Is multiple sclerosis a length-dependent central axonopathy?

The case for therapeutic lag and the asynchronous progressive MS hypotheses.

Gavin Giovannoni[1], Gary Cutter[2], Maria Pia-Sormani[3], Shibeshih Belachew[4], Robert Hyde[5], Harold Koendgen[5], Volker Knappertz[6], Davorka Tomic[7], David Leppert[7], Robert Herndon[8], Claudia A.M. Wheeler-Kingshott[9], Olga Ciccarelli[9,10], David Selwood[11], Elisabetta Verdun di Cantogno[12], Ali-Frederic Ben-Amor[12], Paul Mathews[13], Daniele Carassiti[1], David Baker[1] and Klaus Schmierer[1]

[1]Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK. [2]Department of Biostatistics, University of Alabama at Birmingham School of Public Health, Birmingham, AL, USA. [3]Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy.[4]F. Hoffmann-La Roche Ltd, Basel, Switzerland. [5]Biogen, Cambridge, MA, USA; [6]Teva Pharmaceuticals, Netanya, Israel. [7]Novartis Pharma AG, Basel, Switzerland. [8]University of Mississippi Medical Center, Jackson, Mississippi, USA. [9]NMR Research Unit, Queen Square MS Centre, Department of Neuroinflammation, UCL Institute of Neurology, Queen Square, London, UK. [10]NIHR University College Hospitals Biomedical Research Centre (BRC). [11]Wolfson Institute for Biomedical Research, UCL, London, UK. [12]Merck, Aubonne, Switzerland, [13]Department of Medicine, Imperial College, London, UK.

Correspondence: Professor Gavin Giovannoni, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University London, 4 Newark Street, London E1 2AT. Email: g.giovannoni@qmul.ac.uk, Telephone: +44 (0)20 7882 2579, Fax: +44 (0)20 7882 2180

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Authors' email addresses: g.giovannoni@qmul.ac.uk, cutterg@uab.edu , mariapia.sormani@unige.it , david.leppert@novartis.com, shibeshih.belachew@roche.com, Volker.Knappertz@tevapharm.com , davorka.tomic@novartis.com, rherndon@umc.edu , c.wheeler-kingshott@ucl.ac.uk , o.ciccarelli@ucl.ac.uk , d.selwood@ucl.ac.uk , d.carassiti@qmul.ac.uk , david.baker@qmul.ac.uk, k.schmierer@qmul.ac.uk; harold.koendgen@gmail.com; robert.hyde@biogen.com, p.matthews@imperial.ac.uk, elisabetta.verdun-di-cantogno@merckgroup.com, ali-frederic.ben-amor@merckgroup.com

Abstract

Trials of anti-inflammatory therapies in non-relapsing progressive multiple sclerosis (MS) have been stubbornly negative except recently for an anti-CD20 therapy in primary progressive MS and a S1P modulator siponimod in secondary progressive MS. We argue that this might be because trials have been too short and have focused on assessing neuronal pathways, with insufficient reserve capacity, as the core component of the primary outcome. Delayed neuroaxonal degeneration primed by prior inflammation is not expected to respond to disease-modifying therapies targeting MS-specific mechanisms. However, anti-inflammatory therapies may modify these damaged pathways, but with a therapeutic lag that may take years to manifest. Based on these observations we propose that clinically apparent neurodegenerative components of progressive MS may occur in a length-dependent manner and asynchronously. If this hypothesis is confirmed it may have major implications for the future design of progressive MS trials.

Background

Although multiple sclerosis (MS) typically begins with a relapsing course, over the lifetime of an individual, MS is dominated by the progressive phase of the disease. There is considerable overlap between the relapsing and progressive phases of the disease with a significant number of patients in the secondary progressive phase having relapses (Cohen et al., 2002). A minority of people present with a primary progressive course from the outset (PPMS). Despite presenting without relapses at onset a small proportion of people with PPMS go onto have superimposed relapses (Wolinsky and PROMiSe Trial Study Group, 2004). Against the backdrop of such substantial overlap between clinically described disease phases and/or MS phenotypes we propose to revisit the concept of MS-related disability as driven by three separate, but related, pathological phenomena, which are: 1) inflammation with accompanying demyelination, which can be acute and focal in new lesions/plaques, or more chronic and diffuse and/or meningeal affecting lesions as well as ”normal-appearing” areas of white and gray matter; 2) acute neuro-axonal damage, be it directly due to inflammation or as a result of other processes (see below) (Ferguson et al., 1997; Trapp et al., 1998; Wolinsky and PROMiSe Trial Study Group, 2004); and 3) delayed neuroaxonal loss, or neurodegeneration, of chronically demyelinated or vulnerable neuroaxonal tracts.

Progressive disease as characterized by worsening disability, independent of relapses, is intricately driven by the focal and diffuse inflammation and delayed neuroaxonal loss (pathological processes 1 & 3 above). Limited methodological approaches have been explored in clinical trials to disentangle these two related drivers of progression. At present there are very few options to modify progressive MS; the current approved therapies only have an impact on clinically defined progression if there is evidence of ongoing relapses or focal MRI activity (Hawker et al., 2009). A

significant unmet need persists for people with progressive MS, i.e. treatments targeting the delayed neurodegenerative component.

When exactly the progressive phase of MS begins is subject to debate. Fred Lublin, an eminent neurologist from New York, is often quoted as saying *'I have difficulty defining secondary progressive MS, but I know it when I see it'*; a play on the supreme court judge Stewart Potter's threshold test for what is pornography (Kalven, 1989). The definition of progressive MS disease has remained largely a clinical one based on the qualitative observation of "*steadily increasing objectively documented neurologic dysfunction/disability without unequivocal recovery*". There is currently no consensus metric to identify and quantify progressive disease (Jacques and Lublin, 2015).

Evidence suggests the pathological substrates underlying progressive MS are chronic, widespread, inflammation, microglia activation (Frischer et al., 2009), demyelination, gliosis, and particularly neuroaxonal loss (Lassmann et al., 2012). However, all these processes can be detected from disease onset when disease progression is not clinically apparent. MRI studies indicate progressive brain volume loss, or accelerated brain atrophy, from disease onset and occurs on average at similar rates in the early, and the late, stages of the disease (De Stefano et al., 2010). More recent studies have shown that progressive brain atrophy can even be observed in people with radiologically-isolated syndromes (RIS), or pre-symptomatic MS (Amato et al., 2012; Rojas et al., 2014). These observations underpin the impression that there is no sudden transition, or switch, between the relapsing and progressive phases of the disease. Rather, there is interplay over months and years of pathological processes leading to what we observe as the clinically apparent "chronic disease progression". In reality the clinically detectable emergence of this transition occurs over a period of months to years and is commonly recognised retrospectively (Kremenchutzky et al., 2006).

Evidence suggests that functional domains of the brain and spinal cord have reserve capacity (Mori et al., 2014). Functional domains can sometimes compensate for a substantial degree of neuro-axonal loss before progressive disease becomes clinically apparent (Schwartz et al., 2013). Functional reserve, and partial or complete recovery from focal lesions, both contribute to complexity, and difficulty, in reliably detecting and monitoring disease progression early in the course of the disease.

A widely accepted theory suggests axons that have survived an attack of focal inflammatory demyelination may become vulnerable and degenerate overtime. The proposed mechanisms underlying such delayed axonal degeneration include excitotoxicity, functional energy deficits and mitochondrial impairment, persistent demyelination, delayed axonal transport, age-related

neurodegeneration (Paz Soldán et al., 2015), ongoing focal inflammation that may occur proximal or distal to previous MS lesions and diffuse innate inflammation (Campbell et al., 2014; Friese et al., 2014; Rao et al., 1981). Compensatory axonal sprouting to reinnervate vacated synapses, which is described in animal models (Liu and Chambers, 1958), is hypothesised to increase the metabolic load of surviving axons thereby contributing to premature neuroaxonal death (Herndon, 2002; Liu and Chambers, 1958). This is analogous to what happens to surviving lower motor neurons in the post-polio syndrome (Dalakas, 1995).

Anatomical factors may also play a role. Certain anatomical sites in the brain and spinal cord are claimed to have predilection for developing MS lesions, for example the optic nerves, corpus callosum, paraventricular, deep and subcortical white matter, brain stem, cerebellum and cervical spinal cord (Hallpike et al., 1983). Involvement of these anatomical sites have largely been determined by gross pathological studies and supported by more recent MRI studies using conventional imaging sequences (Moore and Laule, 2012). However, contemporary pathological studies suggest that more than half of the lesion burden in MS occurs in the gray matter, both in the cortical and deep gray matter nuclei (Kutzelnigg and Lassmann, 2014; Rudick and Trapp, 2009). Based on these recent pathological observations it is difficult to link a characteristic anatomical pattern of white matter lesions to the overall distribution of focal and diffuse MS pathology, which challenges the dogma that has been used to classify MS as a white matter disease. The lack of anatomical specificity increases the likelihood of long fibre tracts having a greater disease burden than shorter tracts.

Length-dependent axonopathy

Clinical observations suggest that neuronal domains with longer central axonal projections are more likely to be involved early in the clinically apparent progressive phase of the disease (Figure 1), for example the bladder and corticospinal, or pyramidal, projections to the lower limbs (Kremenchutzky et al., 2006). Involvement of these domains early in the course of the disease predicts a poorer outcome (Simone et al., 2002). If the distribution of MS lesions occurred randomly the functional subdomains, subserved by longer axons, are more likely to accumulate the greatest number of focal inflammatory lesions and hence be at the vanguard of the clinically-apparent progressive phase of the disease (Kremenchutzky et al., 2006). What protects neuronal domains from clinically progressive disease may be reserve capacity, i.e. the ability to compensate for neuroaxonal loss and the capacity for repair (Schwartz et al., 2013). A corollary is the shorter the neuronal pathway the less likely it is to have accumulated sufficient focal lesions and axonal loss to exhaust its reserve capacity (Figure 1 & 2). This might explain why the upper limb, brain stem motor function and the visual system appear to be rarely involved at the

beginning of the clinically-apparent progressive disease. The exception to the latter being progressive visual failure in women carriers of Leber's hereditary optic neuropathy (LHON) who have a clinical phenotype that is indistinguishable from MS (Harding et al., 1992). The latter condition though is arguably not MS, but a separate disease that mimics MS. The progressive visual failure that occurs in carriers of the mitochondrial DNA mutations which cause LHON hints at mitochondria, and cellular energetics imbalance, as playing a major role in delayed axonal loss. Differences in mitochondrial function between the spinal cord and brain may contribute to the selective vulnerability of spinal motor neurons in MS. The majority of upper motor neurons with long projection axons in the spinal cord contain mitochondria deficient in respiratory chain enzymes (Campbell et al., 2014). Spinal cord mitochondria retain considerably less calcium than brain mitochondria, possibly related to an increased set point concentration for calcium uptake (Morota et al., 2007); the different transport and retention capacity of calcium in spinal mitochondria is one mechanism that has been proposed to explain the increased susceptibility of the spinal cord to neurodegenerative processes. Additionally, the combined effects of injury occurring inside and outside the long projection axons at the edges of slowly expanding lesions in the spinal cord, enhance axonal energy failure and axonal degeneration in progressive MS (Campbell et al., 2014).

Another anatomical factor that may make upper limb function more resistant to progression than the lower limbs is the smaller size of the motor units, with a proportionally larger area of the motor cortex, or homunculus, dedicated to hand, arm and shoulder function (Schieber, 2001).

The length-dependent central axonopathy MS hypothesis (Figure 2) is not novel and has been proposed before (Garbern et al., 2002; Herndon, 2002) and has been described in Pelizaeus-Merzbacher disease, a central demyelinating syndrome in which patients lack the major CNS myelin protein, proteolipid protein 1 (Garbern et al., 2002). What we are proposing, however, is that this length-dependent process might be explained by stochastic statistical phenomena that interact with anatomical, pathological and biological factors. In this model shorter axonal pathways may be involved earlier in the disease by chance and manifest clinically with progressive involvement; however, the initial onset of clinically progressive disease in the pathways subserved by shorter axons would be relatively uncommon early in the disease course (Figure 3).

The sensory-motor paradox

It is well known that sensory relapses are more frequent in early, or non-progressive, MS (Weinshenker et al., 1989). In comparison motor, sphincter and cerebellar "attacks", or symptomatic exacerbations identified as such, are more common in older patients and in

progressive disease (Weinshenker et al., 1989). Similarly, sensory, visual and brainstem relapses are known to have a better recovery than motor relapses (Kalincik et al., 2014). Why the paradox between the prognosis of sensory and motor attacks? We propose this paradox may be due to length-dependent mechanisms and the possibility that afferent/sensory systems may have a lower symptomatic threshold compared to efferent/motor systems when affected by MS lesions.

The sensory system has shorter central axons (Webster, 1977) than the motor system (Wiesendanger, 1984), which may protect it if the length-dependent hypothesis is correct. Motor neurons are much longer than sensory neurons and hence more likely to acquire multiple hits that lead to more rapid or earlier exhaustion of functional reserve mechanisms and subsequent susceptibility to neurodegeneration. The architecture of the sensory system is such that afferent inputs go via first, second and third order neurons that communicate via synapses before reaching the cerebral cortex (Webster, 1977). Each relay axon is relatively short and hence this architecture may provide the sensory system relative protection from MS-associated neurodegeneration. The first-order sensory neurones are actually bipolar cells with their cell bodies residing outside the central nervous system in the posterior root ganglia (PRG) (Webster, 1977). A demyelinating plaque affecting the central axonal process is therefore unlikely to kill the neuron residing outside the CNS in the PRG by the process of retrograde neurodegeneration. We know this because multiple sclerosis and sensory radiculopathies that destroy the central neuronal processes before they enter the spinal cord are characterised by preservation of sensory nerve action potentials (SNAPs), which indicates that the neuronal cell bodies and the peripheral axons are intact. The compartmentalisation of first order sensory neuronal cell bodies, outside the CNS, away from the MS pathology, may provide the sensory pathway with a better chance of recovery, via axonal sprouting or axonal regrowth. In comparison pyramidal, or motor, neurons extend their axons from the primary motor cortex to synapse on the anterior horn cells, or their equivalents, in the lateral columns of the spinal cord or brain stem, respectively. Importantly, there are no interneurons, which is why the motor neurones in the corticospinal tract are the longest neurones in the central nervous system. The cell bodies of pyramidal neurones also reside within the CNS, in layer five of the cerebral cortex, and hence are not protected from being directly involved by focal MS pathology.

Why are sensory attacks more common early on in the course of the disease?

In general sensory systems are designed for sensitivity; they need to detect changes in our environment so as to warn of us danger so that we can respond to them. From an evolutionary perspective our afferent/sensory systems are optimised for detection. A very large part of our

cortex is devoted to perceiving the senses. In fact most of the parietal, occipital and temporal lobes are devoted to perceiving, integrating and interpreting sensory inputs. In comparison, the efferent/motor system is optimised for action and much less of our cerebral cortex is dedicated to motor activity. As a result of its size (cortical area), and optimised sensitivity, a small lesion in the sensory pathways may be more likely to cause symptoms than a similar sized lesion in a motor pathway. This may explain why motor attacks have a worse outcome; larger lesions may be associated with greater damage.

Pathological studies indicate that at least half the MS disease burden is found in cortical and subcortical gray matter. A small gray matter lesion in, or near, the sensory cortex could be a very common cause of early attacks. In comparison, a small lesion in the motor cortex may be less likely to cause symptoms. In comparison to the sensory system, the motor system is activated via reflexes, or through conscious volition. The motor system triggers a percept via sensory feedback loops; i.e. we only perceive weakness because the sensory receptors in the joints and muscles tell our brains that there is mismatch between what is meant to be happening and what is happening. The feedback loop results in the motor system having a much higher threshold for detecting change; hence small or relatively minor lesions in the motor system are likely to go unnoticed. This is backed-up by clinical observations; patients with MS are often unaware of detectable, but subtle, weakness in their limbs. This is not the case for the senses.

Small lesions in motor pathways may only cause symptoms if the reserve of the pathway is reduced. Loss of reserve occurs over time and this may explain why motor attacks become more common with time. In comparison, loss of reserve in sensory pathways, and compensatory mechanisms, may reduce the sensitivity of perception and hence have the opposite effect, i.e. small lesions in compromised sensory pathways may be less likely to alter perception and cause symptoms. This could explain why sensory attacks become less frequent in advanced MS.

Asynchronous progressive MS hypothesis

The hypothesis that the neurodegenerative components of the progressive aspects of MS pathology might be a length-dependent, central, axonopathy would have implications for the design of clinical trials testing disease-modifying agents that may target progressive MS. One would assume that once a neuronal system has exhausted its reserve capacity and is gradually losing its function it may be very difficult to modify the processes causing delayed axonal fall-out, by targeting upstream MS-specific disease mechanisms, in particular autoimmune-driven focal

inflammation and demyelination (Coles et al., 2006). In comparison, neuronal pathways that have significant neuronal reserve may be more amenable to disease modification, despite people having entered the clinically apparent progressive phase of MS in another system or domain. We refer to this as the asynchronous progressive MS hypothesis that suggests that neuronal domains might have different disease-course time windows in which disease-modifying therapies may have some benefit (Figure 3 and 4). This hypothesis may explain why so far anti-inflammatory therapies have been, in most phase 3 trials, unsuccessful in progressive MS using the Expanded Disability Status Scale (EDSS) as the primary outcome; particularly above EDSS 3.5, where the EDSS is predominantly an ambulation scale (Hobart et al., 2000). The corollary of the asynchronous progressive MS hypothesis is that trials in which the majority of subjects have a relatively high EDSS, measured over a relatively short time period of time, will only pick-up worsening in the longest neuronal systems at a stage where its reserve capacity is exhausted and less amenable to disease-modifying therapies.

Expanded Disability Status Scale

The EDSS has serious flaws in relation to its robustness as an outcome measure for trials in progressive MS (Hobart et al., 2000); it is non-linear, has ceiling and floor effects, has high intra- and inter-rater variability, represents a functional system-based impairment scale at low scores and a motor disability scale at high scores where it is dominated by ambulation or lower limb function (Hobart et al., 2000). It also involves clinical decision making between the need for and acceptance of a walker versus a cane. The high end of the scale may be its Achilles' heel for progressive MS trials; particularly as it will be more difficult to reverse, or slow down, disability progression in a neuronal system that has already exhausted its reserve capacity using anti-inflammatory disease-modifying agents. Waiting for someone to enter the clinically-apparent progressive phase of MS, which is typically a progressive spastic paraparesis, sets the bar for success very high, particularly if the EDSS is used as the outcome measure. This has previously been referred to as the therapeutic window (Coles et al., 2006); i.e. if anti-inflammatory drugs are started late, too much damage has accrued to prevent the delayed consequences of previous focal inflammatory events running their course. This might imply that trials in the progressive phase of the disease using the EDSS as primary outcome may need to be longer and larger.

Reserve Capacity

Despite its limitations the EDSS may still provide robust enough observations to allow one to generate hypotheses. The length-dependent axonopathy hypothesis (Figure 2) provides an important conceptual framework to generate and test hypotheses. Once a neuronal system has lost it reserve capacity it is expected to be less capable of spontaneous recovery and hence less

prone to improve in function. This concept is supported by observations from the post-hoc analyses of relapse recovery from clinical attacks. In the pivotal natalizumab study (Polman et al., 2006), subjects with an EDSS of less than 3.0 were more likely to make a full recovery from relapses than subjects with an EDSS equal to or greater than 3.0 (Lublin et al., 2014). Even the most potent anti-inflammatory drugs only appear to suppress new lesion formation and have limited effect on reducing pre-existing lesions (Soon et al., 2007; Sorensen et al., 2014). However, the latter may be time dependent; acute or active lesions may be amenable to treatments. For example, relapses under natalizumab treatment not only have a lower severity but also a higher probability of complete confirmed recovery (Lublin et al., 2014). Similarly, fingolimod reduces the likelihood of gadolinium-enhancing lesions present on a baseline scan from converting into persistent black holes on T1-weighted scan (Radue E.W., Sprenger T., De Vera A., Francis G., Rochotte E., Tomic D., Kappos L., 2014); whether these observations are due to anti-inflammatory or neuroprotective effects of natalizumab and fingolimod is a moot point. It does, however, appear that as new focal lesions evolve there is a mix of pathogenic mechanisms whose contribution to damage varies over time; the molecular pathways that prevail in the early stage of a lesion appear to be more amenable to current therapies than those that are present at later stages. However, both these drugs have failed in progressive disease despite their success in relapsing disease.

Therapeutic lag

Neuronal systems with sufficiently preserved functional reserve capacity, may demonstrate therapeutic lag (Figure 4) regarding the time it would take for an anti-inflammatory disease-modifying intervention to impact on the neurodegenerative components of its subsequent progressive disease course. Clinical progression in a neuronal pathway that has no reserve capacity may be primed by focal inflammatory events occurring months or years earlier as some of the pathological mechanisms underlying neuronal degeneration are likely to be delayed. Therefore, anti-inflammatory therapies that reduce new focal MS lesion accumulation may be unlikely to have an impact on neurodegenerative aspects of progression for several years (Figure 4). The effects may also take some time to become detected through imaging modalities due to masking by the effects of pseudoatrophy (early rapid reduction in brain volume) (Koudriavtseva and Mainero, 2016). Therapeutic lag may manifest as a delay for a specific treatment in the slowing down, or a plateauing out, in the rate of progression in a particular pathway. Nevertheless, in subjects with reserve capacity an apparent impact on sustained disease progression may be seen much earlier provided that the anti-inflammatory intervention may either i) decrease more chronic and diffuse inflammation-mediated damage within white and gray

matter tissue, and/or ii) enhance capacity of the affected neuronal pathway to recover, partially or completely, via compensatory mechanisms such as local axonal plasticity, or synthesis of sodium channels across the demyelinated segment to restore conduction, remyelination, distal axonal sprouting and synaptogenesis and cortical or subcortical plasticity.

The concept of a therapeutic lag in progressive MS is supported by the observation of randomised placebo-controlled study of interferon-beta-1b in primary progressive MS (Figure 3); at the end of the 2-year period of observation no clinical or MRI differences were seen between the placebo and active treatment arms (Montalban et al., 2009). However, when the subjects were re-interrogated 5 years after the end of the trial, or 7 years after initial randomisation, clear differences in both clinical and MRI metrics were seen in favour of interferon-beta therapy (Tur et al., 2011). After 5 years without treatment the interferon beta-1b group had significantly better upper limb and cognitive outcomes, respectively, i.e. rather shorter neuronal systems which might have retained higher reserve capacity at time of drug intervention 5-7 years earlier. Consistently, the cohort who were treated with placebo for 2-years showed the greatest decrease in brain parenchymal fraction at year 7 (Tur et al., 2011). Therefore, suppressing inflammation in progressive forms of MS may only manifest after 3-7 years; i.e. when the hypothesised progression primed by previous inflammatory events may have run its course. Therapeutic lag may thus take many years to emerge.

Brain atrophy, an emerging biomarker that is reported to be an integrator of neuroaxonal loss in MS (Bermel and Bakshi 2006) and correlates with T2 and T1 lesion volume (Radue, Barkhof et al. 2015), which are MRI markers of inflammation, demonstrates therapeutic lag (Koudriavtseva and Mainero, 2016). When patients with active MS, as defined by the presence of gadolinium-enhancing lesions, are started on natalizumab brain atrophy rates only plateau-out or ‘normalise’ in year 3 after starting treatment (Sastre-Garriga et al., 2014). This would indicate that the processes responsible for causing neurons and axons to degenerate as a result of an inflammatory insult take many years to play out. Based on these and other observations one may question whether or not the duration of contemporary clinical trials currently underway in progressive forms of MS will be of a long enough duration to overcome the therapeutic lag. In their favour, however, is that some of these studies are event driven and are not of a fixed duration and, hence, will only close once enough clinical events have occurred.

Treatment effects in progressive MS evidenced by non-lower extremity neurological tests

What do the length-dependent MS axonopathy hypothesis and the resulting asynchronous progressive MS (Figure 4) and therapeutic lag (Figure 3) hypotheses, mean for progressive MS? If therapeutic lag exists it would have implications for the optimal duration of doing progressive MS trials. It could mean if the hypothesis was to be confirmed that continuing to focus on longer tract-based neurological domains that may have exhausted their reserve capacity faster as the core components of primary outcome measures would imply that one would have to perform clinical trials that last 3-5 years and possibly longer. We may therefore consider shifting our focus to shorter tract-based pathways that may have higher reserve capacity and hence may be more likely to deliver a positive outcome in a shorter period of time.

A closer look at the outcomes of some of the progressive MS trials supports our hypothesis. In the placebo-controlled, oral methotrexate, chronic progressive MS trial there was less progression of impairment as measured by upper-extremity function tests (9HPT and box-and-blocks test) in the treatment group compared to placebo, and no differences were noted in lower limb outcomes (EDSS and ambulation index) (Goodkin, Rudick et al. 1995). In the placebo-controlled intramuscular interferon (IFN) beta-1a, or IMPACT, SPMS study there was no benefit of IFNbeta-1a on the EDSS, which recruited subjects with an EDSS in range of 3.5-6.0 (Cohen et al., 2002). In comparison the median multiple sclerosis functional composite (MSFC) Z-score change was significantly reduced in the IFNbeta-1a treated subjects compared to placebo-treated subjects (Cohen et al., 2002). This was an effect driven by the 9HPT and the paced auditory serial addition (PASAT) tests, which assessed neuronal domains with shorter axonal pathways and potentially more preserved functional reserve in the target population. In the rituximab primary progressive trial (OLYMPUS study) the EDSS was shown to have a low sensitivity and reliability to detect disability progression (Hawker et al., 2009). When disability was analysed using different disability end points, the timed 25-foot walk test (T25FWT) and 9HPT were more sensitive to detect overall progression events, with the T25FWT being superior to the 9HPT in this respect (Zhang et al., 2014). However, despite a lower observed event rate for 9HPT the rituximab treatment effect was slightly more pronounced on 9HPT progression (hazard ratio [95%CI]: 0.53 [0.31–0.93] for 24-week confirmed progression) than on T25FW progression (hazard ratio [95%CI] 0.60 [0.43–0.83] for 24-week confirmed progression). The observation in the OLYMPUS trial that T25FWT was overall more sensitive to change than 9HPT in both arms though rituximab treatment effect was merely higher on 9HPT, remains compatible with the length-dependent hypothesis. Nevertheless there might have been enough reserve capacity in the lower extremity motor system in this cohort of subjects to provide a positive T25FW read-out within 2 years. It is worth noting

that the majority of subjects enrolled in this study had an EDSS of less than 6.0 (Hawker et al., 2009), which would favour the latter explanation.

More recently in the SPMS natalizumab, or ASCEND, trial in which 63% of study subjects had an EDSS either 6.0 or 6.5, as predicted by the length-dependent axonopathy hypothesis, natalizumab had no effect on lower limb function (EDSS and T25FW), but a statistically significant treatment effect on reducing upper-limb disability progression unrelated to relapse as measured using the 9HPT (Steiner D., Arnold DL, Freedman MS, Goldman MD, Hartung HP, Havrdova E, Jeffery D, Kapoor R, Miller A, Sellebjerg F, Yu B, Forrestal F, Liu K, Amarante D, Cadavid D, n.d.). In comparison, no differential treatment effect was seen between lower and upper limb function with fingolimod, compared to placebo, in the INFORMS PPMS trial (Lublin et al., 2016). Despite fingolimod not having a significant treatment effect on lower limb and upper limb function it did have an impact on inflammation and significantly reduced relapses and focal MRI activity compared to placebo-treated patients (Lublin et al., 2016). Whether this disproves the length-dependent axonopathy hypothesis will require further post-hoc analyses of the data.

In support of some of the aforementioned clinical observations are data from animal models of progressive MS. In chronic relapsing experimental allergic encephalomyelitis (CREAE) in the Biozzi ABH mouse, animals go through a relapsing-remitting phase before entering a secondary progressive phase (Pryce, O'Neill et al. 2005, Al-Izki, Pryce et al. 2011, Al-Izki, Pryce et al. 2012). In this model progressive disease initially manifests in the tail before affecting the hind limbs and finally the forelimbs (Al-Izki, Pryce et al. 2012); a pattern consistent with a central length-dependent axonopathy. Analysis of this model demonstrates that it recapitulates many of the clinical and pathological features seen in MS that have been linked to the progression, such as demyelination, neuroaxonal loss and gliosis and is refractory to peripheral immunosuppression similar to progressive MS (Al-Izki et al., 2012, 2011; Hampton et al., 2013; Pryce et al., 2005). This model provides experimental evidence that a multifocal inflammatory disease of the CNS manifests in a length-dependent manner and that progressive neurodegeneration occurs from disease onset but does not become clinically apparent until substantial loss of neuronal reserve is lost (Al-Izki et al., 2012; Hampton et al., 2013; Pryce et al., 2005). Interestingly, fingolimod did not impact on the progressive phase of this model, despite marked influences on relapsing attacks (Al-Izki et al., 2011), mirroring the observations in the PPMS INFORMS study (Al-Izki et al., 2011; Lublin et al., 2016).

Non-MS related mechanisms contributing to progressive disease

The length-dependent MS axonopathy model is not compatible with the recent results of the positive high-dose simvastatin trial in SPMS (Chataway et al., 2014). This was a randomised double-blind trial that compared simvastatin, 80mg per day, to placebo over 2-years. The primary outcome measure was brain atrophy using volumetric MRI. Secondary outcomes included the EDSS measure. Whole brain atrophy rate in the placebo-treated group was 0.59% per year compared to 0.30% per year on simvastatin; the differences in reductions of -0.25% (95% CI -0.42 to -0.09) was significant ($p = 0.003$). There was also a significant impact on worsening disability ($p < 0.01$); the mean EDSS increased from 5.9 to 6.4 in placebo-treated subjects and to a lesser extent, from 5.8 to 5.9, in the simvastatin-treated group, over 2 years. A length-dependent MS axonopathy model would not predict this outcome, as there was no therapeutic lag. An alternative explanation is that simvastatin may be working downstream of primary inflammatory demyelination and targeting pathological processes responsible for delayed neurodegeneration, or that the placebo arm may have had more activity prior to initiation of the study (Koudriavtseva and Mainero, 2016). If the simvastatin trial results are replicated it may indicate that the progressive phase of MS can be modified, possibly by targeting mechanisms that are not linked to, or are a consequence of, autoimmune-driven inflammatory demyelination.

Conclusions

In conclusion, if we want successful trials targeting the neurodegenerative pathological components of progressive MS disease we may have to rethink what outcome measures are most appropriate to use, with a particular focus on more sensitive and responsive outcome measures in neuronal domains that still retain substantial reserve capacity earlier in the disease course; for example, upper limb, cognitive and visual outcomes. Composite endpoints, including multiple disability measures might be superior to using the EDSS alone; through an increase in sensitivity and possibly specificity. Composite disability endpoints may overcome the over-emphasis of ambulation in EDSS and may therefore be more balanced towards functional domains that have sufficient reserve capacity. Should the length-dependent MS axonopathy hypothesis hold true, one may envisage an era of adaptive studies, for example in which futility analyses are done early using outcome measures that assess neuronal domains that may have higher reserve capacity after 2 years and if positive the study will be extended to look at outcome measures in pathways with more limited reserve capacity, i.e. lower limb motor function.

As a potential driver of clinically asynchronous progression of MS, the length-dependent MS axonopathy hypothesis should be subject to appropriate and rigorous testing to yield evidence-based insights that may lead to customised study designs in the development of

effective treatments for progressive MS. Although the right trial designs, endpoints and futility analyses are critical, appropriate patient populations with more refined inclusion criteria, and/or pre-planned stratification of patients with different severity and duration of progressive disease may be required to improve trial design. The latter may dictate a different length of follow up for certain kind of patient populations to appropriately assess efficacy of putative DMTs. This would usher in an era of truly “patient-centric” and “disease-centric” trials.

Dedication

"If I have seen further, it is by standing upon the shoulders of giants" Sir Isaac Newton.

Since the initial drafting of this paper it came to our attention that the concept of length-dependency underlying the clinical phenotype of multiple sclerosis over time has previously been observed and documented by John Kurtzke (figure 5) (Kurtzke, 2015). We therefore dedicate this paper to the memory of John Kurtzke a true giant in the field of multiple sclerosis who passed away on the 1 December 2015.

Figure 1

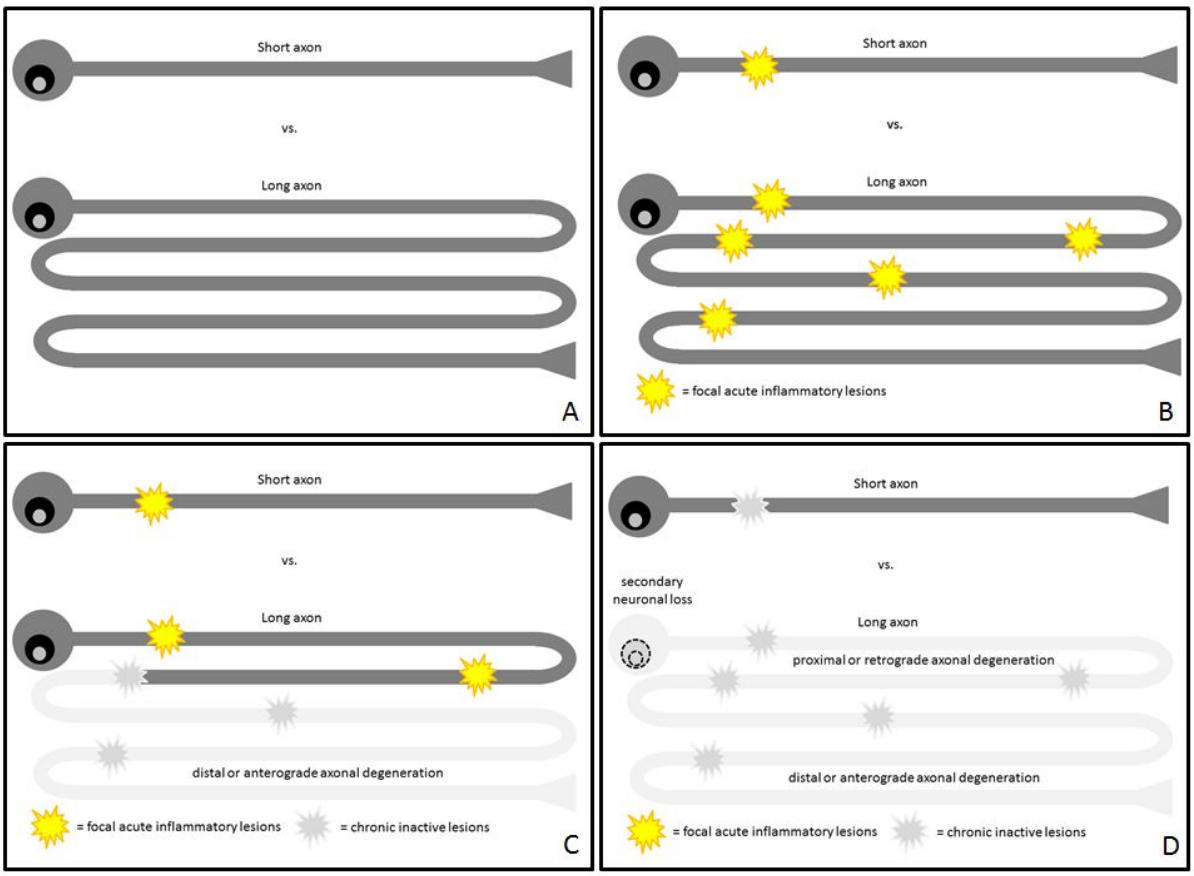


Figure 1a-d: Proposed model underlying MS as a central length-dependent axonopathy

Figures a-d demonstrates that if multiple sclerosis lesions occurred spatially randomly, pathways with longer axons would be affected more frequently by focal acute inflammatory lesions; in the example in figure 1a & b this is five times more likely. The greater lesion burden in the longer pathway will first result in anterograde, or Wallerian, degeneration (figure 1c) followed later by retrograde degeneration and subsequently neuronal loss (figure 1d).

Figure 2

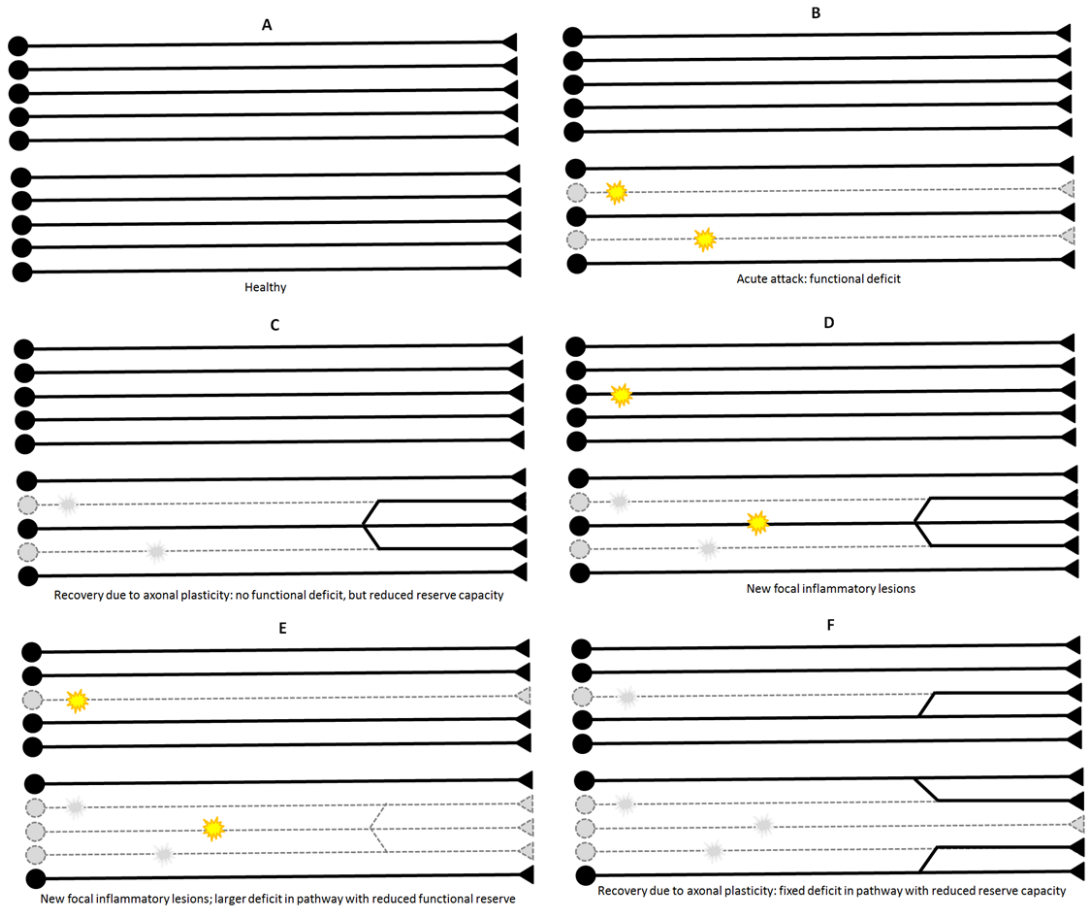


Figure 2a-e: Loss of functional reserve predisposes pathways to manifest earlier with clinically-apparent progressive MS

Schematic example representation of the sequential paths to permanent functional deficit associated with delayed axonal loss following accumulation of focal inflammatory lesions. Compared to normal tissue (Figure 2a) the initial impact of multiple focal inflammatory lesions affecting the lower neuronal pathway (Figure 2b) may trigger a loss of 40% of its functioning neuroaxonal units x, which is able to recover function. Based on animal experiments this is hypothesised to be due to axonal plasticity, and axonal sprouting, from surviving neuroaxonal units (Figure 2c). However, the loss of reserve capacity in the lower pathway makes it more susceptible to damage from further focal inflammatory lesions (Figure 2d), with greater loss of function (Figure 2d and 2e) and the inability to recover completely (Figure 2f) leading to the emergence of clinically-apparent progressive disease.

Figure 3.

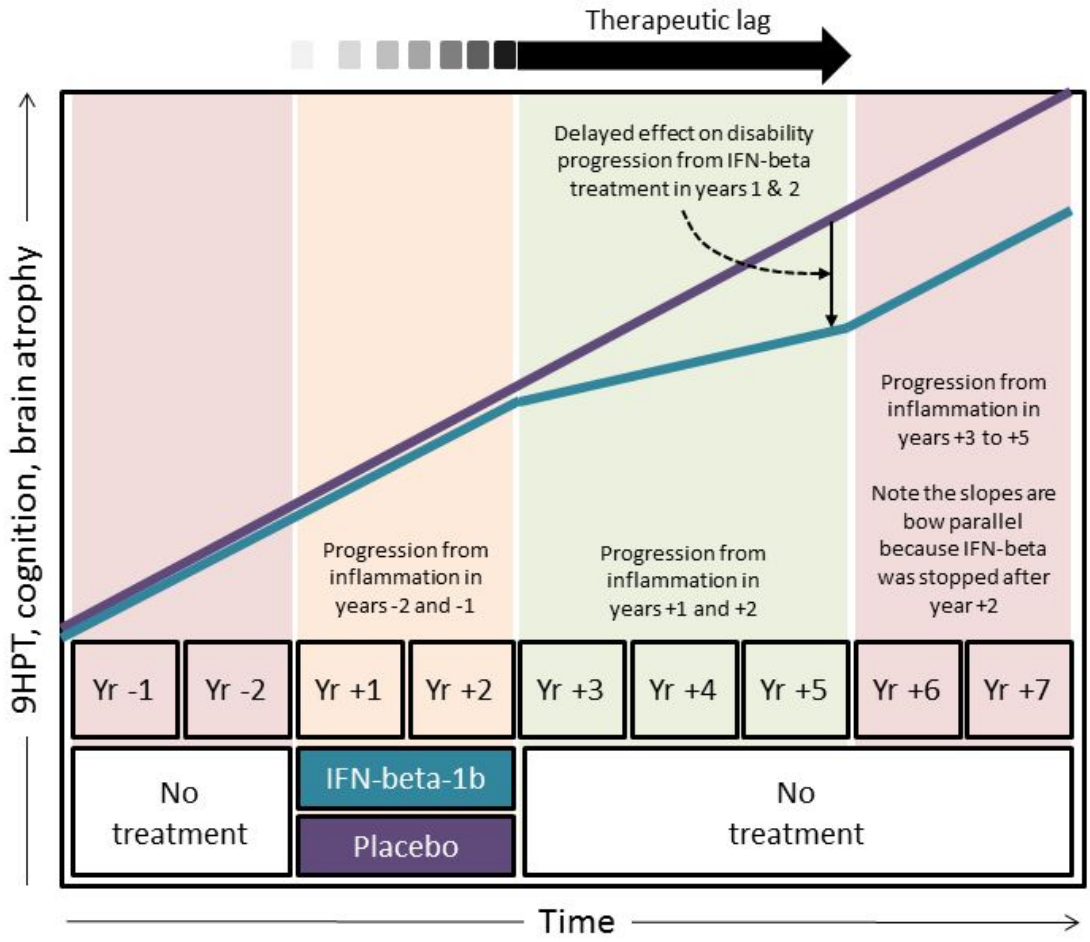


Figure 3: Therapeutic lag

Therapeutic lag refers to the observations that in patients with progressive disease it takes several years for anti-inflammatory therapies to have an impact on disease progression. Figure 3 represents the randomised placebo-controlled study of interferon-beta-1b in primary progressive MS (Montalban et al., 2009). At the end of the two year trial there were no differences between the placebo and active treatment arms. However, at year 7, 5 years after the end of the trial, clear differences were seen in terms of both the clinical and MRI metrics in favour of the initial interferon-beta-1b therapy arm (Tur et al., 2011). Therefore, suppressing inflammation in progressive forms of MS may only translate into a clinically meaningful outcome after lag period of 3-7 years.

Figure 4. Redefining the therapeutic window in progressive MS

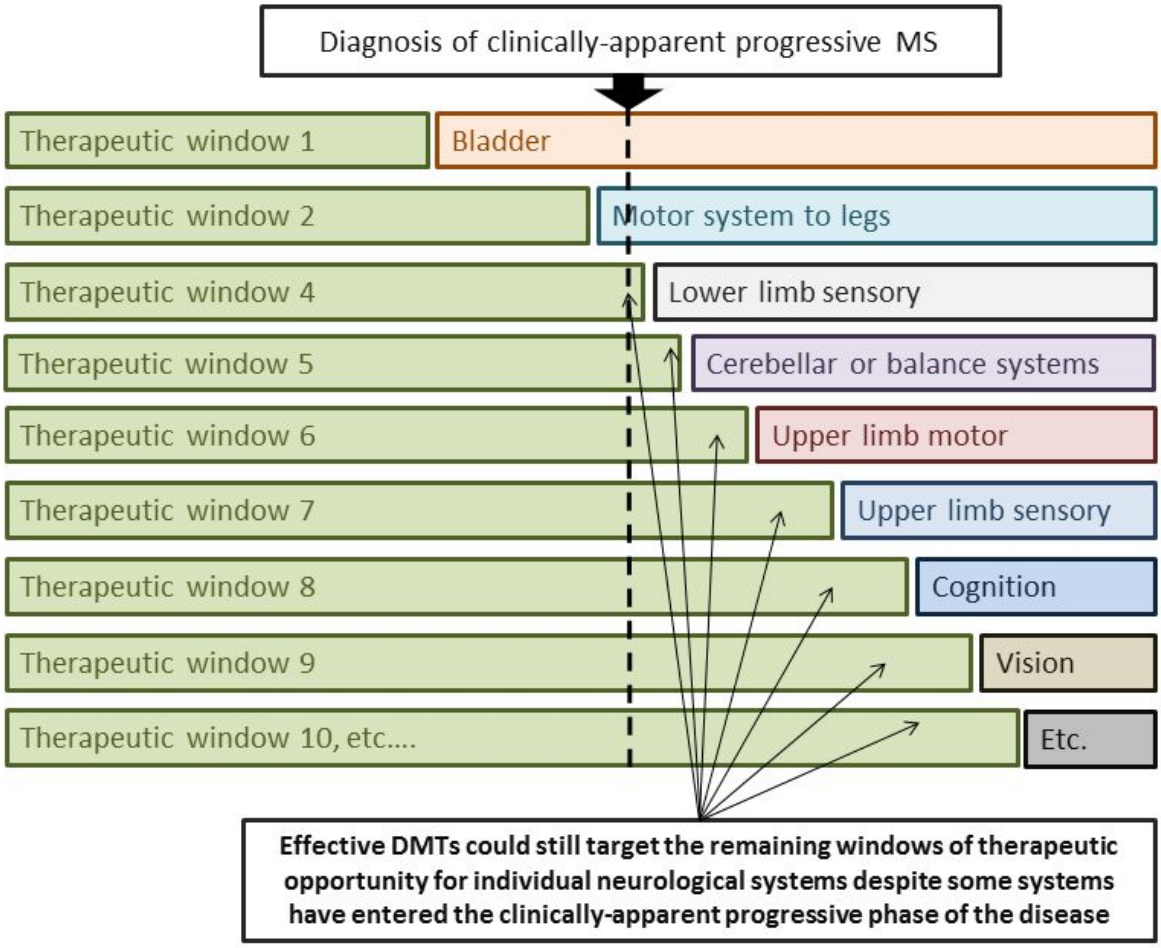
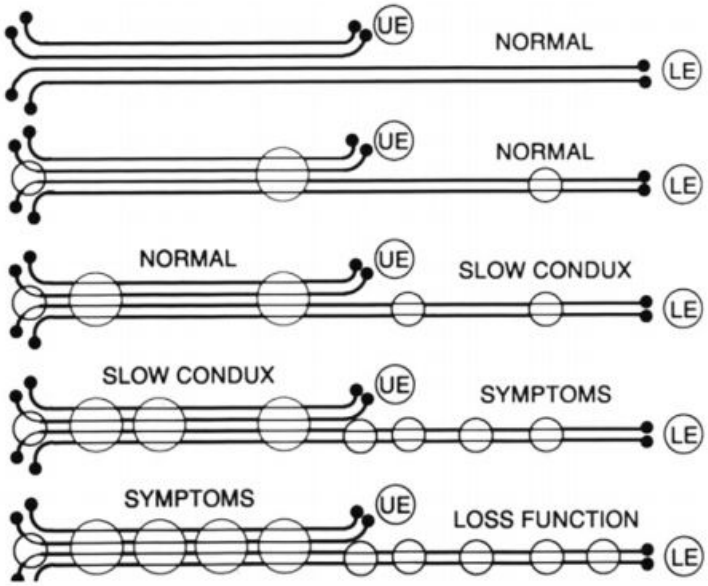


Figure 4: The asynchronous progressive MS hypothesis

This testable hypothesis predicts that neuronal domains may enter the clinically-apparent progressive phase of the disease at different rates depending on the length of the axons in the pathway and the reserve capacity of that pathway, i.e. its ability to compensate for ongoing or future damage. This hypothesis predicts that different neuronal domains will have different length-dependent therapeutic windows in which to respond to anti-inflammatory therapies that suppress ongoing inflammatory demyelinating lesions. The neuronal domains that have not entered the clinically-apparent progressive phase of the disease, due to preservation of functional reserve, may only respond to anti-inflammatory therapies with a delay in the effect due to the delayed onset of clinical expression of neurodegenerative axonal loss; the so-called therapeutic lag. In contrast, the neuronal domains that have already entered the clinically-apparent progressive phase of the disease, due to loss of functional reserve, may fail to respond to anti-inflammatory therapies.

Figure 5: Kurtzke's schematic diagram of MS plaques in the brainstem and spinal cord with resulting clinical symptoms from earlier observations (UE – upper extremity, LE – lower extremity) (Kurtzke, 2015; Tur et al., 2011). Reproduced with permission of MSARD, Elsevier.



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