Dosing	
Treatment(s)	Gabapentin
Dose(s)/Schedule	500 mg/day or 1000 mg/day

BDNF Study Group.

A controlled trial of recombinant methionyl human BDNF in ALS:

Neurology, 1999, 52(7): 1427 - 33.

Hypothesis/Rationale: This trial attempted to replicate encouraging safety and efficacy data from phase I and II trials of rhBDNF.BDNF had been shown to support the survival and growth of neurons in vitro, promoted motor neuron survival in a range of animal models of neurological disorders (including a naturally occurring murine motor neuron disease), and had yielded promising results in early clinical trials.

Location of Study: Miami, FL

Study Results:

Possible benefit.

The study failed to demonstrate a statistically significant survival benefit for the treatment vs. placebo groups. Unusually high survival percentages negatively affected the power of the study; restricting the analysis to certain subpopulations showed statistically significant survival advantages for patients with initial FVC of less than or equal to 91%, and for 20% of people who experience adverse reactions to BDNF within the first two weeks of treatment ("BDNF responders").

Study Design		
Length		9 months
Purpose:		
	✓	Safety
		Dose-ranging
		Pharmacokinetic/molecular
		Efficacy
Controlled?		✓
Crossover?		
Placebo?		✓
Randomized?		✓
Double-blind?		✓
Blind?		
Phase?		
		Pilot
		Phase I
		Phase II
	✓	Phase III

Study Participant Demographics	
Total Participants	1135
Treatment	748
Control	387
% Male	65.2%
Data collected	FVC, ALSFRS, serum chloride, survival

Dosing	
Treatment(s)	BDNF
Dose(s)/Schedule	25 mcg/kg/day or 100 mcg/kg/day

Sommer M, Tergau F, Wischer S, Reimers CD, Beuche W, Paulus W..

Riluzole does not have an acute effect on motor thresholds and the intracortical excitability in amyotrophic lateral sclerosis.

Journal of Neurology, 1999, 246(Suppl 3): 22 - 6.

Hypothesis/Rationale: This study measured the acute effect of riluzole on intracortical excitabilityPrevious studies had yielded inconclusive data on the acute impact of riluzole on intracortical excitability.

Location of Study: Germany

Study Results: Riluzole did not alter motor thresholds or intracortical excitability in ALS patients versus healthy controls.

Study Design		
Length		< 1 month
Purpose:		
		Safety
		Dose-ranging
	✓	Pharmacokinetic/molecular
		Efficacy
Controlled?		✓
Crossover?		
Placebo?		
Randomized?		
Double-blind?		
Blind?		
Phase?		
		Pilot
		Phase I
		Phase II
		Phase III

Study Participant Demographics	
Total Participants	17
Treatment	8
Control	9
Control selection	Age matched healthy controls
% Male	62.5%
Data collected	Transcranial magnetic stimulation/motor evoked potentials

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	100 mg riluzole

Cudkowicz ME, Sexton PM, Ellis T, Hayden DL, Gwilt PR, Whalen J, Brown RH Jr..

The pharmacokinetics and pharmaco-dynamics of Procysteine in amyotrophic lateral sclerosis.

Neurology, 1999, 52(7): 1492 - 4.

Hypothesis/Rationale: The authors conducted a preliminary study of the safety and pharmacokinetics of procysteine.Procysteine was of interest to the authors because of its possible effects on levels of glutathione, an antioxidant found in most cells. The rationale was that encouraging the body to increase production of its own natural antioxidants would be a more effective treatment than the systemic administration of exogenous antioxidant compounds.

Location of Study: Boston, MA

Study Results: **Well tolerated.**

The data suggested that oral administration of Procysteine was safe. Procysteine appeared to enter the CSF after both IV and oral dosing and accumulated to therapeutically significant levels in the CSF. Procysteine did not have a statistically beneficial effect in the patients treated, but observing efficacy was not part of the design of the study.

Study Design	
Length	< 1 month
Purpose:	
	✓ Safety
	Dose-ranging
	✓ Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	13
Treatment	13
Control	0
Data collected	TQNE, FVC, CBC, urinalysis

Dosing	
Treatment(s)	Procysteine
Dose(s)/Schedule	4.5 g over 1 hr IV

Ochs G, Penn RD, York M, Giess R, Beck M, Tonn J, Haigh J, Malta E, Traub M, Sendtner M, Toyka KV..

A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis.

Amyotroph Lateral Scler Other Motor Neuron Disord, 2000, 1(3): 201 - 6.

Location of Study: Germany

Study Design	
Length	3 - 3
Purpose:	
	✓ Safety
	✓ Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	✓ Phase I
	✓ Phase II
	Phase III

Study Participant Demographics	
Total Participants	25
Treatment	20
Control	5
Data collected	Adverse events

Dosing	
Treatment(s)	BDNF
Dose(s)/Schedule	25 to 1000 mcg/day

Beghi E, Chio A, Inghilleri M, Mazzini L, Micheli A, Mora G, Poloni M, Riva R, Serlenga L, Testa D, Tonali P.

A randomized controlled trial of recombinant interferon beta-1a in ALS. Italian Amyotrophic Lateral Sclerosis Study Group.

Neurology, 2000, 54(2): 469 - 74.

Hypothesis/Rationale: The study attempted to demonstrate efficacy of IFN-b in treating ALS by increasing dosage, sample size, and follow-up period. Earlier studies IFN-a and IFN-b yielded only negative results but the authors believed this was due to problems in study design.

Location of Study: Italy

Study Results: **No benefit.**

Although IFN treated patients appeared to have slower declines in FVC scores and faster declines in bulbar scores vs. the placebo group, neither of these differences was statistically significant. There were no differences between treatment and placebo for any of the electrophysiologic measures.

Study Design	
Length	6 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	61
Treatment	31
Control	30
% Male	68.9%
Data collected	Medical research council scale, Norris scale, bulbar scores, FVC, electrophysiologic measures

Dosing	
Treatment(s)	Interferon Beta
Dose(s)/Schedule	12,000,000 IU 3x/wk (subcutaneous)

Roch-Torreilles I, Camu W, Hillaire-Buys D.

Etude de tolérance du riluzole (Rilutek®) dans le traitement de la sclérose latérale amyotrophique.

[Adverse effects of riluzole (Rilutek) in the treatment of amyotrophic lateral sclerosis.]

Therapie, 2000, 55(2): 303 - 12.

Hypothesis/Rationale: The authors conducted a pharmacovigilance study to evaluate the safety and tolerability of riluzole.in a broader population than in initial studies.

Location of Study: France

Study Results: **Well tolerated.**

Riluzole induced one or more adverse effects in 50.3% of patients - most frequently, gastric disturbance, hepatotoxicity, and asthenia. This was assessed as an acceptable safety profile for riluzole.

Study Design		
Length		3 – 36 months
Purpose:		
	✓	Safety
		Dose-ranging
		Pharmacokinetic/molecular
		Efficacy
Controlled?		
Crossover?		
Placebo?		
Randomized?		
Double-blind?		
Blind?		
Phase?		
		Pilot
		Phase I
		Phase II
	✓	Phase III

Study Participant Demographics	
Total Participants	153
Treatment	153
Control	0

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg 2x/day

Pongratz D, Neundorfer B, Fischer W.

German open label trial of riluzole 50 mg b.i.d. in treatment of amyotrophic lateral sclerosis (ALS).

J Neurol Sci, 2000, 180(38657): 82 - 5.

Hypothesis/Rationale: The study analyzed the adverse events reported by German patients who participated in a multinationa, uncontrolled study of riluzole between 1995 and 1997.

Location of Study: Germany

Study Results:

Well tolerated.

The results of the study supported earlier conclusions that riluzole is well tolerated. Among German patients, serious adverse events occurred in 1.7% of patients; these were usually reversible alterations and appeared within the first three months of treatment. Overall, 44.9% of patients reported adverse events, with the most common being reduced lung function, nausea, asthenia, and pneumonia. Many of the adverse events could also be considered symptoms of the underlying disease.

Study Design	
Length	7 – 8 months
Purpose:	
	✓ Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	919
Treatment	919
% Male	58.5%

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg 2x/day

Desnuelle C, Dib M, Garrel C, Favier A.

A double-blind, placebo-controlled randomized clinical trial of alpha-tocopherol (vitamin E) in the treatment of amyotrophic lateral sclerosis. ALS riluzole-tocopherol Study Group.

Amyotroph Lateral Scler Other Motor Neuron Disord, 2001, 2(1): 9 - 18.

Hypothesis/Rationale: The authors based their selection of Vitamin E on increasing evidence on the role of oxidative stress in ALS. Vitamin E had been shown to slow the onset and progression of ALS in the SOD1 transgenic mouse model of ALS.

Location of Study: France

Study Results: **Possible benefit.**

Survival was not influenced by treatment. However, patients given alpha-tocopherol were less likely to progress between disease states in the ALS Health State scale.

Study Design	
Length	12 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	289
Treatment	144
Control	145
Control selection	
% Male	0
Data collected	Norris limb scale, survival, biochemical markers of oxidative stress (in subset)

Dosing	
Treatment(s)	Alpha tocopherol (Vitamin E)
Dose(s)/Schedule	500 mg bid

Mazzini L, Balzarini C, Colombo R, Mora G, Pastore I, De Ambrogio R, Caligari M..

Effects of creatine supplementation on exercise performance and muscular strength in amyotrophic lateral sclerosis: preliminary results.

Journal of the Neurological Sciences, 2001, 191(1-2): 139 - 44.

Hypothesis/Rationale: The study examined the effect of creatine supplementation on exercise performance and muscle strength in ALS patients.

Location of Study: Italy

Study Results: **Short-term benefit.**
The results suggested that creatine supplementation temporarily increases maximal isometric power in ALS patients and may be useful as a symptomatic treatment.

Study Design	
Length	3- 6 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	28
Treatment	28
Control	0
% Male	52.8%

Dosing	
Treatment(s)	Creatine
Dose(s)/Schedule	20 g/day for 7 days followed by 3 g/day for 3-6 months

Groeneveld GJ, van Kan HJ, Torano JS, Veldink JH, Guchelaar HJ, Wokke JH, van den Berg LH.

Inter- and intraindividual variability of riluzole serum concentrations in patients with ALS.

Journal of the Neurological Sciences, 2001, 191(38640): 121 - 5.

Hypothesis/Rationale: The authors investigated whether interindividual variation in riluzole concentrations among ALS patients was greater than intraindividual variation. Although all ALS patients typically receive the same dose of riluzole daily, variation in drug uptake and metabolism could lead to wide variation in therapeutic outcomes and could have implications for clinical practice.

Location of Study: Netherlands

Study Results: The study suggested that riluzole concentrations in the serum of patients with ALS varied greatly from person to person. Peak concentrations showed almost 50-fold differences from patient to patient. This interindividual variation was significant greater than intraindividual variation observed from day to day. The authors hypothesized that interindividual variation might be explained by the genetic variation in patients' ability to metabolize riluzole, or by differences in bioavailability caused by diet or other factors.

Study Design	
Length	< 1 month
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	21
Treatment	21
Control	0
% Male	76.2%

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg bid

Miller RG, Moore DH 2nd, Gelinas DF, Dronsky V, Mendoza M, Barohn RJ, Bryan W, Ravits J, Yuen E, Neville H, Ringel S, Bromberg M, Petajan J, Amato AA, Jackson C, Johnson W, Mandler R, Bosch P, Smith B, Graves M, Ross M, Sorenson EJ, Kelkar P, Parry G, Oln.

Phase III randomized trial of gabapentin in patients with amyotrophic lateral sclerosis.

Neurology, 2001, 56(7): 843 - 8.

Hypothesis/Rationale: The study expanded on an earlier Phase II study which suggested that gabapentin slowed the rate of strength decline in ALS patients.Gabapentin is a glutamate-blocking drug; preclinical studies had suggested gabapentin could prolong motor neuron survival.

Location of Study: San Francisco, CA

Study Results:

No benefit.

The authors found no differences in the rate of muscle strength deterioration between treatment and placebo groups. In fact, analysis of the combined data from the Phase II and Phase III trials suggested that patients treated with gabapentin had a significantly more rapid decline in FVC.

Study Design	
Length	9 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	128
Treatment	65
Control	63
% Male	61.5%

Dosing	
Treatment(s)	Gabapentin
Dose(s)/Schedule	3,600 mg/day

Debove C, Zeisser P, Salzman PM, Powe LK, Truffinet P.

The Rilutek (riluzole) Global Early Access Programme: an open-label safety evaluation in the treatment of amyotrophic lateral sclerosis.

Amyotroph Lateral Scler Other Motor Neuron Disord, 2001, 2(3): 153 - 8.

Hypothesis/Rationale: The purpose of the study was primarily to expand safety data on riluzole, and to provide open label access to the drug for all patients who had earlier participated in clinical trials.

Location of Study: Aventis Pharma

Study Results: **Safe and well tolerated.**
Only 1.9% of serious adverse events were considered to be related to the study drug. The safety results were consistent with those previously reported from placebo-controlled trials.

Study Design	
Length	20 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
✓	Phase III

Study Participant Demographics	
Total Participants	7916
Treatment	7916
Control	0

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg bid

Bensimon G, Lacomblez L, Delumeau JC, Bejuit R, Truffinet P, Meininger V; Riluzole/ALS Study Group II.

A study of riluzole in the treatment of advanced stage or elderly patients with amyotrophic lateral sclerosis.

Journal of Neurology, 2002, 249(5): 609 - 15.

Hypothesis/Rationale: The purpose of this study was to investigate the efficacy of ALS in populations excluded from previous studies - namely, the elderly and those suffering from advanced stages of the disease.

Location of Study: France

Study Results:

Well tolerated.

Riluzole was well tolerated in the patient population, with adverse events appearing with a similar frequency as in earlier studies. The study did not reach an adequate power to detect differences in survival between the two groups, and no major difference was observed. The authors concluded that riluzole was well tolerated, even in patients in advanced stages of the disease.

Study Design	
Length	18 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
✓	Phase III

Study Participant Demographics	
Total Participants	168
Treatment	82
Control	86
% Male	48.8%
Data collected	Survival, manual muscle testing, Clinical Global Impression of Change, ventilatory function, self-assessment

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg bid

I. Niebroj-Dobosz, P. Janik, H. Kwiecinski.

Effect of Riluzole on serum amino acids in patients with amyotrophic lateral sclerosis.

Acta Neurologica Scandinavia, 2002, 106(1): 39 - 43.

Hypothesis/Rationale: The authors attempted to determine whether the statistically observed clinical impact of riluzole administration (that the drug slightly extends the time to tracheostomy) can be correlated with changes induced by riluzole in serum amino acid concentration

Location of Study: Poland

Study Results: Before starting treatment with riluzole, the majority of ALS patients had elevated levels of glutamate, GABA, and total amino acids. (The elevated levels of GABA were the most statistically significant vs. healthy controls.) After 6 months of treatment, glutamate and total amino acid levels decreased, but later returned to or exceeded their initial values. This effect appeared to be limited mainly to advanced stage ALS patients.

Study Design	
Length	18 months
Purpose:	
	Safety
	Dose-ranging
✓	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	37
Treatment	17
Control	20
Control selection	Healthy volunteers (10 male, 10 female)
% Male	70.6%
Data collected	Levels of amino acids in blood serum

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	100 mg/day

Lacomblez L, Bensimon G, Leigh PN, Debove C, Bejuit R, Truffinet P, Meininger V; ALS Study Groups I and II..

Long-term safety of riluzole in amyotrophic lateral sclerosis.

Amyotroph Lateral Scler Other Motor Neuron Disord, 2002, 3(1): 23 - 9.

Hypothesis/Rationale: The authors assessed the long-term safety of riluzole in light of its recent regulatory approval and in order to contribute to a broader understanding of the safety and tolerability of the treatment.

Location of Study: France

Study Results:

Safe and well tolerated.

No particular adverse event, or frequency of adverse event, appeared to be related to the dose level of the previous double-blind riluzole treatment. Drug is well tolerated for long periods of up to almost 7 years.

Study Design		
Length		14 – 43 months
Purpose:		
	✓	Safety
		Dose-ranging
		Pharmacokinetic/molecular
		Efficacy
Controlled?		
Crossover?		
Placebo?		
Randomized?		
Double-blind?		
Blind?		
Phase?		
		Pilot
		Phase I
		Phase II
	✓	Phase III

Study Participant Demographics	
Total Participants	516
Treatment	516
Control	0
% Male	62.4%

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg bid

Lacomblez L, Dib M, Doppler V, Faudet A, Robin V, Salachas F, Bensimon G, Meininger V..

Tolérance du riluzole dans un essai ouvert (phase IIIb). [Tolerance of riluzole in an open-label Phase IIIb study.]

Therapie, 2002, 57(1): 65 - 71.

Hypothesis/Rationale: The authors conducted a multi-center open label study in order to assess the safety of riluzole in a broader clinical population than had been addressed in previous studies.

Location of Study: France

Study Results: **Well tolerated.**

Safety data was similar to that in previous clinical trials; the most frequent adverse effects were asthenia, nausea and elevation of serum transaminase levels.

Study Design	
Length	3 – 24 months
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
✓	Phase III

Study Participant Demographics	
Total Participants	2069
Treatment	2069
Control	0
% Male	55.4%

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg 2x/day

H. Ryberg, H. Askmark, and L.I. Persson.

A double-blind randomized clinical trial in amyotrophic lateral sclerosis using lamotrigine: effects on CSF glutamate, aspartate, branched-chain amino acid levels and clinical parameters.

Acta Neurologica Scandinavia, 2003, 108(1): 1 - 8.

Hypothesis/Rationale: The study assessed whether lamotrigine in a normal therapeutic dose could positively impact the clinical parameters of ALS or levels of excitatory amino acids in the CSF of ALS patients.Lamotrigine, an anti-epileptic drug, had been shown to inhibit glutamate release and deactivate voltage-gated calcium channels in vitro.

Location of Study: Sweden

Study Results: **No benefit.**
There were no significant differences in the clinical scores of patients during treatment with Lamotrigine vs. placebo. At the end of the treatment period, there were higher levels of LTG in the serum and CSF of patients in the placebo-lamotrigine group

Study Design	
Length	6 months
Purpose:	
	Safety
	Dose-ranging
✓	Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	✓
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	30
Treatment	30
Control	30
% Male	61.5%
Data collected	Clinical scales, CSF levels of glutamate, aspartate, branched-chain amino acids, and Lamotrigine

Dosing	
Treatment(s)	Lamotrigine
Dose(s)/Schedule	300 mg/day

Kalra S, Genge A, Arnold DL.

A prospective, randomized, placebo-controlled evaluation of corticoneuronal response to intrathecal BDNF therapy in ALS using magnetic resonance spectroscopy: feasibility and results.

Amyotroph Lateral Scler Other Motor Neuron Disord, 2003, 4(1): 22 - 6.

Hypothesis/Rationale: The authors supplemented an ongoing trial of BDNF in ALS by tracking N-acetylaspartate levels as a marker of neuronal survival (higher levels would indicate higher numbers of surviving neurons.) This study was part of an effort to identify a suitable biomarker for screening drug effects in human subjects.

Location of Study: Canada

Study Results: **No benefit.**

Lack of change in NAA correlated with the lack of clinical efficacy and supports the validity of NAA/Cr as a surrogate in this setting. MRSI a feasible and safe method to evaluate intrathecal therapies in ALS.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
✓	Phase III

Study Participant Demographics	
Total Participants	11
Treatment	5
Control	6

Dosing	
Treatment(s)	BDNF
Dose(s)/Schedule	25 or 150 mcg/day via continuous infusion

G.J. Groeneveld, J.H. Veldink, I. van der Tweel, S. Kalmijn, C. Beijer, M. de Visser, J.H. Wokke, H. Franssen, L.H. van den Berg..

A randomized sequential trial of creatine in amyotrophic lateral sclerosis.

Annals of Neurology, 2003, 53(4): 437 - 45.

Hypothesis/Rationale: The authors attempted to determine whether creatine supplementation has a measurable effect on patient survival or symptoms.The choice of creatine as an experimental treatment is based on evidence related to the involvement of mitochondrial dysfunction in the processes leading to cell death in neurodegenerative disorders.

Location of Study: Netherlands

Study Results: **No benefit.**

The trial was stopped after 34 patients died and it could be demonstrated that there was no statistically significant difference in survival between the two groups. The final analysis of the data includes secondary endpoint data from all 175 patients.

Study Design	
Length	16 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	175
Treatment	88
Control	87
% Male	67.4%
Data collected	Survival, clinical scores

Dosing	
Treatment(s)	Creatine
Dose(s)/Schedule	5 gm (powdered) 2x/dy

Cudkowicz ME, Shefner JM, Schoenfeld DA, Brown RH Jr, Johnson H, Qureshi M, Jacobs M, Rothstein JD, Appel SH, Pascuzzi RM, Heiman-Patterson TD, Donofrio PD, David WS, Russell JA, Tandan R, Pioro EP, Felice KJ, Rosenfeld J, Mandler RN, Sachs GM, Bradley WG.

A randomized, placebo-controlled trial of topiramate in amyotrophic lateral sclerosis.

Neurology, 2003, 61(4): 456 - 64.

Hypothesis/Rationale: The study investigated whether topiramate slowed disease progression, and whether the drug was safe and well-tolerated in ALS patients.Topiramate, and FDA approved treatment for epilepsy, has antiexcitotoxic properties andcan diminish glutamate release from neurons and prevent activation of the AMPA glutamatergic excitatory amino acid receptor. In vitro studies suggested that topiramate protects motor neurons in a dose-dependent fashion.

Location of Study: Boston, MA

Study Results:

No benefit.

Topiramate did not appear to have a beneficial effect for ALS patients. Patients treated with topiramate had a faster decline in arm strength during the treatment period, and use of the treatment was associated with an increase in a range of adverse events, including anorexia, depression, diarrhea, ecchymosis, mausea, kidney calculus, and paresthesia. The authors concluded that further studies of topiramate were probably not warranted.

Study Design	
Length	12 months
Purpose:	
	Safety
	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	294
Treatment	197
Control	97
% Male	64.3%
Data collected	MVIC, FVC, ALSFRS, vital signs, physical exam, adverse event reports

Dosing	
Treatment(s)	Topiramate
Dose(s)/Schedule	800 mg/day

Pattee GL, Post GR, Gerber RE, Bennett JP Jr.

Reduction of oxidative stress in amyotrophic lateral sclerosis following pramipexole treatment.

Amyotroph Lateral Scler Other Motor Neuron Disord, 2003, 4(2): 90 - 5.

Hypothesis/Rationale: The authors selected pramipexole based on its anti-oxidant properties and a growing number of studies suggesting the involvement of oxidative stress in ALS.

Location of Study: Lincoln, NE

Study Results: **Possible benefit.**

2,3-DHBA serum levels reduced by 45% and DHBA/salicylate ratios declined by 59% following treatment with pramipexole, although the study design did not allow these results to be correlated with the outward clinical signs of the disease.

Study Design		
Length		1 month
Purpose:		
		Safety
		Dose-ranging
		Pharmacokinetic/molecular
	✓	Efficacy
Controlled?		
Crossover?		
Placebo?		
Randomized?		
Double-blind?		
Blind?		
Phase?		
		Pilot
		Phase I
		Phase II
		Phase III

Study Participant Demographics	
Total Participants	12
Treatment	12
Control	0

Dosing	
Treatment(s)	Pramipexole
Dose(s)/Schedule	up to 6 mg/d

Groeneveld GJ, Van Kan HJ, Kalmijn S, Veldink JH, Guchelaar HJ, Wokke JH, Van den Berg LH.

Riluzole serum concentrations in patients with ALS: associations with side effects and symptoms.

Neurology, 2003, 61(8): 1141 - 3.

Hypothesis/Rationale: The study investigated interindividual variation in riluzole serum concentrations, controlling for body mass.

Location of Study: Netherlands

Study Results: The authors observed inter-individual variation in riluzole serum levels that was higher than previously observed. However, adverse events were more likely to be reported by patients with high serum concentrations, suggesting that dosing could be increased for patients with low serum concentrations without the risk of serious side effects. In addition, fasciculations and muscle stiffness occurred less frequently among patients with higher riluzole concentrations.

Study Design	
Length	< 1 month
Purpose:	
	Safety
	Dose-ranging
	✓ Pharmacokinetic/molecular
	Efficacy
Controlled?	
Crossover?	
Placebo?	
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	169
Treatment	169
Control	0
% Male	68.8%

Dosing	
Treatment(s)	Riluzole
Dose(s)/Schedule	50 mg 2x/day

Steele J, Matos LA, Lopz EA, Perez-Pinzon MA, Prado R, Busto R, Arheart KL, Bradley WG.

A Phase I safety study of hyperbaric oxygen therapy for amyotrophic lateral sclerosis.

Amyotrophic Lateral Sclerosis & Other Motor Neuron Disorders, 2004, 5(2): 107 - 17.

Hypothesis/Rationale: Hyperbaric oxygen treatment had been shown to delay the onset of weakness in the wobbler mouse.

Location of Study: Miami, FL

Study Results: **Clear benefit.**

Four patients reported decreased fatigue, while one dropped out due to increased fatigue. Maximum isometric voluntary contraction of all muscle groups except right hand grip improved significantly by up to 97%.

Study Design		
Length		< 1 month
Purpose:		
		Safety
		Dose-ranging
		Pharmacokinetic/molecular
	✓	Efficacy
Controlled?		
Crossover?		
Placebo?		
Randomized?		
Double-blind?		
Blind?		
Phase?		
		Pilot
	✓	Phase I
		Phase II
		Phase III

Study Participant Demographics	
Total Participants	5
Treatment	5
Control	0
% Male	0

Dosing	
Treatment(s)	Hyperbaric oxygen therapy
Dose(s)/Schedule	100% oxygen @ 2 atmospheres pressure for 60 min daily, 5d/wk

Meininger V, Bensimon G, Bradley WR, Brooks B, Douillet P, Eisen AA, Lacomblez L, Leigh PN, Robberecht W..

Efficacy and safety of xaliproden in amyotrophic lateral sclerosis: results of two phase III trials.

Amyotroph Lateral Scler Other Motor Neuron Disord, 2004, 5(2): 107 - 17.

Hypothesis/Rationale: Xaliproden was chosen because it was a non-peptidic compound that nevertheless exhibits neurotrophic properties. Earlier trials using neurotrophic proteins had shown promising pre-clinical and pilot results, but large-scale investigations ultimately proved futile due to toxicity and pharmacokinetic issues related to the high molecular weight and morphology of the compounds used.

Location of Study: France

Study Results: **Possible benefit**.

Although differences in primary outcome measures did not reach statistical significance, in both studies certain measures trended toward a beneficial effect of Xaliproden. In the first study, time to VC <50% was significantly reduced for patients treated with Xaliproden.

Study Design	
Length	0 - 0
Purpose:	
✓	Safety
	Dose-ranging
	Pharmacokinetic/molecular
	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	
Blind?	
Phase?	
	Pilot
	Phase I
	Phase II
	Phase III

Study Participant Demographics	
Total Participants	2077
Treatment	1038
Control	1039
Data collected	Survival, time to VC<50%

Dosing	
Treatment(s)	Xaliproden
Dose(s)/Schedule	1 or 2 mg/d orally OR 1 or 2 mg/d plus 50 mg riluzole 2x/day

Gordon PH, Moore DH, Gelinas DF, Qualls C, Meister ME, Werner J, Mendoza M, Mass J, Kushner G, Miller RG.

Placebo-controlled phase I/II studies of minocycline in amyotrophic lateral sclerosis.

Neurology, 2004, 62(10): 1845 - 7.

Hypothesis/Rationale: The authors conducted two clinical trials designed to test the safety and tolerability of minocycline at different doses.Minocycline had in earlier studies been shown to have neuroprotective effects in animal models of neuronal injury, Parkinson's disease, and rodent models of ALS. Although the exact mechanism of minocycline in ALS was not clear, it had been hypothesized that minocycline acted primarily as an anti-inflammatory and anti-apoptotic.

Location of Study: New York, NY

Study Results: **No benefit.**

There were no significant difference in adverse events between the treatment and placebo groups in the first trial, or the treatment and placebo periods in the second. The mean tolerated dose was 387 mg/day.

Study Design	
Length	6 – 8 months
Purpose:	
	✓ Safety
	Dose-ranging
	Pharmacokinetic/molecular
	✓ Efficacy
Controlled?	✓
Crossover?	✓
Placebo?	✓
Randomized?	✓
Double-blind?	✓
Blind?	✓
Phase?	
	Pilot
	✓ Phase I
	✓ Phase II
	Phase III

Study Participant Demographics	
Total Participants	42
Treatment	33
Control	9
% Male	59.5%

Dosing	
Treatment(s)	Minocycline
Dose(s)/Schedule	100 mg 2x/day OR 100 mg 2x/day increasing each week by 50 mg bid up to 400 mg/day

Lacomblez L, Bensimon G, Douillet P, Doppler V, Salachas F, Meininger V.

Xaliproden in amyotrophic lateral sclerosis: early clinical trials.

Amyotroph Lateral Scler Other Motor Neuron Disord, 2004, 5(2): 99 - 106.

Hypothesis/Rationale: The authors selected Xaliproden based on the fact that it is a non-peptidic compound that nevertheless exhibits neurotrophic properties. Earlier trials using neurotrophic proteins had shown promising pre-clinical and pilot results, but large-scale investigations ultimately proved futile due to toxicity and pharmacokinetic issues. The authors hypothesized that using a non-peptidic compound would help alleviate these issues and would exert a long-sought neurotrophic effect in ALS.

Location of Study: France

Study Results: **Possible benefit.**

While the 6-month analysis showed no significant differences between treatment and control, the final analysis showed a significant 43% slower rate of deterioration in FVC in xaliproden-treated patients but not in functional and MMT scores.

Study Design	
Length	8 months
Purpose:	
	Safety
✓	Dose-ranging
	Pharmacokinetic/molecular
✓	Efficacy
Controlled?	✓
Crossover?	
Placebo?	✓
Randomized?	
Double-blind?	✓
Blind?	
Phase?	
	Pilot
	Phase I
✓	Phase II
	Phase III

Study Participant Demographics	
Total Participants	54
Treatment	27
Control	27
Data collected	FVC, limb functional score, manual muscle testing score

Dosing	
Treatment(s)	Xaliproden
Dose(s)/Schedule	2 mg/day

Current Research Topics in ALS

June 2005

A Report to the Department of Public Health, State of Massachusetts on behalf of the ALS Therapy Development Foundation

Jennifer Clark
Doctoral Candidate, Department of the History of Science, Harvard University

CURRENT RESEARCH TOPICS IN ALS

The previous report, *Clinical Trials in ALS*, discussed the state of clinical research on ALS over the past six decades, tracing the rise and fall of etiological hypotheses and trial design paradigms over time and discussing major issues in the conduct of clinical research in ALS. Clinical investigations, however, provide only a small window of insight into the body of clinically relevant research being conducted in ALS. For one thing, clinical investigations are dependent on the availability and safety of investigational drugs. However, not all potential targets for therapeutic intervention can be modified by known chemical compounds, and not all compounds that affect a particular target are safe or feasible for use in clinical investigations. In addition, the vanguard of published clinical investigations does not necessarily correspond with the vanguard of laboratory research in a disease – conducting clinical research is dependent on obtaining funding, designing a trial, enrolling participants, conducting the trial itself (which may take a year or longer), and preparing trial data for publication. Thus, even the most up-to-date clinical trial data released through conference papers or publication may have been inspired by laboratory research conducted 2, 5, or even 10 years earlier. Understanding the future direction of clinical research in ALS requires close attention to the basic, translational, and pre-clinical research that will ultimately guide future clinical investigations.

Basic and pre-clinical research on ALS has increased dramatically in recent years. More than half of the 8,182 research papers published on ALS between 1945 and mid 2005 were published in the final decade of

that period, and almost a third of all scientific papers on ALS have been published within the past two years.[1] For obvious reasons, the thousands of publications reporting basic and pre-clinical research activities in ALS cannot be reviewed with the same degree of detail as the relatively small number of published clinical studies. Instead, this chapter provides a thorough (but not exhaustive) overview of major contemporary laboratory research topics in ALS that have the potential to impact future clinical therapeutic research. Topics covered include emerging understandings of the biology of ALS, the ongoing search for reliable biochemical markers of disease, the cellular and animal models of disease most frequently used to conduct preclinical studies, and a review of selected emerging etiological hypotheses.

Section I. ALS Clinical Characteristics & Biomarkers

At first glance, clinical perspectives on the basic outward signs and symptoms of ALS appear to have changed little in the past 130 years. Despite drastic changes in diagnostic practices, therapeutic options, palliative care technologies, and understandings of the biological processes behind the symptoms and signs of ALS, textbook descriptions of the core clinical syndrome remain remarkably similar to Charcot's initial definition of ALS – an unusual phenomenon in light of drastic changes in the clinical definitions of other neurological disorders during same time period, particularly multiple sclerosis and Alzheimer's Disease.[2] Yet despite this consistency in defining the traditional clinical signs & symptoms of the syndrome, research in recent years has suggested that the biological impact of ALS may extend beyond motor neurons and the central nervous system.

Elevated levels of glutamate, dysfunction in glutamate uptake mechanisms, increases in oxidative stress, and general alterations in immune system function – all factors believed to contribute to the degeneration of

motor neurons - have all been observed systemically.[3] Other phenomena bear a less direct connection to outward symptoms - researchers have long observed alterations in ALS patients' collagen metabolism, which may help explain the reduced incidence of bedsores among bedridden ALS patients.[4] Additional systemic changes observed in ALS include hypermetabolism and possible sub-clinical involvement of neurons other than the motor neurons, including the autonomic and sympathetic nervous systems.[5]

More importantly, recent studies have suggested a significant overlap between the syndrome of frontotemporal lobar dementia (FTLD) and ALS, with between 1/3 and 1/2 half of all ALS patients also showing signs consistent with FTLD. Traditionally, ALS has been considered a neurological disorder that spares cognition, but the increasing evidence on the overlap between ALS and FTLD challenges this notion. The symptoms of FTLD can be subtle (partial aphasia, behavioral changes) and can easily be overlooked or attributed to the stress of living with ALS by caregivers and patients, which may explain why cognitive involvement was initially ruled out as a component of the clinical syndrome of ALS.[6]

The search for sub-clinical manifestations of ALS and comorbid conditions is relevant to therapeutic research on ALS for several reasons. Many of the etiological hypotheses that have been proposed for ALS deal with more or less systemic phenomena with pathological effects that manifest themselves only in a specific population of neurons. Subclinical pathological changes outside of the spinal cord and central nervous system provide additional pieces of evidence against which to evaluate these etiological hypotheses.

In addition, phenomena external to the central nervous system may provide additional (and possibly more practical) sites for making a definitive diagnosis of ALS. These diagnostic parameters can also be used to assess how accurately animal models mimic human disease.

In addition to elucidating sub-clinical systemic and peripheral changes in ALS, research has increasingly focused on

identifying one or more biochemical markers of disease progression that coincide with or are predictive of observed clinical symptoms. Amyotrophic lateral sclerosis is at a disadvantage compared to many other diseases, because it is difficult to locate, verify, and quantify disease progression in living patients and even more difficult to do so over short periods of time. As discussed in the previous chapter, one effect of these obstacles has been that clinical trials in ALS tend to be quite lengthy, especially given the short survival times of most ALS patients, and must rely on survival rates as the ultimate measure of the clinical effectiveness of a given treatment. In addition, although clear guidelines exist for the diagnosis of ALS through various clinical tests of muscle strength, movement, and reflexes, these diagnostic practices often involve long periods of latency in between observations (in order to check for progression of symptoms) and it may take up to a year or more to verify a diagnosis of ALS.

These characteristics have left clinical practice and research on ALS at a disadvantage compared to other diseases with well established biomarkers, such as prostate cancer and HIV/AIDS. In addition to simply confirming a diagnosis, if ALS does indeed have multiple biological causes which result in the same clinical syndrome, a panel of diagnostic biomarkers could offer the possible ability to distinguish among a range of variant causes of pathology and in so doing determine the appropriate therapeutic intervention.[7]

A more ambitious task envisioned for biochemical markers of ALS pathology is finding a chemical or chemicals whose levels correlate to disease progression. Such markers, at the very least, provide an additional endpoint for clinical trials, and can validate or help standardize clinical observations. Under ideal circumstances, such biomarkers could also be used to significantly reduce the length of clinical trials – even allowing researchers to conduct short screening trials in which changes in key biomarkers after a brief treatment period give an immediate indication of potential therapeutic value.[8]

Unfortunately, the search for biomarkers in ALS has not yet produced a definitive or

widely accepted biomarker or panel of biomarkers for ALS. Although researchers can point to a long list of observed biochemical changes in ALS, these changes have been useful only in distinguishing population-based differences between ALS and other neurodegenerative diseases or healthy controls – not in diagnosing ALS or in improving the accuracy of diagnosis. An overview of these observations, as reported in major review articles on ALS, is provided in Appendix A.[9] Other notable studies in recent years have shown increased levels of the DNA repair enzyme PARP,[10] increased levels of the pro-inflammatory prostaglandin PGE2,[11] increased levels of Substance P,[12] and decreased levels of ICE/Caspase I in the cerebrospinal fluid of ALS patients.[13] Studies of muscle tissues in both ALS patients and mSOD1 mice have also suggested that the differential expression of two isoforms of Nogo, a protein which appears to inhibit neurite outgrowth, may serve as a potential diagnostic marker for ALS.[14]

A number of studies have focused on connecting oxidative stress to disease progression or therapeutic effectiveness; suggesting that the clinical progression of ALS is associated with increases in malondialdehyde,[15] lipid peroxidation,[16] manganese superoxide dismutase (mnSOD) nitration,[17] and total antioxidant status (TAS).[18] However, most of these observations are not unique to ALS, and are likely to be a feature of a range of diseases involving neuronal distress. Apoptosis and peripheral immune system activation have also been proposed as possible areas in which to seek an ALS biomarker, but similar to oxidative stress, the activation of these biochemical pathways are unlikely to be exclusive to ALS.[19] Other notable studies include those showing increased serum levels of APOE,[20] increased serum levels of matrix metalloproteinase MMP-9,[21] and increased serum ICE/Caspase-1,[22] although distinguishing these findings as specific to ALS is a more difficult task.[23]

There have, however, been two promising trends in researchers' biomarker search strategies over the past several years. The first is a growing interest in combining the search for biochemical markers with the adoption of sophisticated neuro-imaging techniques. Unlike multiple sclerosis,

pathological changes in ALS cannot be detected using traditional MRI techniques - the only way to directly measure disease state is through brain or spinal cord biopsies, which for obvious reasons are clinically unfeasible.[24] While lower motor neuron involvement can be fairly reliably determined in the diagnostic process using electromyography, upper motor neuron involvement presents a particular diagnostic dilemma because it traditionally has only been able to be determined using marginally reliable clinical examination techniques that in some cases date back to the late 1800's.[25] In recent years, a number of researchers have turned to proton magnetic resonance spectroscopy as a means of measuring both clinical progression and upper motor neuron involvement.[26] This technique has the advantage of measuring neuronal survival *in vivo* using the ratio of n-acetyl aspartate (a brain metabolite found only in neurons) to creatine as a surrogate marker of neuronal survival – the higher the NAA to creatine ratio, the more neurons surviving in the area analyzed.[27] This approach has been used to validate the observed clinical results of both rilutek and BDNF.[28] It also has diagnostic utility in distinguishing ALS from other neurological diseases.[29]

Although recent studies have cast some doubt on the short term precision of this marker, novel neuroimaging techniques directed at measuring neuronal death over time nevertheless offer a compelling additional surrogate marker of progression for use in clinical investigation.[30]

Another notable trend in the search for biomarkers for ALS is the trend toward searching for 'signatures' – patterns of protein or metabolite expression that can be predictive of ALS diagnosis – rather than one single biomarker. These attempts have taken several forms, from standard blood chemistry studies[31] and HPLC-based studies of specific CSF and plasma metabolite expression[32] to more complicated high-throughput protein fingerprinting techniques.[33] Ramstrom et al. analyzed protein expression in ALS patients' CSF via liquid chromatography and Fourier transform mass spectrometry. They found no specific single biomarker but were able to identify 80% of the ALS patients in the study based on their patterns of protein

expression.[34] Ranganathan et al.'s study of the CSF proteome in ALS patients versus healthy controls, which used surface enhanced laser desorption/ionization mass spectrometry and pattern recognition software, was 92% effective in distinguishing ALS patients from other samples.[35]

However, in most cases these initial protein fingerprinting efforts have been limited to distinguishing between healthy volunteers and ALS patients, while in typical diagnostic practice clinicians must separate authentic cases of ALS from cases of syndromes that mimic ALS. In addition, any utility these protein fingerprints might have is limited to diagnostic purposes, since interpreting changes in hundreds of protein levels is an even more labor intensive approach to measuring disease progression than the 100-item clinical examinations currently used for the same.

It is likely that future investigations into biomarkers in ALS will attempt to overcome these issues. A key question for future investigations is whether diagnostic protein 'fingerprints' can distinguish different variants of ALS (familial vs. sporadic, or even variants within sporadic and familial.) Based on clinical trial, diagnostic, and histopathological data, several researchers have suggested that the causes and downstream events in ALS may be considerably more varied than the relatively uniform outward clinical signs and symptoms. However, aside from data on genetic mutations responsible for ALS, laboratory investigations have not yet been able to distinguish among these purported variants.

Another key question for research is the sensitivity of biomarkers or biomarker panels which track disease progression over time – ideally, researchers hope to find a biomarker or panel of biomarkers that can indicate within a few weeks whether a particular drug has therapeutic potential in ALS. To date, the most promising biomarkers can still only be considered as additional endpoints in standard clinical trials, rather than the primary endpoint in a three-week screening study. Finally, even protein fingerprinting techniques which have successfully distinguished ALS patients from healthy controls face

significant challenges if they are to be transformed from a stand-alone laboratory study into a standard diagnostic test. Recent progress in identifying possible biomarkers and novel biomarker research techniques, in combination with the growth in basic research on ALS, offers hope of overcoming these challenges.

Section II. In Vitro Models in ALS

The establishment of reliable, relevant laboratory models of disease is an important step in investigating both the underlying biology of and possible treatments for diseases. In ALS, the earliest such models were *in vitro* cell culture models, typically in which motor neurons (or cells with similar properties) were subjected to a specific chemical, viral, or bacterial insult which led to their death. As a primarily sporadic disease of idiopathic origin, creating these models posed a slight problem in that rather than infecting cells with a specific pathogenic agent (as done in infectious diseases), researchers had to model the effects of the unknown pathogenic agent using other agents and chemicals, even though testing treatments in a model which mimicked the downstream effects of the disease might have little relevance to alleviating the ultimate cause of disease. Thus, a great number of *in vitro* studies have been based on models in which excitatory compounds are administered to healthy neurons in toxic doses – a model of disease which, despite rather effectively causing motor neuron death, replicates only a portion of the observed biological phenomena in ALS. The issue of constructing *in vitro* models is made more difficult by the clinical impossibility of creating cultures from the affected cells – obviously, *in vitro* models of ALS must use mouse, rat, or fetal cells and, by virtue of involving neurons, must find a way to induce neurons to divide without losing the properties that make the cell culture a relevant model of disease.[36]

Despite these obstacles, a wide range of assays have been developed for ALS. In the absence of a reliable animal model of disease, *in vitro* models were for a long time the primary disease model for testing

possible treatments, and despite the availability of animal models for ALS, the use of *in vitro* models has increased exponentially in recent years with the onset of high-throughput screening programs. In 1995, the number of drugs tested through *in vitro* assays was estimated at 50 (based on published scientific literature); this number has increased to thousands of drugs in recent years, although exact figures and results have not yet been published. *In vitro* results have also been the laboratory basis for at least 6 clinical trials.[37]

Because of the wide number of *in vitro* cell culture models proposed for ALS, and their only partial replication of disease pathology, it is nearly impossible to point to any one standard cell culture model in ALS. Typically, researchers investigating drugs' effects *in vitro* have used a variety of assays – not all of which are necessarily ALS-specific – in order to identify drugs which have high level of activity against a range of processes known to cause or exacerbate neurodegeneration. In general, assays which operate by exposing neurons to excitotoxic compounds or which induce cells to express mSOD1 are accepted approaches to disease modeling, although the details of the cell cultures themselves – the selection of the cell population, means of delivering the toxic substance, and so on – varies significantly from model to model.

The best barometer to date of the 'standard' accepted *in vitro* models are the assays selected for inclusion in NINDS neurodegenerative screening program in which 27 different laboratories screened 1,046 possible neuroprotective compounds in a range of assays related to Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, and ALS. Eleven assays out of the 29 conducted were viewed as relevant to ALS – four relied on mSOD1 to replicate disase, four relied on excitotoxic compounds, and three focused on inducing mitochondrially-mediated apoptosis as a means of inducing neuronal death.[38]

While the earliest in vitro models focused on a single type of cell (usually motor neurons or neurons that mimicked the behavior of motor neurons in some way), over the past several decades, research has gradually uncovered the fact that motor neurons may not be only cell type involved

in ALS pathogenesis. A recent trend in *in vitro* models of ALS has been an increasing focus on organotypic co-cultures of motor neurons and supporting cells (astrocytes, microglia, or both.) Zhao et al., for example, studied both co-cultures of human microglia & motor neurons and microglia, motor neurons, and astrocytes, to trace the role of microglia in initiating motor neuronal injury. [39] In another recent study, Cassina et al. exposed spinal cord astrocytes to fibroblast growth-factor I (FGF-1, which when expressed in astrocytes may play an indirect role in inducing neuronal death) and then cultured embryonic motor neurons on top of the pretreated astrocytes in order to probe the role of reactive astrocytes in inducing motor neuron apoptosis.[40] Other studies have even attempted to preserve the specific environment in which these cells exist by culturing slices of spinal cord.[41] Appendix B summarizes the most recently used *in vitro* models in the six months prior to the publication of this paper. These new organotypic cultures are considerably more complex than the earliest *in vitro* models of ALS, and this added complexity may help to better model the complex disease processes occurring *in vivo*.

Section III. In Vivo Models of ALS

In vivo models, in which diseases are modeled in complete organisms rather than in cultured cells, provide a much more holistic approach to modeling disease compared to *in vitro* models. Animal models of disease have the potential to provide insight not only on the possible therapeutic value of certain drugs, but also on their potential systemic side effects and on the biology underlying the disease being studied. This latter point is especially true for neurological disorders, since the nature of the central nervous system makes it nearly impossible to examine pathological changes in a live patient (in the absence of animal models, researchers must rely on post-mortem pathology findings for their knowledge on disease processes – a process which is biased toward revealing end-stage

disease pathology and obscuring earlier disease events.)

The earliest *in vivo* models for neurodegenerative diseases like ALS were general neuronal injury models created by injuring or administering a neurotoxin to the animal being studied, and hereditary models in which animals expressed naturally occurring mutations that caused ALS-like symptoms. As the genetic basis of familial ALS became better understood in the early 1990s, a number of transgenic models of ALS were created and quickly became the standard for pre-clinical investigations. Recent years have seen the introduction of several 'knockout' models of ALS (models in which a gene is deleted or under-expressed) and the introduction of invertebrate models of ALS which, while asymptomatic, nevertheless may provide a means of creating truly high-throughput *in vivo* drug screening programs. Important questions still remain regarding the exact correlation between the most popular animal model of ALS (the SOD1 mouse) and the human version of the disease. However, the general consensus is that the animal models developed in the past decade represent a powerful research tool in ALS, and that emerging data on possible differences between animal and human manifestations of familial ALS add to rather than detract from the usefulness of these models.[42]

This section reviews the major animal models of ALS, focusing on mouse models of disease. Although bovine, equine, and canine models of ALS exist, most larger animal models of ALS are irrelevant to or impractical for laboratory and pre-clinical research.[43] It is important to note that even with respect to murine models of disease this is by no means a comprehensive account – by 1990 alone, researchers in ALS could point to nearly 40 different animal models which had been proposed for ALS.[44] The discovery of several genes associated with familial ALS in the 1990s, and the subsequent creation of animal models based on those genes, has only increased that number.

However, many of the models that have been proposed for ALS fall far short of modeling human ALS pathology and as a result have not been widely adopted by the

research community. Instead, this section focuses on the most widely used naturally occurring, transgenic, and knockout mouse models of ALS, paying specific attention to the mSOD1 transgenic mouse. (This descriptor refers not to a single mouse strain, but rather to a range of mice expressing different mutant human SOD1 genes at different levels - variations in mutation and copy number result in a wide range of variations in disease phenotype.) The concluding pages of this section cover invertebrate animal models of ALS and general issues related to interpreting the clinical relevance of studies conducted on animal models of ALS.

Naturally occurring rodent models

A number of naturally occurring mutations have been shown to cause ALS-like syndromes in mice. One of the most widely used naturally occurring animal models of ALS – especially prior to the discovering of the involvement of SOD1 in familial ALS - is the Wobbler mouse, which had been identified in the mid-twentieth century but first appeared in the literature on ALS in the early 1980s.[45] Technically, the Wobbler mouse does not have ALS – instead, it expresses a phenotype that has been compared to both progressive muscular atrophy (a variant of ALS affecting primarily lower motor neurons) and Werdnig-Hoffman disease (a form of hereditary infantile spinal muscular atrophy.)[46]

Despite these differences, the Wobbler mouse was initially used to probe the possible causes of ALS – when research shifted to focus on excitotoxicity, however, studies of Wobbler mice revealed significant differences in the types of amino acid abnormalities observed in the brains of Wobbler mice and ALS patients.[47] In addition, the type of glutamate dysfunction observed in the Wobbler mouse was different from that observed in ALS patients – researchers found an increase, rather than a reduction, in NMDA and kainite binding sites in the spinal cord of Wobbler mice.[48] These differences suggested that the neurodegenerative phenotype in Wobbler mice had a different origin than that expressed in human ALS. Later studies have revealed additional pathological changes that support the use of the Wobbler mouse as a model for ALS, including

evidence of cerebral pathology and mitochondrial respiratory chain dysfunction.[49]

Riluzole, thyrotropin-releasing hormone (TRH), insulin-like growth factor I (IGF-I), hyperbaric oxygen therapy, N-acetyl-L-cysteine, various steroids, 7-nitroindazole, leukaemia inhibitory factor (LIF), and SOD1 supplementation have all been tested using Wobbler mice, often with significant benefit. The Wobbler mouse's positive response to a wide range of treatments that have proved futile in clinical trials has cast doubt on its appropriateness as a model for ALS.[50]

Although the Wobbler mouse is arguably the most widely used naturally occurring animal model of ALS, there are several other mutations which can cause ALS-like symptoms. The progressive motor neuronopathy (pmn) mouse, for example, was introduced in the early 1990s and, like the Wobbler, expresses a syndrome that is similar to but not an exact match with human ALS.[51] The pmn mouse has been used in preclinical investigations of several drugs, including GDNF, CNTF, riluzole, and xaliproden.[52] Another naturally occurring model, the MND mouse, expresses a mutation on chromosome 8 which causes late-onset, progressive degeneration of upper and lower motor neurons.[53] The MND mouse initially appearing to be a promising model for ALS, but has been also shown to have additional pathology outside that traditionally associated with ALS and may be a more appropriate model for Batten's disease.[54]

The legs at odd angles (Loa) mouse has a dominant mutation in dynein, a protein responsible for intracellular transport in neurons, and expresses progressive motor dysfunction with age.[55] The Han-Wistar spastic rat has also been used as animal model to study upper motor neuron degeneration in ALS.[56]

Naturally occurring models of ALS can present both an advantage and a disadvantage to pre-clinical research. It is possible that transgenic models of ALS, which are based on relatively rare human mutations, are too restrictive a pathology and inadvertently cause researchers to identify as 'promising' those treatments which have the greatest relevance to less than 2% of all ALS patients. In this case,

naturally occurring models can serve as additional models in which to test possible treatments and a means of ensuring that treatments are acting on biological phenomena common to all forms of ALS. On the other hand, naturally occurring models might produce red herrings in the search for a treatment for ALS – the combination of murine biology and the novel chromosomal location of the mutant gene might combine to create a causative chain that bears little relation to anything encountered in human patients, and which hinders attempts to connect preclinical results to expected results in humans.[57]

Transgenic & knockout rodent models

Transgenic models of disease provide a promising means of overcoming the limitations of naturally occurring disease models. In transgenic models, the observed disease phenotype is caused by the same gene or genes that cause disease in human patients. Despite crucial genetic, physiological, and immune system differences between mice & humans, the mutated genes that cause diseases in people will frequently cause same diseases in animals. Transgenic technology also offers the possibility of accelerating the onset date of late-onset diseases – by inserting multiple copies of a disease-causing gene, researchers can significantly reduce the time it takes for that mutation to cause disease and increase the pace of research.

The first transgenic mouse model for ALS was created through over-expression of the human neurofilament heavy (NF-H) subunit.[58] The observed phenotype in this mouse bears many similarities to human ALS, and only requires a twofold expression of the human gene.[59] Experiments with over- and under-expressing other human and murine neurofilament subunits have yielded interesting and at times puzzling results. Mice over-expressing human NF-H could be 'rescued' from their disease phenotype by a similar over-expression of human NF-L, suggesting that the *balance* between the two neurofilament subunits – not some pathological property of human NF-H – was responsible for the disease.[60]

Normal (1x) expression of human neurofilament medium (NF-M) subunit also

caused ALS-like symptoms, whether NF-M was expressed in addition to or as a replacement for mouse NF-M.[61] Mutant neurofilament light (NF-L) subunit and NF-L knockout mice also showed motor neuron degeneration.[62] It is clear that the various human neurofilament subunits can play a large role in creating ALS-like symptoms in mice, but it is not as clear whether this is relevant to human ALS. One study suggested that mutations in NF-H may lead to ALS, but a separate study failed to identify any abnormal NF sequences in a sample of 100 familial ALS patients known not to carry mutations in the SOD1 gene.[63]

While neurofilament mice have proven a resource in investigating the possible causes of ALS, they have not become widely used as animal model for therapeutic research – mostly because their phenotype does not exactly mimic human ALS. The transgenic SOD1 mouse, created shortly after discovery that mutations in SOD1 can cause familial ALS, has become the animal model of choice for conducting pre-clinical investigations in ALS.[64] It was immediately clear that the SOD1 mouse mimicked human disease not just in symptoms but in biochemical phenomena, including neurofilament-rich axonal swellings, Lewy body-like NF inclusions, Golgi fragmentation, and mitochondrial dysfunction.[65] Early on, researchers also showed that a mutant form of human SOD1 was necessary to cause disease symptoms – overexpression of wild-type human SOD1 only caused mild, sub-clinical damage to neurons.[66]

The particular mutation in the SOD1 expressed by transgenic mice can have a large impact on the phenotype expressed – as in familial ALS, different mutations can lead to different disease courses. The G37R and L38V mutations, for example, have been associated with an earlier age of onset, while the G37R, G41D, and G93C mutations are associated with longer survival times.[67] In mouse models, the G93A mutation is most widely used.[68] Although G93A is not the most common human mutation, it was one of the first to have clinical progression reported in detail, and has short timeline of disease.[69] Massive loss of functional motor units begins at 47 days of age and precedes by 6 weeks the onset of clinical signs.[70] High copy G93A mice tend to show intra-

cytoplasmic vacuoles (which indicate increased damage to the mitochondria) but these do not appear in low copy mice or in familial ALS patients, suggesting a slightly different etiology in high copy mice. Other mutations include the G86R,[71] G85R (which produces a rapidly progressive degeneration and early behavioral changes),[72] G37R, and H46R.H48Q.[73] Despite the availability of a wide range of SOD1 mutations, more than 65% of all published studies using SOD1 mice use mice expressing the G93A mutation.[74]

In addition to tracking the variation caused by different mutations in SOD1, researchers have also probed the effect of additional transgenic modifications on disease progression. These studies have at times yielded puzzling results. For example, although human wild-type (non-mutant) SOD1 does not cause disease in mice, hWT-SOD1 accelerates disease progression when expressed along with mSOD1(G93A), but has no effect when expressed along with mSOD1(G85R).[75] Even more puzzling, genetic aberrations which have been shown to cause ALS-like symptoms independently may actually produce a less severe disease when expressed simultaneously. Thus, deleting murine NF-L in SOD1(G85R) mice slows disease progression rather than hastening it as expected.[76] Similarly, NF-H over-expression slows progression in SOD1(G37R) mice.[77] Dual expression of the naturally occurring Loa mutation and SOD1(G93A) results in a similarly unexpected alleviation of disease progression and extension of lifespan.[78] These surprising results provide insight into the complexity of the biological processes responsible for ALS, and highlight the promising role transgenic animals may play in elucidating the etiology of ALS.

A number of knock-out mice (mice in which specific genes are deleted or underexpressed) have also proven to have ALS-like symptoms. For example, deletion of the hypoxia-response element of the VEGF promoter induces ALS-like symptoms in mice, an interesting finding which expanded possible roles for VEGF (once considered only as angiogenic factor relevant to cancer.)[79] Most recently, mice under-expressing Alsin, a cytoskeletal protein implicated in a juvenile form of familial ALS, were found to develop

progressive motor dysfunction with age.[80] Knockout models – especially those initiated without any intention of modeling ALS (as in the case of VEGF) – may provide a promising means of discovering new proteins implicated in ALS.

Induced rodent models and invertebrate models of ALS

Though less frequently used in published studies, there are a range of contemporary induced models of ALS in which a virus or toxin is used to mimic disease. For example, mice bred to be genetically susceptible to infection with lactate dehydrogenase-elevating virus (LDV) develop ALS-like symptoms shortly after being exposed to the virus.[81] Another induced model of disease uses cycad toxin to create a disease similar to the ALS-Parkinson's Disease complex (ALS-PDC) observed on Guam.[82] The advantages of induced models include lower costs and greater control over the timing of pre-clinical research (studies on transgenic and naturally occurring disease models are necessarily constrained by the age at which animals begin showing symptoms of disease.) However, because there is little evidence that human ALS is caused by a virus or exogenous toxin (except in the case of ALS-PDC), the relevance of these induced models to clinical research is unclear.

Another means of alleviating some of the time constraints imposed by transgenic and naturally occurring mouse models of disease is the creation of invertebrate transgenic models. Invertebrate models offer the advantages of modeling disease in a whole organism, while also offering a study timeline and volume of model organisms similar to those offered by tissue culture models of disease. So far, SOD1 transgenic C. elegans[83] and Drosophila[84] models have been created. Although neither model demonstrates an ALS-like phenotype, mSOD1 does appear to make these models more susceptible to a range of injuries and may yet prove to be an effective model for high-throughput *in vivo* screening programs.

Despite the wide range of animal models available for ALS, and the degree of knowledge on the correlation between their various phenotypes and human ALS, there

are still some general problems which must be overcome.[85] Although the scientific evidence for the clinical relevance of SOD1 mouse study results is strong, this connection has yet to be borne out by the results of clinical trials. Despite the popularity of the SOD1 mouse, positive results in the mouse have only correlated to positive clinical results in humans on one occasion, and those results came before researchers had a sophisticated understanding of pre-clinical study design considerations related to the SOD1 mouse.[86]

Like human ALS patients, mice expressing mutant human SOD1 transgenes demonstrate a wide variation in disease onset and survival time, and also show sex-specific differences in average onset and progression. The difference between female and male SOD1 mice is great enough that segregating treatment and control groups by sex is likely to produce as great an effect as many of the treatments which have been shown to have benefit in the SOD1(G93A) mouse. As work on animal models proceeds, researchers must decide between replicating the characteristics of the human ALS population (which would require more stringently designed pre-clinical studies using larger numbers of animals) and screening studies in which animal populations are specifically selected to have minimal variation in disease onset and progression in order to enable smaller studies and less complicated statistical analysis.

Section IV. Emerging Theories on Disease Etiology

Crucial to the assessment of existing animal models of disease and the creation of new models is an understanding of the specific biological chain of events that precipitates the outward clinical symptoms of disease. The previous chapter of this report discussed several etiological assumptions underlying clinical trials in ALS, including a virus of unknown origin, a deficit of a motor neuron-specific neurotrophic factor,

autoimmune disease, glutamate excitotoxicity, and oxidative stress. Because of the long timelines that often precede clinical investigation, there are a number of theories relating to disease etiology which are currently being investigated in laboratory and pre-clinical studies, but which in most cases have not yet transitioned into clinical investigation. This section provides a brief overview of several of these emerging etiological hypothesis, addressing in turn research on the possible role of protein aggregation, disrupted axonal transport mechanisms, mitochondrial defects, abnormal cell cycle signaling, and proteosomal dysfunction in pathogenesis of ALS. For each etiological hypothesis, general findings that have implicated that particular pathway are reviewed and an explanation of both the general hypothesis (as it relates to all neurodegenerative diseases) and the specific process by which pathology is localized in motor neurons is discussed when available.

Protein Aggregation

Protein aggregates (toxic clumps of misfolded proteins) are a hallmark of a range of neurodegenerative diseases.[87] While the specific molecular content of these aggregates varies among the various neurodegenerative diseases, most aggregates contain misfolded cytoskeletal elements, including filamentous aggregates of neuronal intermediate filament proteins or inclusions with the microtubule-associated protein tau.[88] The general explanation of protein aggregates' role in disease causation is that they cause disease by disrupting cells' quality control systems.[89] However, in nearly all neurodegenerative diseases cells in which protein aggregates are visible and the specific cells that degenerate do not overlap completely. Thus, researchers believe it is likely that readily observable protein aggregates (in the form of microscopically visible inclusion bodies) are a downstream byproduct of pathogenic events that take place on the level of smaller aggregates or perhaps individual misfolded proteins.[90] In addition, although a wide range of protein aggregates are observed in neurodegenerative diseases, protein aggregation and inclusion bodies are also observed in non-symptomatic subjects; for this reason, researchers stress the

importance of distinguishing toxic, disease-causing aggregates from nontoxic ones.[91]

Ubiquitinated inclusions are the most frequently observed types of proteim aggregates observed in sporadic ALS, but the biochemical basis for the formation and the possible toxicity of these aggregates is still not well understood.[92] One area of increasing focus in understanding protein aggregation has been the study of cellular mechanisms for processing and disposing of these aggregates.[93] While inclusion bodies have been observed in the motor neurons of sporadic ALS patients, the most significant data on protein aggregation in ALS comes from the most common genetic cause of ALS: gain of function mutations in SD1. Mmature mSOD1 is highly stable, but the earliest disulfide-reduced polypeptides in SOD1 assembly pathway are highly destabilized and predisposed to forming toxic protein aggregates.[94] Not only does mutant SOD1 demonstrate a tendency to misfold and aggregate, but mSOD1 appears to be able to attract and recruit other proteins typically found in sporadic ALS inclusions.

In addition, although mSOD1 is expressed systemically in affected patients, the localization of pathology to motor neurons can be explained by the high expression of mSOD1 in these neurons, and by the unique cell cycle status of neurons, which ensures that cell death as a last-resort method of quality control results in the irreversible loss of motor neuronal signaling.

It is important to note that protein aggregation on its own does not constitute a free-standing etiological hypothesis. Protein aggregation is nearly always considered in conjunction with the specific cellular processes that are disrupted by (or whose disruption causes) protein aggregation. The following sections address several cellular processes which have been suggested to be dysfunctional in ALS; the role of protein aggregation either as a cause or byproduct of this dysfunction is discussed in each section.

Axonal transport

Disruptions in axonal transport – the process by which various cellular organelles and cytoskeletal elements are moved across the long, thin chamber that makes up more than 99% of the length of neurons – have

long been observed in amyotrophic lateral sclerosis. As early as 1976, researchers were beginning to uncover impairment in axonal transport in ALS patients, and by 1985 the phenomenon was widely recognized as a key event in the pathogenesis of ALS, either as a proximal cause or a downstream event.[95] Axonal transport is particularly important to the survival of neurons, since nearly all cellular materials necessary to axonal functioning must first be synthesized in neuronal perikarya and then transported into (and eventually out of) the axon itself. The processes by which this transport occurs have historically been divided into fast and slow forms of transport based on the rate at which cargo travels through the cell. Organelles and other membrane-based cellular components are transported via fast axonal transport; cytoskeletal elements are typically transported through slow axonal transport.[96] While the earliest studies on axonal transport in ALS mainly revealed alterations in fast axonal transport (specifically, reductions in anterograde and retrograde fast axonal transport velocity and density), later studies in the late 1980's demonstrated that slow axonal transport is also impaired in ALS.[97]

Axonal dysfunction in ALS is not limited to cytoskeletal behavior – ALS-affected axons show distinct changes in overall appearance versus those in healthy neurons, including both a reduced overall axon caliber (diameter) and, often, the presence of giant axonal swellings filled with cytostructural and axonal transport protein aggregates and various axonal transport cargo.[98] These large axonal swellings have been observed to include massive accumulations of kinesin (one of two major molecular motors responsible for fast axonal transport) and highly phosphorylated neurofilaments (cytoskeletal components that regulate axonal caliber), but appear to only rarely contain cytoplasmic dynein (the other major molecular motor involved in fast axonal transport.)[99]

While predicting the effect of serious disruptions in axonal transport is fairly straightforward, hypotheses on the ultimate cause of these changes in ALS are varied. One hypothesis is that oxidative stress deranges neurofilament phosphorylation and assembly, which in turn affects slow axonal transport and leads to accumulation

of neurofilament components and axonal swelling at the site of these accumulations.[100] Others have hypothesized that the hyperphosphorylation of neurofilament subunits is due to the increases in Cyclin-dependent kinase 5 (CDK5) levels which are often observed in neurodegenerative diseases.[101]

In recent years, a variety of transgenic animal models of motor neuron disease have provided new insights into issues of axonal transport, although not entirely clear that results in them translate into humans with the disease. In addition to mutations in neurofilament subunit [H], kinesin, and dynein all causing motor neuron disease-like symptoms in animal models of disease, transgenic mice expressing mSOD1 – a familial-ALS causing mutation in a gene not directly related to either axonal transport or cytoskeletal proteins – also show characteristic alterations in axonal transport.[102] In the low-copy mSOD1 G93A mouse, researchers detected significant decreases in neurofilament proteins, a reduction in axon caliber, and impairment of fast & slow axonal transport coincidental to the appearance of neurofilamentous aggregates and inclusions.[103] High copy mSOD1 G93A mice, which typically have a much more rapid progression of disease, also show similar changes – although in high copy mice impairment of fast axonal transport is more pronounced than impairment of slow axonal transport.[104]

Transgenic mice expressing mutant SOD1 have also provided researchers with insight into the relevance of axonal transport defects at various points in the timeline of disease pathology. In general, reduced transport of specific slow transport cargoes appears to occur long before observable neurodegeneration.[105] In mice expressing mSOD1 G86R, researchers observed a distinct upregulation of proteins related to fast axonal transport and an early decrease in microtubule-associated proteins up to 5 months before the onset of symptoms – changes which were limited to the spinal cord and did not appear in the brain.[106] Expression of mSOD1 has also been observed to alter the cellular localization of the fast axonal transport molecular motor dynein by attracting and incorporating dynein into mSOD1 aggregates.[107] In mice,

both mHuSOD1 and endogenous mouse SOD1 are transported via slow axonal transport in motor and sensory axons of the sciatic nerve, suggesting that mSOD1 may act locally during transport to damage motor axons.[108]

Despite the insights that murine models have provided on a possible role for axonal transport disruption in the pathology of ALS, sorting out the ultimate cause and relevance of these changes may prove more difficult that first expected. Researchers recently crossed mSOD1 mice with Loa/+ mice (legs at odd angles, a naturally occurring mutation in dynein associated with axonal transport impairment and motor neuron degeneration), expecting that the cross would result in exacerbation of motor neuron disease symptoms. Counterintuitively, mice expressing both mSOD1 and Loa/+ mutations had delayed disease progression and a longer lifespan vs. those expressing only mSOD1. The mice also appeared to have a complete recovery in axonal transport deficits vs. those expressing only mSOD1 or Loa/+.[109]

Mitochondrial defects / mitochondrial permeability transition pore

In recent years, increasing attention has been paid to the possibility that defects in mitochondrial function – specifically inappropriate activation of the mitochondrial permeability transition pore that directs mitochondrially-mediated apoptosis – might play a pathogenic role in ALS.[110] In addition to performing critical roles related to aerobic energy production and intracellular Ca2+ buffering, neuronal mitochondria play a key role in neurodegeneration and in both apoptotic and necrotic cell death.[111] While mitochondrially-induced cell death was initially interpreted to be a downstream event of some other pathological process, recent research has focused on the possibility that mitochondrial defects or dysfunction lead to aberrant apoptotic or necrotic cell death.

Researchers have focused on this latter role and the specific changes that mitochondria undergo when regulating or inducing apoptotic or necrotic cell death – in particular, they have focused on the formation of the mitochondrial permeability

transition pore (mtPTP) – a Ca2+ dependent pore in the inner membrane of the mitochondrion. Formation of the mtPTP triggers the release of calcium and other molecules that interact with regulatory proteins involved in inducing apoptosis, and also disrupts the energy-producing functions of the mitochondrion.[112] The formation of the mtPTP also induces massive swelling of mitochondria, which correlates with microscopic findings on mitochondria in ALS.[113] Recent research has cast some doubt on whether the massive mitochondrial vacuolation observed at the onset of ALS is indicative of classical mitochondrial permeability transition or a new form of vacuolation.[114]

Whether mitochondrial involvement in neuronal apoptosis occurs through 'normal' formation of the mtPTP, through abnormal formation of the same, or through derangements in mitochondrial function that lead to mtPTP-like disruptions in mitochondrial membranes, mitochondrially-mediated cell death has been an increasing focus of therapeutic intervention.

Several theories attempt to link 'normal' mtPTP formation with other biochemical phenomenon in ALS. For example, researchers have theorized a link between copper imbalances and mitochondrial dysfunction through the increased production of mtPTP-inducing reactive oxygen species.[115] Others have shown that disrupting spinal mitochondrial function predisposes motor neurons (but not other neurons) to glutamate-receptor mediated toxicity.[116] Because specific patterns of mitochondrial distribution throughout neurons are critical to both neuronal survival and synaptic function, there may also be a link between disruptions in cellular transport mechanisms and mitochondrial dysfunction.[117]

Cell cycle dysfunction

While some researchers consider phenomena like the formation of the mitochondrial permeability transition pore and other apoptotic signaling to be aberrant processes, it is also possible that apoptotic signaling occurs *appropriately* in reaction to other abnormal cellular occurrences. Researchers in recent years have focused on

the possibility that aberrant cell cycle signaling leads to neuronal apoptosis.

Most cells in the human body go through a process of growth and division governed by a series of proteins known as cyclin-dependent-kinases (CDKs), which play a role in cell growth, division, differentiation, senescence, and apoptosis.[118] Unlike most cells, neurons are typically in a quiescent state in most adults, meaning they do not divide or proliferate. Because of this, almost all CDKs are silenced. However, there is a variety of evidence that motor neurons in ALS are activated to reenter the cell cycle and transition from the G(1) to S phase, a shift which may be either the result or the cause of programmed cell death. The evidence in favor of reentry into the cell cycle includes hyperphosphorylation of retinoblastoma protein, increased levels of cyclin D, and redistribution of E2F-1 into cytoplasm of motor neurons & glia.[119]

It has been suggested that cell cycle signaling may be related to changes in the regulation of Cdk5, the only cyclin-dependent kinase which is not typically silenced in adult neurons. (Cdk5 is essential to the development of neurons and is also crucial to the survival of adult neurons.)[120] Researchers have observed Cdk4-mediated cell cycle signaling at the G1-S checkpoint subsequent to Cdk5 deregulation in SOD1(G37R) mice.[121] While Cdk5 and cycling Cdks have no relation in healthy people, in neurodegenerative diseases they may work together to contribute to neuronal death.[122]

Proteasomal dysfunction

Another area of growing interest in ALS research is the possible involvement of the ubiquitin-proteasome system in disease pathology.[123] The proteasome, a barrel-shaped complex of proteins with an interior chamber that contains several active proteolytic sites, is involved in a range of protein degradation pathways, including the regulation of cell cycle protein levels, the degradation of misfolded proteins, and the processing of proteins for antigen presentation.[124] Ubiquitin, a crucial element of proteasomal function, marks proteins for degradation by the proteasome and has long been observed to be a feature of the types of protein aggregates and inclusion bodies observed in ALS.[125]

There is evidence that proteasomal function may be impaired or aberrant in ALS, a phenomena which might explain the presence of these ubiquitinated inclusion bodies.[126] Cells appear to be highly sensitive to even small changes in proteasomal function. For example, a single mutation in active site of 20S proteasome beta5 subunit caused impairment of chymotrypsin-like activity and hypersensitized cells to oxidative stress, triggering accumulation and aggregation of ubiquitinated proteins, and eventually cell death.[127] The source of proteasomal inhibition in ALS is likely to be motor neuron specific rather than systemic, since treatment with proteasomal inhibitors induced general, rather than motor neuron-specific neurodegeneration in organotypic spinal cord cultures.[128]

In certain cases of familial ALS, mSOD1 may be a direct or indirect cause of this proteasomal inhibition. Impairment of proteasomal function (as measured by chymotrypsin-like activity) is observed in mSOD1 mice quite early in the course of the disease.[129] Studies have yielded conflicting data on whether proteasomal impairment is due to simply a decrease in constitutve proteasome levels or whether it represents an abnormal shift in proteasomal function toward the immunoproteasome.[130] Aggregated SOD1 has been shown in a range of studies to be a byproduct of proteasomal inhibition, but may also be implicated directly in that process.[131] It is possible that mutant SOD1 activates the immunoproteasome or otherwise impairs the 20S component (the core 'barrel' component) of the proteasome.[132] Another possibility is that proteasomal inhibition also leads to decreased degradation of Cdk5, inducing its deregulation and setting in motion a chain of events leading to neuronal death. Of the etiological hypotheses discussed in this section, the role of proteasomal dysfunction in ALS is perhaps the least comprehensively explored to date; further research is needed in order to elucidate the specific biochemical phenomena that lead to proteasomal inhibition in motor neurons and to understand the changes in proteasomal conformation that cause the observed inhibition.

Section V. Conclusion and Policy Considerations

Laboratory research and scientific knowledge on ALS has expanded considerably in recent years, providing hope that clinical or laboratory breakthroughs may be imminent. Expanding clinical understanding of the clinical, sub-clinical, and biological manifestations of ALS – even those which appear to have little direct role in disease pathology – has led researchers to increasingly consider models of disease in which peripheral and systemic phenomena cause pathology that is localized to motor neurons, rather than models in which the disease-causing agent originates only in the affected cells. The search for biological markers of disease in both the CNS and the periphery is also beginning to yield promising results with the shift toward more complex and system-based approaches to measuring changes in protein expression. Researchers have a well-characterized array of *in vitro* and *in vivo* models that in many cases closely mimic human ALS, and are increasingly aware of the study design considerations necessary to conduct and interpret the clinical relevance of research using these models. Transgenic mouse models of ALS have also helped point to novel biological pathways that appear to be involved in ALS pathogenesis but which are often involved too early in the disease process to be observed adequately in humans. In the past four years, researchers' knowledge on the possible role of misfolded proteins and protein aggregates in the pathogenesis of ALS has increased exponentially. This new knowledge base promises to provide a strong basis for selecting and testing investigational drugs in future clinical trials.

The purpose of this report was not only to provide a concise overview of major research trends in ALS – most of which are already highly familiar to members of the ALS research community – but to do so in order to introduce policymakers, lay advocates, and non-specialists to the recent history of research in ALS. In addition to exponential growth in research activities, ALS has also

experienced a recent surge in public awareness through both the efforts of celebrity spokespeople and ALS voluntary organizations, and through somewhat troubling rises in the number of people affected by the disease. The attention now directed toward ALS by state agencies, newly formed voluntary organizations, corporations, and patient advocates has the potential to positively impact clinical and laboratory research on ALS, provided that these efforts take into full consideration the history of research in ALS. Policy and advocacy efforts which interpret ALS's status as a medical mystery to mean that there has been a total lack of medical attention to the disease are likely to engage in efforts that either replicate past research or fail to take full advantage of the sophistication of contemporary research on ALS. This report has attempted to communicate not only the promise of current research on ALS but also the complexity and depth of the laboratory research that has been thus far devoted to attempting to solve the medical mystery that is ALS.

There are a number of considerations which organizations interested in entering into the field of ALS research funding may wish to consider. Despite being populated with researchers who care deeply about finding a cause and cure for ALS, the ALS research community is subject to the same pressures as other research communities –pressure to publish, pressure to claim priority on a new discovery, pressure to compete with other research groups. Because of the relatively small size of the ALS research community, these pressures can lead to fragmentation and can detract from the progress of research. Members of the ALS research and policy community have increasingly sought ways to circumvent these pressures, whether through NIH-funded drug screening consortia or less formal working groups organized on a topical or geographical basis. Organizations creating new programs or funding opportunities in ALS should be careful to assess, with the input of members of the ALS research community, whether these new programs create new opportunities for collaboration rather than new opportunities for competition.

Another consideration is the involvement or creation of incentives for the involvement of corporate and other outside interests in ALS research. Although the impact of corporate interests on academic research has been criticized, ALS presents a unique situation in which the benefits of corporate involvement may far outweigh the costs. Especially with the increasing interest in screening programs, in which large numbers of biologically active compounds are tested for an effect against molecular, cellular, and in vivo models of disease, pharmaceutical corporations offer access to large libraries of investigational drugs.

Though these drugs may take years to reach the clinic, they may yet be able to help researchers better understand the etiology of ALS based on their ability to impact various disease models. The involvement of research organizations focused on diseases other than ALS may also benefit screening programs by helping understand differences and similarities among diseases based on the data from screening drugs in models of multiple diseases.

Ultimately, the future of laboratory research in ALS is directly relevant to issues of public health – both now and in the past. Despite Lou Gehrig's death from ALS in 1941 and the public awareness his struggle raised, in the late 1940's it was ultimately multiple sclerosis – not ALS – that became known as one of the nation's top neurological problems. Multiple sclerosis became the focus of intense research effort - one of the first times that a disease that was neither infectious in origin nor widely prevalent (MS affected less than a quarter million people at the time) became the focus of a major public health effort on the local, state, and national level.

Although Gehrig's death had been expected to spur research on ALS, his name was ultimately used to raise money for multiple sclerosis research - largely because ALS was considered a very rare disease at the time compared to multiple sclerosis. Researchers now know that nearly as many people are diagnosed with ALS each year as with MS. The difference in lifespan after diagnosis, however, leads to drastic differences in the prevalence of

the two diseases - it is possible that if ALS became a chronic, treatable condition, that ALS and MS might affect roughly equal numbers of people. Increased government and nonprofit interest in ALS as a public health issue in recent years, along with the promising state of ALS research, provides hope that the public health movement Lou Gehrig's diagnosis was expected to inspire may yet come to pass.

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Appendix A: Studies of Biomarkers in ALS

Overview of Possible Biomarkers for ALS[1]

Sit & Pathway	Observations Unique to ALS
Cerebrospinal fluid	
Routine Analyses[2]	Elevated protein levels associated with increased survival Positive banding and protein >75 mg% associated with paraproteinemia
Excitatory Amino Acids[3]	Possibility of transglutaminase levels as a marker of disease stage
Oxidative stress/injury[4]	8-hydroxy-2'-deoxyguanosine 3-nitrotyrosine 3-nitro-4-hydroxypheol acetic acid
Cytoskeletal markers[5]	Low molecular weight neurofilament (NF)
Blood	
Oxidative stress/injury[6]	Increased red blood cell protein (RBC) carbonyl content as disease progresses
Immunological markers[7]	Increased IL-6 (associated with epidermis IL-6 staining) Auto-antibodies against fetal and juvenile CNS vasculature
Cell death / apoptosis[8]	Increased ICE/caspase levels
Skin	
General morphology[9]	Enhanced elastosis Edematous dermis Irregular collagen fibrils Enhanced laminin immunostaining in epithelial and blood vessel basement membrane. Enhanced type III procollagen staining of collagen bundles associated with increased urinary levels of type III procollagen Reduced type IV collagen immunostaining of basement membrane Increased cystatin in epithelial basement membrane
Immunological markers[10]	Increased IL-6 staining of basement membrane
Cell death / apoptosis[11]	Increased cell death following exposure to SIN-1 or H_20_2

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Appendix B: In Vitro Models of ALS

Cited in articles published between Jan 2005 and Jun 2005

Source	Types of Cells	Disease-causing agent
Mouse	NSC34 (hybrid of motor neurons and neuroblastoma cells)	mSOD-1 expression[1]
Mouse	N2a (neuroblastoma cells)	mSOD-1 expression[2]
Mouse	Spinal motor neurons	mSOD-1 expression[3]
Rat/Mouse	ND7 (hybrid of post-mitotic rat neonatal dorsal root ganglion neurons and mouse neuroblastoma cells)	mSOD-1 expression[4]
Rat/mouse	VSC 4.1 (rat motor neurons/mouse neuroblastoma cells)	mSOD-1 expression[5]
Rat	Embryonic ventral spinal cord cells seeded onto neonatal Schwann cells[6]	
Rat	Motor neurons & astrocytes	AMPA toxicity (to motor neurons only)[7]
Human	Fetal cerebral cells	TNF-alpha toxicity[8]
Human	Fetal cerebral cells	Tat and gp120 (HIV proteins) toxicity[9]
Rat	Astrocytes	Glyoxal (glutamate transporter-1 inhibitor) toxicity[10]
Rat	Spinal cord astrocytes	FGF-1 toxicity[11]
Rat	Lumbar spinal cord cultures	THA (threohydroxyaspartate, a glutamate uptake blocker) toxicity[12]
Rat	Lumbar spinal cord cultures	PDC (L-trans-pyrrolidine-2,4-dicarboxylate, a glutamate uptake blocker) toxicity[13]
Human	Glial cell precursors	Chronic echovirus 6 infection[14]
Mouse	Hippocampal slice cultures	Increased expression of EAAT2 via recombinant virus infection[15]

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Selected Additional Resources on ALS

Section I. Organizational Resources:

Major ALS Clinics and Research Centers

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Forbes Norris MDA/ALS Research Center, California Pacific Medical Center
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San Francisco, CA 94115
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Medical Director: Robert G. Miller, M.D.

UCLA ALS Clinic and Research Unit
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Los Angeles, CA 90095-6975
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Medical Director: Michael C. Graves, M.D.

Yale University MDA/ALS Clinic
Yale University School of Medicine
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Kessenich Family MDA ALS Center
University of Miami School of Medicine
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Phone: 1-800-690-ALS1 (690-2571)
Website: http://www.miami-als.org
Medical Director: Walter Bradley, D.M., F.R.C.P.

The Lois Insolia ALS Center
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Directors: Raymond P. Roos M.D., Betty Soliven M.D.

Indiana University Medical Center ALS Program
Department of Neurology RG-6
Indiana University School of Medicine
1050 West Walnut Street
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University of Kentucky and Veterans Affairs Medical Centers ALS Clinic
Department of Veterans Affairs Medical Center
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Lexington, Kentucky 40502-2236
Phone: 606-281-4920
Website: http://www.mc.uky.edu/neurology/neur.htm
Medical director: Edward J. Kasarskis, M.D., Ph.D.

John Hopkins University School of Medicine - MDA/ALS Neuromuscular Clinic
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Medical director: Leo McCluskey, M.D.

MDA/ALS Center of Dallas Clinic
University of Texas Southwestern Medical Center
Department of Neurology
5161 Harry Hines Blvd.
Dallas, Texas 75235
Phone: 214-648-6419
Co-directors: Wilson W. Bryan, M.D., Jeffrey L. Elliott, M.D.

Baylor College of Medicine MDA/ALS Center
Department of Neurology
6501 Fannin Street NB 302
Houston, Texas 77030
Phone: 713-798-4073
Medical Director: Stanley H. Appel, M.D.

ALS/MS Clinical Research Center
University of Wisconsin Hospital and Clinics
600 Highland Avenue, Room H6/563 CSC
Madison, WI 53792-5132
Phone: 608-263-9057
Medical Director: Benjamin Rix Brooks, M.D.

Nonprofit (Non-Academic) Research Centers

ALS Therapy Development Foundation
215 First Street
Cambridge, MA 02142
Phone: 617 441 7200
Website: http://www.als.net

Section II. Patient Information Resources

Information for Newly Diagnosed Patients

What is ALS?
ALS Therapy Development Foundation
URL: http://www.als.net/als101/whatisals.asp

Tips for Newly Diagnosed ALS Patients
Will Hubben / ALS Association
URL: http://alsa.org/community/article.cfm?id=383&

ALS – A Guide for Patients
Dr. Eric Livingston
URL: http://home.earthlink.net/~jakesan/pages/guide1.html

ALS - A Beginner's Manual
Cecil Neth
URL: http://www.alscecilneth.net/

What is ALS? For Kids
ALS March of Faces
URL: http://www.march-of-faces.org/KIDS/moe1.html

Local (Massachusetts) Support Groups

MDA-sponsored Support Groups
Canton area support groups: 781-575-1881
Boston area support groups: 617-348-2155

Athol Support Group
Athol Memorial Hospital
2033 Main St., Athol, MA
Meets the last Thursday of each month from 6:30 p.m. – 8:00 p.m.

Dedham Support Group
Traditions Assisted Living
735 Washington Street, Dedham, MA.
Meets on the 4th Wednesday of every month from 7:30 p.m. - 9:00 p.m.

Methuen Support Group
Holy Family Hospital
70 East Street, Methuen, MA
Meets on the last Wednesday of every month from 6:30 p.m. – 8:30 p.m.

North Dartmouth Support Group
The Cedars
628 Old Westport Road, Dartmouth, MA
(508) 366-0690
Meets on the 4th Thursday of every month from 6:00 p.m. to 8:00 p.m. (does not meet July or August.)

Peabody Support Group
Lahey Clinic Northshore
One Essex Center Drive, Peabody, MA
978-538-4300
Meets on the 4th Thursday of every month from 7:00 p.m. - 8:30 p.m.

Online/Email Support Groups

ALS Digest
Email-based forum and support group.
How to join: email bro@met.fsu.edu to subscribe.

ALS Chat Rooms
A list of and instructions on participating in MDA-sponsored online chat rooms on topics relating to ALS.
How to join: Visit http://als.mdausa.org/chat/index.cfm

Living with ALS
Online support group for people with ALS and their caregivers. The group is primarily directed toward sharing information and ideas on using palliative and assistive technologies to ease the burden of living with ALS.
How to join: Visit http://health.groups.yahoo.com/group/living-with-als/

Braintalk Forums: ALS
Online support group and forum for people with ALS and their caregivers. The Braintalk forum is highly focused on emerging therapies, alternative medicine, new research, and other topics related to the treatment and possible cure of ALS.
How to join:
http://neuro-mancer.mgh.harvard.edu/cgi-bin/forumdisplay.cgi?action=topics&forum=ALS&number=3

Information on Clinical Trials in ALS

Clinical Trials & Studies of Neuromuscular Disease
MDA USA (ALS Division)
URL: http://www.mdausa.org/research/ctrials.cfm

Drug Development Update
ALS Association
URL: http://www.alsa.org/patient/drug.cfm?CFID=906529&CFTOKEN=47942698

Current Clinical Trials
ALS Therapy Development Foundation
URL: http://www.als.net/research/studies/currentClinicalTrialList.asp

Section III. Major Fundraising and Advocacy Organizations

ALS Association
www.alsa.org
The ALS Association is a national not-for-profit health agency providing patient and community services, public education, patient advocacy and research. The Association's affiliate network includes chapters in communities throughout the nation.

ALS March of Faces
www.march-of-faces.org
Promotes ALS awareness and advocacy and is operated by ALS patients and caregivers.

Hope for ALS
http://www.hopeforals.org
Hope for ALS is a Houston-based nonprofit with a mission to raise research funds necessary to find effective treatments for those suffering from ALS today.

Hope Happens (formerly: ALS Hope)
www.alshope.org
Hope Happens' aids the search for a cure for ALS by funding progressive research and hopes in the process to create a new methodology for funding, researching, and developing treatments for other neurological disorders.

Les Turner ALS Foundation
www.lesturnerals.org
The Les Turner ALS Foundation is devoted to the treatment and elimination of amyotrophic lateral sclerosis (ALS), better known as Lou Gehrig's disease, and is based in Chicago.

Muscular Dystrophy Association, ALS Division
http://als.mdausa.org
Since the early 1950s, when Eleanor Gehrig served as a national volunteer leader of MDA, the Association has assisted those affected by ALS. MDA's ALS Division offers a comprehensive range of services for patients and caregivers, and aids the search for a treatment or cure through an aggressive, international research program.

Project A.L.S.
www.projectals.org
Project A.L.S. is a not-for-profit organization dedicated to finding a cure for ALS, finding an effective treatment for people living with A.L.S., and raising awareness about A.L.S. Current research projects focus on gene chip technology, accelerated drug testing, and neural stem cell replacement.

Ride for Life
www.rideforlife.org
Holds an annual fundraising event in which ALS patients ride their electric wheelchairs down the highways and byways to raise funds for a cure and raise awareness. During the past 7 years, the ride has raised more than 1 million dollars.