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Issues for Clinical Drug Development in Neurodegenerative Diseases

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Abstract

Neurodegenerative diseases pose specific challenges for drug development. These diseases typically have a slow and variable clinical course, an insidious onset, and symptom expression is only observed when a significant proportion of neurons are already lost. It is important to identify vulnerability factors and other determinants of clinical course in order to be able in the future to select patient populations for clinical trials with a predictable prognosis. The neurodegenerative process itself is not amenable to direct observation and, thus, cannot be monitored in clinical trials. For this reason, surrogate biomarkers are required for use as outcome parameters. In this respect, magnetic resonance imaging has proved valuable for assessing disease activity and progression in multiple sclerosis. Rating scales are of use as outcome measures but, as these generally measure symptom severity, they are most appropriate for use in assessing symptomatic treatments. Survival has been used with success as an outcome measure in trials in amyotrophic lateral sclerosis, where disease progression is rapid. The optimal outcome measure, the sample size required and the treatment duration need to be chosen in relation to the phase of the disease. Potential new treatments can be chosen based upon new knowledge of the genetics and physiopathology of neurodegenerative diseases and, in some cases, screened in transgenic mouse models, although it should be recognised that the validity of these models in terms of treatment response has yet to be established empirically.

Traditionally, neurology has been a branch of medicine characterised by the extent of unsatisfied medical needs for the treatment of relatively frequent pathologies. This is particularly the case for neurodegenerative diseases, where, until recently, symptomatic treatments were all that could be provided. This situation has changed somewhat with the introduction of immunomodulatory treatments for multiple sclerosis and of riluzole for amyotrophic lateral sclerosis (ALS). These drugs may slow the rate of deterioration in many patients, but are far from ideal in terms of the size of the treatment effect. More recently, the introduction of acetylcholinesterase inhibitors for the treatment of Alzheimer's disease represents another important advance, although these drugs do little if anything to modify the course of the disease.

The limited progress in developing new drugs for the treatment of neurodegenerative diseases is related to the complex pathophysiology of these disorders and methodological difficulties in demonstrating pertinent treatment effects. This review addresses these issues with examples from recent development programmes.

1. The Specific Features of Neurodegenerative Diseases

Neurodegenerative diseases have several specific features that render estimation of drug effects on disease progression very difficult. First, in terms of natural history, disease onset is insidious, and clinical course variable and usually slow. With the exception of ALS, in which average survival time from first symptoms to death is thought to be around 3 years, the other common neurodegenerative diseases evolve over decades (table I).

Table I. Average survival time following symptom onset in the most common progressive degenerative diseases of the nervous system

Disease	Survival time (y)
Alzheimer's disease	~10
Amyotrophic lateral sclerosis	~3
Huntington's disease	~15
Multiple sclerosis	~30
Multisystem atrophy	~6–7
Parkinson's disease	~20

Table II. Extent of neuronal loss at symptom onset in the most common progressive degenerative diseases of the nervous system

Disease	Neuronal loss (%) [reference]
Alzheimer's disease	20[1]
Amyotrophic lateral sclerosis	80[2]
Huntington's disease	57[3]
Multiple sclerosis	35[4]
Parkinson's disease	50[5]

Secondly, the underlying pathophysiology of neurodegenerative diseases is poorly understood and inaccessible for direct observation. These diseases probably have a multifactorial aetiology and involve a multitude of interacting neurochemical processes that contribute to neuronal death. The situation is generally compounded by the absence of validated surrogate markers of disease progression, although in this respect the use of magnetic resonance imaging (MRI) may be useful in multiple sclerosis.

Thirdly, the relationship between neuronal loss, disease progression and symptom presentation varies between both diseases and patients. In Parkinson's disease and Alzheimer's disease the core symptoms are relatively consistent. On the other hand, for motor neuron diseases, multiple sclerosis and, to some extent, Huntington's disease, initial symptom presentation can be very variable. Moreover, given the built-in redundancy of the nervous system, symptoms may only appear when a considerable proportion of neurons have already been lost (table II). The consequences of this are that potential neuroprotective treatments are introduced when neuronal loss is well advanced and the potential for repair is only limited. Ideally, it would be possible to measure neuronal loss early, before symptoms appear, so that treatments could be introduced in a preventive fashion to retard the moment when the critical threshold of neuronal loss for clinical manifestation of disease is reached (see table II).

2. Evaluating Progression in Neurodegenerative Diseases

The specific features of neurodegenerative diseases impose a number of constraints in the design of clinical trials. The slow progression of most of

these diseases necessitates a long study observation period in order to measure a significant change in disease evolution or differences between two potential treatments. Because of the absence of validated surrogate measures, there is no short-cut to measuring the disease process itself. The development of such markers represents a major challenge for clinical research in this area and would facilitate greatly the implementation of clinical trials. This is illustrated by a randomised clinical trial of glatiramer acetate in multiple sclerosis that used MRI metrics as the primary outcome measure,[6] in which a difference in outcome between the two treatment groups was observed before a difference in clinical outcomes.

In addition, the high inter-individual variability in clinical course necessitates the use of large sample sizes. Many negative studies of potential neuroprotective agents carried out in the past included too few patients to achieve the necessary statistical power to demonstrate a difference in outcome between two groups. The situation is compounded by the fact that add-on studies rather than placebo-controlled studies are now required in certain diseases where validated reference treatments exist, for example multiple sclerosis (interferon [IFN]-β and glatiramer acetate) or ALS (riluzole). The sample size also depends on the outcome measure used and the planned study duration. For example, a pilot study using MRI metrics as an outcome measure in multiple sclerosis would require 50–75 patients per group compared with 150–250 using a clinical measure of disability such as the Expanded Disability Summary Scale (EDSS). Similarly, in ALS, 400–500 patients per group would be necessary to demonstrate an effect in combination with riluzole and using survival as an endpoint compared with only 75 patients per group when riluzole was evaluated alone.[7]

An evaluation of disease progression in a large cohort of 960 patients with Huntington's disease[8] attempted to identify variables predictive of the disease progression rate measured with the Unified Huntington's Disease Rating Scale (UHDRS) that could be used to determine sample sizes in clinical trials. For example, it was found that a higher Total Functional Capacity (TFC) scale score at baseline, indicating less functional impairment, was associated with a faster annual decline in TFC score. For this reason, including only patients with a high TFC score at baseline allows for smaller sample sizes in clinical trials (figure 1).

Another example of the importance of disease stage for the demonstration of a treatment effect has come from the trials with riluzole in ALS. In a placebo-controlled randomised study of 168 patients with advanced-stage disease, no beneficial effect of treatment was observed,[9] whereas in a previous randomised trial including a similar number of patients with less deteriorated ventilatory function, a clear effect on survival was demonstrated.[10]

3. Outcome Measures

A number of different classes of outcome measures have been used in studies of neurodegenerative diseases (e.g. biomarkers, symptomatic effects scales, disease progression scales, neuroprotection and survival measures), all of which have different limitations.

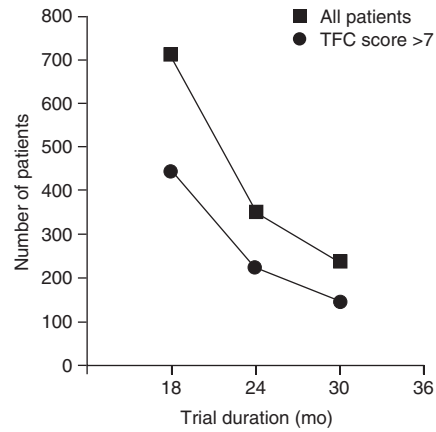

Fig. 1. Number of patients required per treatment arm in clinical trials in Huntington's disease in order to detect the effect of a drug estimated to slow the decline in Total Functional Capacity (TFC) scale scores using TFC by 40% with a statistical power of 80%. Estimations are compared for the inclusion of all patients and for inclusion only of patients with a baseline TFC score >7.[8]

3.1 Biomarkers

Biomarkers have been defined as "A characteristic that can be measured objectively and estimated as an indicator of physiological or pathological process, or the action of medication".[11] Two types of biomarker are of potential interest as surrogate markers for clinical trials of neuroprotective drugs, namely biochemical and imaging markers.

Generally speaking, biochemical markers have been of little use in neurodegenerative diseases for two reasons. First, an appropriate marker needs to be proposed based on a robust biochemical hypothesis of the disease process and, in general, the pathophysiology of these diseases is not sufficiently understood to identify such markers. An exception is for diseases with an important autoimmune inflammatory component, such as multiple sclerosis, in which measurement of immunoglobulins in the cerebrospinal fluid (CSF) is useful, or myasthenia gravis, where circulating anti-acetylcholine receptor antibodies can be measured. However, in classical neurodegenerative diseases, insufficiently characterised biochemical markers may even be misleading. An example of this is a clinical trial of tocopherol (vitamin E) supplementation in ALS,[12] designed to test the hypothesis that attenuation of oxidative stress, believed to contribute to motor neuron death, would reduce mortality or slow functional decline. This trial clearly showed that tocopherol supplementation could improve a biochemical marker of oxidative stress, namely serum thiobarbituric acid reactive species (TBARS), but had no effect on survival.

For the future, measures of soluble amyloid-β (Aβ) peptide in serum or CSF may be a promising biological marker to measure disease progression in Alzheimer's disease. Several studies have demonstrated a reduction in the levels of $A\beta_{1–42}$ protein in CSF of patients with Alzheimer's disease, hypothetically due to reduced clearance from the nervous system.[13-15] There is evidence that this reduction appears before a clinical diagnosis of Alzheimer's disease can be made, suggesting that CSF $A\beta_{1–42}$ protein levels could be used as a biomarker for prodromal Alzheimer's disease in patients with mild cognitive impairment.[16] In addition, there appears to be a progressive reduction in $A\beta_{1–42}$ protein over the course of the disease,[17,18] raising the possibility of using the levels of this protein as a surrogate marker for clinical trials, although much further validation is needed. Nonetheless, CSF $A\beta_{1–42}$ protein levels have been used as surrogate outcome markers in one trial of prophylaxis of Alzheimer's disease with statins (HMG-CoA reductase inhibitors).[19] No effects on $A\beta_{1–42}$ levels were observed, although CSF levels of amyloid precursor protein degradation products were reduced. Tau protein in the CSF has also been described as a potential diagnostic marker for Alzheimer's disease[20-22] but does not seem to be sufficiently sensitive to measure disease progression.[23] For the moment, these two biomarkers, particularly when used in combination, appear to be more interesting as biomarkers for the differential diagnosis of Alzheimer's disease than as outcome markers for clinical trials.[16] Another CSF constituent, 14-3-3 protein, classically considered a relatively specific biomarker for Creutzfeld-Jakob disease,[24] has also been described in certain patients with Alzheimer's disease, in which it seems to be associated with rapidly progressing dementia and poor prognosis.[25,26]

A second problem relates to access to the CNS. Sequential CSF sampling poses certain ethical problems for clinical trials, although it has occasionally been used. One of the aetiological hypotheses for ALS proposed that spinal motor neurons degenerate as a result of excitotoxicity provoked by high concentrations of glutamic acid in the CSF following failure of astrocytic uptake of this amino acid.[27] Indeed, this was one of the arguments stimulating the development of riluzole in this indication. Thus, changes in CSF glutamic acid concentrations may be of potential interest as a biochemical marker of disease progression in ALS. This has been assessed in a clinical trial of lamotrigine performed in Sweden.[28] The study demonstrated that CSF glutamic acid levels did indeed change as the disease progressed, but no effect of lamotrigine was observed on either CSF glutamic acid or on disease progression measured with a rating scale.

Imaging markers, either using MRI or radiotracers with positron emission tomography (PET) or single photon emission computerised tomography (SPECT), on the other hand, have already demonstrated their utility in clinical trials and, once individual methodologies have been sufficiently validated, are likely to become a cornerstone of future trials in neurology. MRI is principally of use in diseases with gross structural damage, and has been used in multiple sclerosis, vascular dementia and leukoariosis, neoplastic brain disease and Alzheimer's disease. In multiple sclerosis, T2-weighted images allow accumulation of lesion burden to be estimated and both IFNβ and glatiramer acetate have been demonstrated to decrease accumulation of lesions on T2-weighted scans. Lesions visible on gadolinium-enhanced images from T1-weighted scans correspond to new active lesions where blood-brain barrier breakdown and inflammation is occurring. Treatment with IFNβ leads to a rapid and complete disappearance of these new gadolinium-enhancing lesions. A retrospective analysis of data generated in a large placebo-controlled trial of glatiramer acetate[6] has shown that these two MRI metrics fulfil established criteria (Prentice criteria) for use as a surrogate marker for relapse rate.[29] Current diagnostic guidelines for multiple sclerosis now accept MRI evidence for diffusion in time and space as an alternative to clinical evidence from relapses.[30] This allows earlier diagnosis of disease and more rapid initiation of treatment. In the future, MRI techniques could be used to measure the impact of new treatments in very early disease.

MRI techniques can be used to visualise loss of cortical tissue in Alzheimer's disease and vascular dementia. Thus, it is possible to quantify the extent and rate of progression of brain atrophy, and of disease progression, through serial MRI measures.[31,32] In theory, this could be used to assess the impact of treatments. This hypothesis has not yet been tested extensively, since the current specific treatments for Alzheimer's disease, acetylcholinesterase inhibitors, are believed to be symptomatic rather than disease-modifying treatments. Nonetheless, one pilot randomised, placebo-controlled study has assessed the effect of donepezil on the rate of hippocampal atrophy using MRI,[33] in which a lower decrease in hippocampal volume over 6 months was demonstrated in the donepezil-treated patients.

In contrast with the rather global measures of neurodegeneration that can be obtained from classical MRI, PET/SPECT allows neurotransmitter-specific damage to be visualised. This is important for neurodegenerative diseases in which a minority of the population of neurons are lost, for example the midbrain dopaminergic neurones in Parkinson's disease and cortical and spinal motor neurones in ALS. These structures are difficult to identify using classical MRI. Another use of SPECT is for determination of glucose metabolism, which is decreased in areas of neuronal loss and can thus be used as an overall measure of neurodegeneration, in much the same way as brain volume measurement with MRI.

Perhaps the best characterised application of PET/SPECT in neurodegenerative disease is to measure loss of dopaminergic neurons in Parkinson's disease using specific markers for dopamine uptake into nerve terminals, such as [^{18}F]-DOPA or [^{123}I]-CIT.[34,35] This can be used to track the loss of neurons in the basal ganglia over the course of the disease and has provided an estimate of the threshold of neuronal loss at which clinical symptoms first appear (figure 2). This technology can theoretically be used as an outcome measure in clinical trials to detect potential neuroprotective effects.[36] Two comparative trials of dopamine agonists and

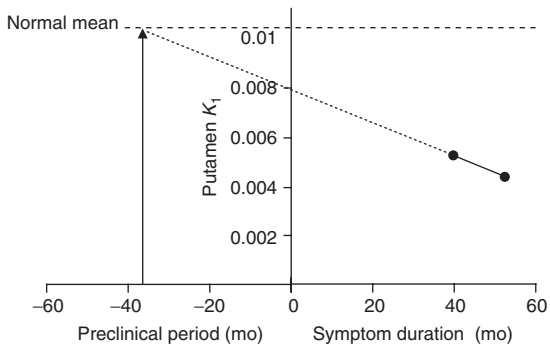

Fig. 2. Loss of dopaminergic neurones in Parkinson's disease using [^{18}F]-positron emission tomography, illustrating disease progression (reproduced from Cesaro,[41] with permission. © Masson, Paris, 2002).

levodopa in Parkinson's disease suggested that pramipexole and ropinirole may slow the loss of dopaminergic neurons consistent with a potential neuroprotective effect.[37,38] However, this conclusion needs to be re-evaluated in light of a recent study demonstrating that levodopa appears to accelerate the decline in fixation of the SPECT marker over time.[39] However, all of these treatments are symptomatic treatments and there is no *a priori* reason to think that they should have neuroprotective effects. A trial of riluzole, which is potentially neuroprotective, in Parkinson's disease,[40] which reported no effect of treatment on clinical milestones, evaluated [^{18}F]-DOPA PET in a subpopulation of patients; however, data from this analysis are not yet available.

Other uses of PET/SPECT ligands as potential surrogate markers of neurodegeneration include evaluation of loss of acetylcholinesterase in Alzheimer's disease and evaluation of a number of postsynaptic markers of GABAergic neurons in the basal ganglia in Huntington's disease, including dopamine, opiate and benzodiazepine binding sites.[35]

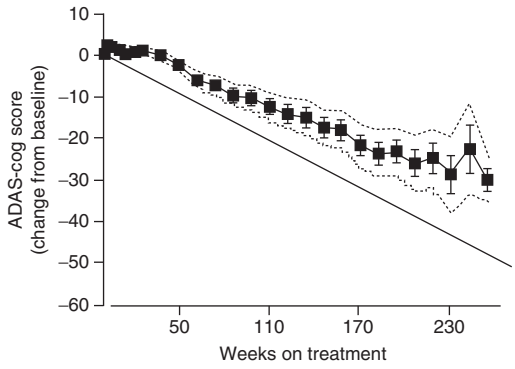

Fig. 3. Example of use of a symptomatic scale in drug development trials. Mean changes from baseline in Alzheimer's Disease Assessment Scale – cognitive subscale (ADAS-cog) scores over 254 weeks in 133 patients with Alzheimer's disease treated with donepezil. The bold line represents the rate of decline in ADAS-cog score in a group of untreated historical controls (reproduced from Rogers et al.,[45] with permission from Elsevier BV and the European College of Neuropsychopharmacology).

3.2 Rating Scales

Rating scales have been developed and validated for all the principal neurodegenerative diseases encountered in clinical practice. Many of these quantify symptom severity and have been developed to monitor natural history of the disease or the efficacy of symptomatic treatments. Examples include the Parkinson's Disease Rating Scale (PDRS),[42] the UHDRS[43] or the Alzheimer's Disease Assessment Scale – cognitive subscale (ADAS-cog).[44] These scales are the best adapted outcome measures for determining potential symptomatic effects of new treatments and are sufficiently sensitive to enable such trials to be performed over relatively short evaluation periods and with relatively low sample sizes (figure 3). The scales can also be useful for distinguishing between a symptomatic effect and a neuroprotective effect, since symptomatic rebound can be observed when treatment is interrupted. The majority of new treatments introduced into neurological practice over the last 2 decades have been shown to be effective and have been approved on the basis of clinical trials using symptomatic rating scales.

However, inclusion of these scales into studies of neuroprotective drugs can also reveal treatment effects on symptoms that were not anticipated *a priori*. This allows pilot studies to be performed more rapidly and with fewer subjects (and, thus, at lower cost) than would be necessary with a clinical study programme dedicated to evaluating a neuroprotective effect. An example is the beneficial effect of riluzole on chorea scores on the UHDRS in Huntington's disease, which has now been reported in three small studies (figure 4). Although such studies should not be considered as proof-of-concept studies, since they evaluate a different treatment objective than the primary one (symptomatic rather than disease-modifying effects), they do nonetheless allow much larger and longer trials to be initiated with greater confidence.

Symptomatic rating scales have also been used to assess potentially neuroprotective treatments, the decline in score being taken as a surrogate measure of neurodegeneration. This was the approach adopt-

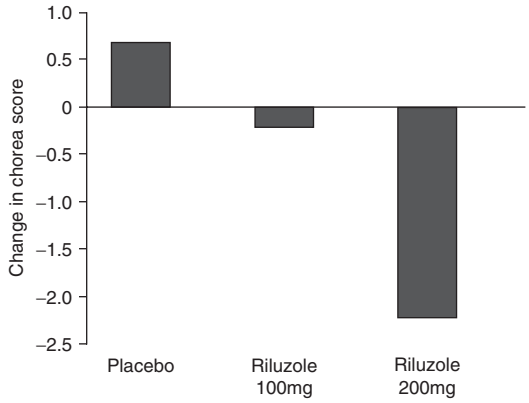

Fig. 4. Effect of riluzole on chorea symptoms in Huntington's disease measured with the Unified Huntington's Disease Rating Scale.[46]

ed in the trial of immunotherapy of Alzheimer's disease evaluating vaccination with Aβ(42) peptide.[47] Although the trial was stopped because of the development of meningoencephalitis in a significant number of patients, preliminary results showed that patients who generated antibodies against Aβ(42) peptide presented significantly slower rates of decline of cognitive function measured with the Mini Mental State Examination or the Disability Assessment for Dementia. This approach is only pertinent when the treatment tested, as in this vaccination study, is not expected to have a symptomatic effect and, thus, not affect rating scale scores independently from an effect on disease progression. Where this is not the case, interpretation of the study can be confounded by symptomatic effects of treatment, as was observed in the DATATOP (Deprenyl and Tocopherol Antioxidative Therapy of Parkinson's Disease) study of selegiline and tocopherol in Parkinson's disease (see the discussion of this study in section 3.3).

Another class of rating scales have been developed to measure disease progression. These scales are often categorical milestone-related measurement devices that are not ideally suited for quantitative evaluation of treatment effect sizes. Although many such scales have been used as outcome measures in clinical trials, the vast majority have not been able to discriminate treatment effects. In such cases, it is difficult to unequivocally attribute the lack of effect observed to a real inactivity of the test drug or to the metric shortcomings of the scale. An exception to this is the EDSS,[48] which evaluates the accumulation of disability in multiple sclerosis. Although this scale is a composite of categorical milestones such as the inability to walk unaided and, thus, is hardly a linear measure of disease progression, it has been shown to be sensitive to treatment effects. This scale was used as the primary outcome measure in the original pivotal clinical trial with intramuscular IFNβ-1a in relapsing-remitting multiple sclerosis.[49] An attenuation of the rate of accrual of disability as measured with the EDSS has since been observed in some, but not all, subsequent trials with all three types of IFNβ,[50-52] as well as with glatiramer acetate,[53] in both relapsing-remitting and secondary progressive disease.

Scales such as the EDSS are more pertinent than symptomatic rating scales for tracking long-term changes in disease status, as their sensitivity does not vary with changes in symptom presentation during the course of the disease. An example is the analysis of disability outcome after 10 years of treatment in the open-label, long-term extension of the pivotal randomised clinical trial performed with glatiramer acetate[54] (figure 5). This showed that early accrual of disability in the placebo group during the double-blind period is never recovered following subsequent switch of patients to glatiramer acetate.

At the moment, the EDSS is the only disability progression scale to have been shown to be useful in demonstrating treatment effects, and such scales need to be validated for other neurodegenerative diseases. One promising approach in this area is the use of a basket of disability measures to determine disease progression in ALS. In a recent randomised clinical trial of topiramate in ALS, a variety of disability scales were used as secondary outcome measures.[56] Although the trial was not able to discern any treatment benefit associated with topiramate, it did allow metric validation of an algorithm associating these scales into a sensitive and quantitative measure of disease progression.[57]

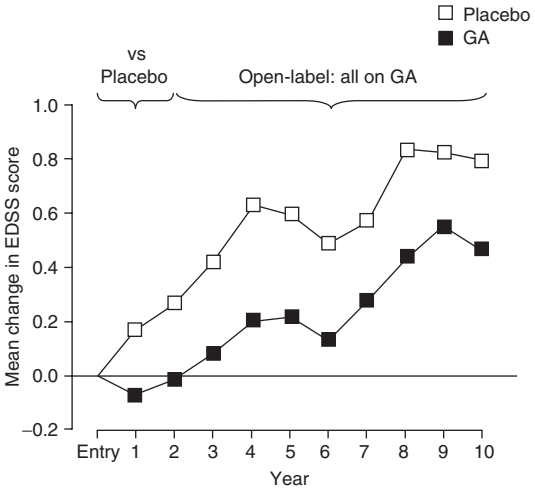

Fig. 5. Effect of glatiramer acetate (GA) on accumulation of disability in multiple sclerosis measured with the Expanded Disability Summary Scale (EDSS). The early accrual of disability in the placebo (PL) recipients during the double-blind period is never recovered after subsequent switch to GA.[55]

3.3 Neuroprotection

Direct measures of neuroprotection are obviously not feasible at the moment and no unambiguous demonstration of a neuroprotective effect has been observed in any drug trial in any neurodegenerative disease.

Two approaches have been used to measure neuroprotection. The first approach involves using neuroimaging to track loss of specific populations of neurons, and this approach has been used in clinical trials of ropinirole and pramipexole (see section 3.2). However, such studies are extremely costly and have so far generated results that do not provide an unequivocal demonstration of neuroprotection. As such, neuroimaging studies are not recognised by the principal regulatory authorities as providing adequate proof of clinical efficacy.

An alternative approach, pioneered in early 1990s in the DATATOP study of selegiline and tocopherol in the treatment of Parkinson's disease,[58] is to use time-to-event analysis in an attempt to demonstrate inflections in the course of the disease. This study evaluated the effect of treatment on the time required to reach a specified target event, the need to initiate treatment with levodopa, in treatment-naive patients with early Parkinson's disease. Although treatment did increase the time required to reach this event, the interpretation of the results is confounded by a symptomatic effect of treatment which influences the decision to initiate levodopa. Moreover, the milestone chosen, unlike survival (see section 3.4) is a rather 'soft' endpoint, which depends more on the subjective judgement of the physician than any unequivocal event in the natural history of disease. The interest of selegiline treatment in Parkinson's disease has also been mitigated by concerns about increased mortality.[59,60] Nonetheless, the need to initiate levodopa treatment was again used as the primary outcome measure in a large clinical trial aimed at demonstrating a neuroprotective effect of riluzole in Parkinson's disease.[40] The elimination of potential artefacts due to symptomatic effects of the drug was attempted through the use of a substantial wash-out period before assessment. Once again, the study failed to provide unequivocal evidence for a beneficial effect of riluzole on the time required to reach this milestone. A somewhat similar approach was used in a study of the potential neuroprotective effect of selegiline and bromocriptine in Parkinson's disease, this time looking at the proportion of patients who had evolved to a given score on the motor component of the PDRS at study end, after washout of study medication.[61] The treatment effects observed are again difficult to interpret because of potential symptomatic effects persisting through the washout period.

Institutionalisation has been proposed as an endpoint for time-to-milestone studies in Alzheimer's disease. This is a pertinent endpoint, as it is a determining event in the disease course in terms of quality of life, carer impact and cost.[62] Nonetheless, it remains as a 'soft' endpoint, as there are no objective criteria to determine when a given individual should enter dedicated residential care. It is possible that such decisions are not made identically for patients participating in clinical trials compared with those in standard care. A recent randomised placebo-controlled trial of donepezil in which entry to institutional care was used as the

primary endpoint failed to reveal any significant treatment effect on time to this milestone.[63] On the other hand, another trial of donepezil, this time using a predefined criterion of functional impairment derived from a basket of symptomatic rating scales, did provide evidence for a beneficial effect of donepezil using time-to-event analysis.[64]

A major issue with the studies that attempt to determine neuroprotective effects indirectly through delay in milestone timing for progression markers is that it is extremely difficult to distinguish effects on symptoms from effects on progression. This illustrates the critical importance of matching the endpoint measure used to the hypothesis under scrutiny. For evaluating a potential symptomatic treatment, the use of a symptomatic rating scale such as the PDRS or the UHDRS is appropriate. In contrast, when testing a potential neuroprotective effect, use of such scales as a surrogate marker of disease progression can introduce significant imprecision, particularly when the treatments also affect symptoms and, thus, reduce the probability of observing a significant treatment effect.

3.4 Survival

Survival has many advantages as an endpoint in trials of neurodegenerative disease. This is a robust and pertinent endpoint with low measurement error and inter-rater variability. Statistical analysis of survival data is well codified using time-to-event analysis with a simple binary outcome criterion, allowing unambiguous interpretation of the results. Although survival is widely used as an endpoint in clinical trials in cardiology, the only neurological condition in which this approach has been applied successfully to date is ALS. This was no doubt made possible because of the shorter median survival time in this disease than in other neurodegenerative diseases (table I).

However, even for Alzheimer's disease, survival analysis may be a feasible outcome measure in clinical trials. A large, prospective epidemiological study (the CHSA [Canadian Study of Health and Aging])[65] evaluated 5-year outcome in 823 patients with a case-ascertained diagnosis of Alzheimer's disease of known date of onset. This identified a median survival time, adjusted for other known mortality risk factors, of 3.3 years from disease onset. This is considerably shorter than previously believed, which the authors explained by the inclusion of patients with rapidly evolving disease who are normally absent from studies where patients are not followed from initial onset because of rapid mortality. If sufficient patients could be included at disease onset, it would be possible to use the survival hazard ratio as a primary outcome criterion in clinical trials of relatively short duration. Moreover, since survival time in Alzheimer's disease is strongly influenced by age,[65,66] survival trials could be conducted in older patients.

Since the pathogenesis of neurodegenerative diseases is unknown, and it is therefore currently not possible to use aetiology-based treatments in such pathologies, using survival as an endpoint is particularly pertinent, as it recapitulates the entire disease process and can identify potential treatment effects

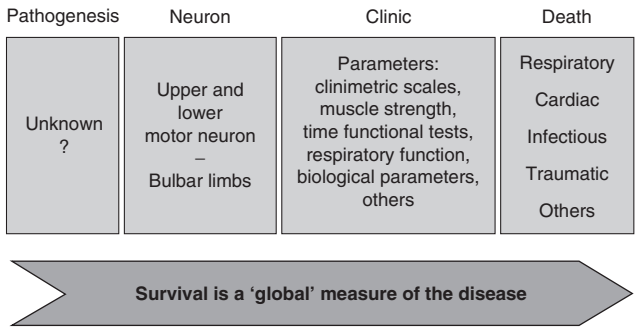

Fig. 6. Survival as a global measure of disease process in amyotrophic lateral sclerosis.

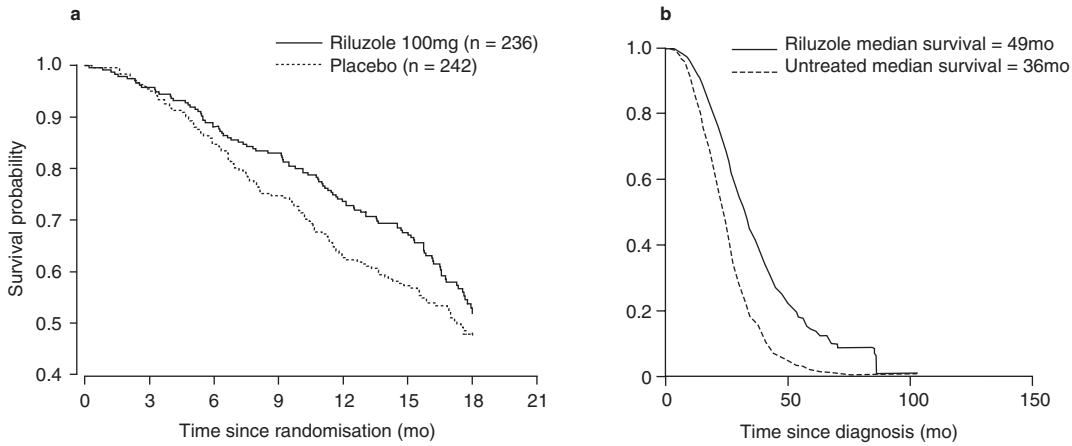

Fig. 7. Effect of riluzole on survival in amyotrophic lateral sclerosis, presented as Kaplan-Meier survival curves. (**a**) Data from a randomised placebo-controlled trial (reproduced from Lacomblez et al.,[67] with permission from Elsevier). (**b**) Data from a patient registry (reproduced from Turner et al.,[70] with permission).

wherever in the disease process they are implicated (figure 6).

Two randomised placebo-controlled clinical trials have demonstrated that riluzole significantly extended survival time in patients with ALS.[10,67] Data from the larger of these two trials are presented in figure 7. Importantly, survival data are generally collected systematically in patient registries and this allows the results from the clinical trials to be compared with survival rates observed under everyday standards of care. An association between longer survival and use of riluzole has been described in a number of these patient registries[68-71] (figure 7), arguing for the effectiveness of the treatment under naturalistic conditions of care. Such a conclusion could not have been obtained so simply had a more experimental outcome endpoint been chosen, such as MRI or CSF analysis, neither of which are used systematically in routine care.

More recently, survival analysis has been applied to the long-term outcome data from the DATATOP trial in Parkinson's disease.[72] Although this analysis was not performed to detect a survival advantage associated with a specific treatment, it nevertheless identified a number of determinants of survival in Parkinson's disease. In particular, a robust response to early treatment with levodopa was associated with an increased probability of survival in the mid-term. This demonstrates the feasibility of performing pertinent survival analyses in neurodegenerative diseases that progress relatively slowly, and provides an elegant demonstration of the association between short-term treatment benefits in terms of symptomatic effects and longer term survival benefits.

4. Genetics of Neurodegenerative Disease and Hypothesis Generation

Genetic markers are of no use as outcome measures in clinical trials of neurodegenerative diseases as they are trait- rather than state-dependent markers. Although genetic susceptibility to neurodegenerative disease is undoubtedly important, none of the major diseases, with the exception of Huntington's disease, has a primary genetic aetiology. There are a several rare hereditary neurodegenerative diseases in which a primary underlying genetic defect has been identified, including Kennedy's disease, Machado-Joseph disease and Wilson's disease, but their prevalence is very low. Even in diseases where mutations responsible for familial transmission of diseases has been described, such as mutations in the genes encoding presenilin and amyloid precursor protein in Alzheimer's disease, of the gene encoding superoxide dismutase-1 (SOD-1) in ALS and of the *parkin* gene in Parkinson's disease,

these mutations are found in only a small minority of affected individuals.

Nonetheless, there are a number of potential uses for genetic markers in neurodegenerative diseases (table III), some of which have already proved their utility. Given the limited contribution of single genes to the aetiology of neurodegenerative diseases as a whole, genetic markers have limited interest as diagnostic aids. However, determination of polyglutamine repeats in the *Huntington* gene may be useful for genetic counselling in families at risk for Huntington's disease. Identification of such repeats in the gene encoding the androgen receptor can be used in the differential diagnosis of Kennedy's disease and other motor neuron diseases, an important consideration given the very different prognosis and treatment implications of such a diagnosis.

Genetic markers come into their own as drivers of hypotheses for identifying candidate drugs for development in neurodegenerative diseases. A case in point is the identification of gain-of-function mutations in the *SOD-1* gene in certain familial cases of ALS.[73] This was rapidly exploited to develop a transgenic mouse model of ALS in which the mutated human *SOD-1* gene was expressed.[74] These mice develop a progressive and fatal neurodegenerative disease that resembles human ALS in many ways, and have provided for the first time an accessible and pathophysiologically credible model in which to test drug candidates for human ALS. Although the demonstration of the efficacy of riluzole in ALS predates the development of these mice, the observation that this drug slowed disease progression and increased survival in these transgenic mice[75] is a pertinent argument in favour of the use of this animal model. Certain drugs also fail to modify disease progression and have not been shown to be of benefit in clinical trials in humans, including brain-derived neurotrophic factor and topiramate.[56,76] The SOD-1 transgenic mouse is now used in several large collaborative programmes to identify potential drug candidates for the treatment of ALS, several of which are currently under clinical investigation. However, optimism should be cautioned by the identification of one false-positive in the animal model,[77] creatine, which subsequently failed to demonstrate efficacy in the human disease.[78,79] Since the development of the SOD-1 mouse, transgenic mouse models have been developed for use in screening programmes for both Huntington's disease[80] and Alzheimer's disease.[81]

Table III. Potential uses of genetic markers in clinical drug development

Diagnosis (polyglutamine repeat diseases and genetic diseases)
Development of animal models
Identifying new therapeutic targets
Prognosis: prediction of disease progression
Prognosis: identification of vulnerability factors and development of risk assessment scores
Identifying and implementing prevention strategies
Pharmacogenomics
Therapeutic indices (prediction of treatment response and identification of responder populations)
Development of alternative outcome criteria (surrogate markers)

The study of such transgenic mice allows valuable information to be gained on disease pathology and process that is relatively inaccessible in the corresponding human disease. Nonetheless, it is important to bear in mind, first, that these are only animal models and not the disease itself and, secondly, that the organisation of the mouse nervous system differs in important ways from the human nervous system, and therefore disease expression may differ between the two species. Dissecting the different biochemical pathways that are perturbed in these animal models allows new components of the pathology to be identified, which can inspire novel treatment strategies. An example of this is the observation that expression of the Nogo protein, an important determinant of neurite outgrowth, is perturbed in the SOD-1 transgenic mouse, an observation that was subsequently reiterated in human ALS.[82] This raises the possibility that drugs targeting signalling pathways involved in neuritic outgrowth may be of interest as therapeutic agents in ALS.

A quite different application of genetic markers to drug development has led to the investigation of potential prophylactic treatments for Alzheimer's disease. This involved the use of a pharmacoepidemiological approach rather than the development

of animal models. In 1993, Roses and colleagues[83] demonstrated that the ε4 allele of the apolipoprotein E gene was a vulnerability factor for Alzheimer's disease. Apolipoprotein E is responsible, *inter alia*, for the transport of cholesterol into the CNS. An association between elevated plasma cholesterol and the incidence of Alzheimer's disease was subsequently demonstrated as well as, more importantly, an association between exposure to cholesterol-lowering drugs and a reduced relative risk of developing Alzheimer's disease. On the basis of these findings, several prospective longitudinal cohort studies are underway to assess the ability of prophylactic statin use to prevent the development of Alzheimer's disease.[84]

5. The Right Development Plan for the Right Disease

There are several factors that need to be taken into consideration when planning a clinical trial to evaluate the efficacy of a novel treatment, such as hypothesis to be studied, outcome measure, sample size, patient characteristics, study duration and tolerability of study medication. It is important to get the hypothesis to be tested right when the study is designed. New drugs should ideally be based on a credible mechanistic hypothesis. Although certain treatments for neurodegenerative diseases have been discovered serendipitously, for example the use of glatiramer acetate in multiple sclerosis, there are many examples of successful drug development programmes that have originated in mechanistically based hypothesis, including the use of IFNβ in multiple sclerosis, riluzole in ALS and acetylcholinesterase inhibitors in Alzheimer's disease. However, a sound mechanistic hypothesis is not a guarantee of success, and the past 2 decades have witnessed many failures of apparently logical hypotheses, such as free radical scavengers and glutamate receptor antagonists in stroke,[85,86] nicotinic agonists in Alzheimer's disease[87] and growth factors in ALS.[88]

The hypothesis to be tested should also determine the endpoint used in the clinical trial. A drug likely to have a symptomatic effect should be evaluated with a symptomatic rating scale, whereas a drug that is likely to have a disease-modifying effect should use an outcome measure that reflects this. The failure to demonstrate unequivocal neuroprotective or disease-modifying effects in Parkinson's disease, for example with selegiline and riluzole, can no doubt be attributed in part to the use of inappropriate symptomatic rating scales as outcome measures.

Timing of the intervention should also be taken into account with respect to the postulated mechanism of action. It is important that the event that the treatment is supposed to forestall has not actually occurred when treatment is given. For example, the chances of demonstrating significant efficacy of anti-ischaemic agents are likely to be higher in the treatment of subarachnoid haemorrhage, where treatment can be given while the ischaemic episode is still underway, than in thromboembolytic stroke, where irreversible damage is likely to have occurred when treatment can be administered. This was indeed demonstrated for the antioxidant drug tirilazad, which has been shown to be efficacious in the acute treatment of subarachnoid haemorrhage but not of ischaemic stroke. Another example is the efficacy of riluzole in ALS, aimed at attenuating motor neuron loss. If the drug is given too late in the disease process, the neurons remaining to be rescued may be too few to allow a relevant impact of treatment. This has indeed been observed in patients with late-stage disease and severely impaired ventilatory function, in whom no significant treatment effect was observed.[9]

The outcome measure needs to be chosen with care, to be sufficiently sensitive to identify treatment effects in the timescale of the study, in order to be robust and as independent as possible of measurement error or investigator bias. In this respect, survival with death as the endpoint is clearly the measurement of choice, but may be difficult to implement in studies of neurodegenerative diseases that evolve only slowly, such as Parkinson's disease. Surrogate endpoints should not be considered validated in most neurodegenerative disease (a possible exception being MRI markers of lesion activity and burden in multiple sclerosis), and their use can give

rise to misleading results. An example where this was observed was a randomised placebo-controlled trial of tocopherol in ALS,[12] in which survival was used as the primary outcome measure but a plasma marker of oxidative stress (TBARS) was evaluated as a potential surrogate marker. Even though tocopherol treatment led to significantly elevated plasma tocopherol levels and a concomitant fall in plasma TBARS, no effect on survival was demonstrated.

Sample sizes need to be adapted to the study outcome measure to ensure sufficient statistical power to be able to measure the anticipated treatment effect. Many previous drug trials in ALS were insufficiently powered to detect a significant treatment effect (e.g. those of dextromethorphan and lamotrigine), and this may have led to potentially interesting treatments being rejected without adequate evaluation. For this reason, the last decade has seen the emergence of large multinational collaborative trials in ALS, such as those studying riluzole, insulin-like growth factor-1, gabapentin or xaliproden, which have made it possible to recruit sufficient patients for adequate hypothesis testing. This issue becomes even more critical for rare neurodegenerative diseases for which bringing together sufficient patients to perform a meaningful trial is a major challenge.

A related issue is study duration, which needs to be of sufficient length for unambiguously anticipated treatment effects to be revealed. Although not relevant for the evaluation of symptomatic treatments, it is an important challenge for measuring potential disease-modifying effects in diseases that progress slowly or have important inter-individual variability. Such studies necessitate long treatment periods and, thus, a large number of patients to allow for attrition as a result of premature study discontinuation.

Finally, it is important that the tolerability of new treatments be carefully evaluated before undertaking clinical trials. Patients with neurodegenerative diseases are usually elderly and often sufficiently physically impaired that they may be more at risk for adverse events than healthy volunteers. Clinical trials of ciliary neurotrophic factor and other growth factors in ALS have revealed that these agents actually increased mortality compared with placebo,[89] and such treatments may be too aggressive for use in individuals with neurodegenerative disease.[90] Similar concerns about increased mortality have been raised about the use of selegiline in Parkinson's disease.[59]

6. Conclusions and Perspectives

Advances in our understanding of the pathophysiology of neurodegenerative diseases have identified a limited number of core biochemical events that appear to intervene in many neurodegenerative diseases. These include oxidative stress, apoptosis, cytoskeletal dysfunction, excitotoxicity and inflammation. It may be the case that what distinguishes the different neurodegenerative diseases from each other is not so much the underlying disease process but rather the target population of neurons affected, which may be determined by as yet unidentified vulnerability factors.[91] This has important consequences for drug development. First, it implies that drugs need to be developed for different stages of the pathophysiological process, which could be used in combination to provide a more incisive effect on disease progression than any individual agent alone. Moreover, the different pathophysiological mechanisms may be involved to different extents in different patients, opening the way to matching treatments to patients, and contributing to the significant level of non-response observed in many clinical trials in neurodegenerative diseases. The idea that responders and non-responders to specific treatments can be identified is supported by recent analyses of registry data in multiple sclerosis[92] and of MRI data in Alzheimer's disease.[93] Secondly, this hypothesis suggests that different drugs may be effective in more than one neurodegenerative disease. This is currently under investigation with riluzole, which is being evaluated in randomised clinical trials of Huntington's disease, multiple system atrophy and adrenoleukodystrophy. It is also supported by recent findings that the anti-inflammatory drug glatiramer acetate, known to be effective in multiple sclerosis, has neuroprotective effects in a range of animal

models of neurodegeneration in which the primary pathophysiological mechanism is believed to be free radical damage, trauma or excitotoxicity rather than inflammatory.[94] A corollary of the notion that the same drugs could be effective in many diseases is that novel drugs could initially be tested in those neurodegenerative diseases best adapted to provide a rapid and unambiguous evaluation of the test hypothesis.

The observation that elevated plasma cholesterol levels may be a vulnerability factor for Alzheimer's disease is exciting as it opens the way to prevention studies in Alzheimer's disease with statins, which are currently in progress. Further identification of genetic or environmental vulnerability factors is an important challenge for the future, as it will allow prevention strategies to be put in place.

Another important need is to identify signs, symptoms or markers of prodromal stages of neurodegenerative diseases which would allow diagnosis to be made earlier. This is necessary for the initiation of neuroprotective treatments as early as possible in the disease process while there remain a maximum number of neurones to protect.

Finally, we need to develop well validated biological surrogate markers for disease progression in neurodegenerative disease that can be implemented in smaller pilot studies to evaluate experimental treatments. Currently, such proof-of-concept studies are unfeasible because of the absence of such markers, necessitating large, long and costly studies for each evaluation. A rapid system of screening promising therapies in pilot studies would speed up the drug development process considerably. Recent advances with MRI in the field of multiple sclerosis suggest that the development of valid surrogate markers can indeed be envisaged.

In conclusion, the lessons of the last decade about clinical drug development in neurodegenerative disease have the potential to shape clinical trials of the future and optimise the chances of identifying therapies that will attenuate progression of these very disabling disorders.

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