

# Clinical trials in multiple sclerosis: potential future trial designs

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**Abstract:** Clinical trials of new treatments in multiple sclerosis (MS) currently require large sample sizes and long durations in order to yield reliable results. The differential responses of an already heterogeneous population of MS patients to individual disease-modifying therapies (DMTs) will further complicate future trials. MS trials with smaller samples and faster outcomes are conceivable through the substitution of current clinical and MRI outcomes with objectively measurable genomic and proteomic biomarkers. Currently, biomarkers that could be utilized for diagnosis and monitoring of MS disease activity are in the early validation phase. The power of single biomarkers or multiple correlated biomarkers to predict prognosis and response to treatment could initially be compared with currently accepted methods. These prospectively validated disease biomarkers could then be used to subcategorize the spectrum of MS patients into a finite number of endophenotypes with demonstrable different molecular pathogenesis and DMT response profiles. Newly developed DMT could potentially be assessed within specific endophenotypes and compared with pharmacogenomically relevant active comparator DMT. This approach may increase the efficiency of MS trials through homogenization of patient population and minimization of nonresponders in study groups, providing the potential for the development of targeted therapies.

**Keywords:** Multiple Sclerosis, Endophenotypes, Clinical trial, Trial design, Disease Modifying therapy, Pharmacology

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## Introduction

Clinical trials in multiple sclerosis (MS) have been the cornerstone of drug development, leading to the introduction of an arsenal of disease-modifying therapies (DMT) that has provided neurologists with a range of options for the treatment of MS, thereby improving chances for a beneficial neurological outcome.<sup>1</sup>

The ultimate goal of a curative treatment for all patients with MS has not been achieved. Even today, with accurate early diagnosis, aggressive treatment, and vigilant clinical and paraclinical monitoring for breakthrough disease activity, a proportion of MS patients inevitably accumulate neurological disability and transition into a progressive disease course that is currently not responsive, or only marginally responsive, to

approved pharmacotherapies. This calls for continued research to better understand the immunopathological phenomena that drive disease activity, and to develop more effective interventions based on this newly acquired knowledge. One approach toward understanding the pathogenesis of MS is to investigate therapies targeted to specific patients in the context of clinical trials. A major question that remains is whether candidate treatments that do not seem to benefit MS patients are truly not effective, or whether the benefits are seen in areas not currently measured. There is a difference between those patients who fail based on current measures and those who may not appear to achieve benefits. Thus, the design of time-limited and budget-efficient phase III MS clinical trials faces unprecedented challenges that are due to the nature of the disease,

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the availability of numerous DMTs, and the standard outcomes used.

### The status quo and limitations of current trial designs

#### *MS heterogeneity*

Clinically, MS is a very heterogeneous disease with regard to age of onset, neurological manifestations, relapse rates, and the rate at which neurological disability accumulates.<sup>2,3</sup> Also, the disease course appears to differ between ethnic and racial patient populations.<sup>4</sup> There is also heterogeneity in the diversity of pharmacological and immunological candidate putative molecular and cellular targets.<sup>5,6</sup> Consequently, the challenge to identify the correct pharmacological agent for the correct patient population during the correct disease stage can be daunting.

It is currently thought that genetic susceptibility<sup>7-10</sup> and environmental risk factors<sup>11-15</sup> play a role in pathophysiology of MS. The extent of the involvement of specific factors in the initiation and perpetuation of MS disease activity remains to be elucidated. Presumably, the initiation of MS is not predicated on a singular and sudden event, but rather a series of genetically and proteomically permissive and environmentally triggered events that become chronic and variable. The relative importance and combination of contributing genetic and environmental risk factors likely drive the variability of disease phenotypes among MS populations on individual and global scales. A meaningful and clinically significant genetic association for central nervous system (CNS) autoimmunity among humans is currently limited to HLADRB1\*15:01, which may also impact disease activity.<sup>16</sup> Large genome-wide or exome screening assays have not been able to identify other risk alleles that have a meaningful impact on clinical disease onset or phenotype.<sup>17-19</sup>

Given the heterogeneity of clinical disease in patients with MS, different environmental factors may be critical in different populations. Globally, MS incidence varies across macro-geographical areas,<sup>20</sup> and may be modulated by latitude and sun exposure,<sup>21</sup> the degree of urbanization,<sup>22</sup> the prevalence of certain infections,<sup>23</sup> smoking,<sup>24</sup> and other factors.<sup>25,26</sup> Variability in neurological signs, relapse frequency, and the rate of disease progression

results in a wide range of clinical phenotypes from benign relapsing MS with very limited progression over decades<sup>27-29</sup> to primary progressive MS with relentless accumulation of disability and premature death.<sup>30-32</sup> The MS spectrum is further complicated by the introduction of subclinical MS, as seen in radiologically isolated syndromes (RIS)<sup>33</sup> and the concept of MS prodrome.<sup>34-37</sup> Differential treatment responses to DMTs in clinical trials and post-drug approval also constitute disease heterogeneity.<sup>38</sup> This heterogeneity among MS patients illustrates an unmet need for more individualized treatment approaches through improved pharmacogenomic methods, which in turn will require objective molecular categorization of subclasses of MS patients.

#### *Current problems in the clinical and paraclinical assessment of MS*

Clinical and paraclinical MS has thus far been examined on a macro-anatomical scale both in terms of diagnosis and follow up of disease activity. MS is diagnosed when there is overt clinical disability accompanied by corroborating MRI abnormalities. Obvious flaws of this approach are delays in diagnoses and relatively frequent misdiagnoses.<sup>39-41</sup>

Current disease phenotypes are mostly based on clinical characteristics, and magnetic resonance imaging (MRI) of the brain as the paraclinical biomarker for further definition of these clinical phenotypes.<sup>42</sup> However, currently utilized clinical and paraclinical tools for detection and monitoring of MS are limited.

The Expanded Disability Status Scale (EDSS), as the main clinical scoring scale for MS disability assessment and estimation of disease progression, has a skewed reliance on measurable physical disability and primarily reflects ambulation status as the readout of physical deficits. However, neurological deficits secondary to MS span across functional areas that are not easy to capture, including cognitive decline, sensory disturbances, and loss of motor dexterity.<sup>43</sup> The EDSS only registers overt deficits and misses the subtle clinical deteriorations in the course of the disease as it is inherently limited by the sensitivity of the clinical examinations that constitute this scale.<sup>44</sup> Finally, the EDSS records clinical information retrospectively, but yields no information on disease progression.<sup>43,45</sup>

Other disability scales, including the Multiple Sclerosis Functional Composite (MSFC), a three-part, standardized, quantitative assessment instrument that was developed to address some of the problems of the EDSS,<sup>46</sup> was criticized by regulators for the uncertain clinical meaningfulness of the composite measure.

MRI as the main paraclinical assessment method in MS is also flawed due to its dependence on relatively large anatomical structural changes within the CNS. It is very likely that disease progression continues on a micro-level, driven by cellular and molecular events beyond the sensitivity of MRI.<sup>44</sup>

Analyses of CSF specimens show oligoclonal bands (OCBs) in most patients. Composed of immunoglobulins, OCBs may suggest a pathogenic role that autoreactive antigen-specific B cells play in MS. The presence of OCBs is a valuable tool in confirmation of MS diagnosis.<sup>47–49</sup> However, having no or little variability with disease activity or clinical disability, OCBs have little utility as a biomarker in clinical treatment trials.

Previous studies have assessed molecules for their ability to diagnose MS or provide predictive data. However, aside from recent novel observations on serum neurofilament light chain levels as a marker of disease activity and a tool to monitor treatment responses,<sup>50,51</sup> most other biomarkers that have been studied lack sensitivity, specificity, and predictive value.<sup>44</sup>

#### *Current problems with trial design in MS*

Given the heterogeneity and diagnostic methods of MS, extensive clinical trial programs that entail very large numbers of patients and long durations are needed for each new agent to be tested and approved. In addition, with the availability of already-approved effective treatments, active comparator-controlled trial designs are now commonly employed, resulting in the need to recruit even larger cohorts with longer follow-up periods per trial than placebo-controlled studies before meaningful clinical and statistical differences can be reliably detected. As mentioned above, another issue is the lack of sensitivity and utility of presently employed clinical and paraclinical assessments, which contributes to needing large numbers of patients and long-duration clinical trials in MS.

Clinically, and presumably pathogenetically, diverse patient populations are recruited to current MS clinical trials based on standard inclusion and exclusion criteria. Consequently, negative or positive results may be driven by a particular subgroup, as suggested by post-hoc analyses.<sup>52,53</sup> Nonresponders in study cohorts not only fail to receive the benefits of therapeutic interventions, but they also dilute positive signals from the responders in their cohort. Furthermore, data obtained from these studies are utilized to identify active comparator controls for future trials. Therefore, any bias from unrecognized effects of nonresponders on clinical trial outcomes often carry over to future assessments of novel therapies.

Finally, all investigations of new therapies are accompanied by a burden of risk for the study participants through unexpected adverse reactions, or through the substantial delay in drug approval that may result from the current trial designs.

#### **Requirements for novel trial designs**

First, tangible goals should be articulated that allow the identification of well-defined study populations and treatment responders through biological markers. These markers should allow the identification of likely treatment responders and nonresponders within clinical trial cohorts, and the verification of a response and nonresponse in validation cohorts. Such markers can be viewed as dynamic and sensitive surveillance methods that allow detection of positive response to DMTs in a given patient. Currently available examples include the visualization of MRI brain lesions and the quantification of cerebrospinal fluid (CSF) and blood neurofilament by digital enzyme-linked immunosorbent assay (ELISA).

Safety and effectiveness are the two main deliverables of the clinical trials process. Pharmacological safety is relatively easily defined by the incidence of adverse effects and side-effects of a therapeutic intervention. The remainder of this article focuses on the definition of effectiveness, which is considerably more challenging in MS.

To achieve approval of therapeutic interventions that are ideally suited for a defined patient population, the goals should be the identification of biological markers that predict a meaningful clinical response to each pharmacological agent in patient subgroups, to reduce the sample size of

the study population, to shorten the duration of the clinical trial, and to accelerate drug approval. Ideally, investigators will be able to group MS patients into objectively differentiable subclasses through these biological markers. Admittedly, this is a tall order in the face of the many treatments available, and the lack of modern natural history data.

Novel candidates for MS biomarkers should meet the following criteria before they can achieve any advantage over current methods. A potential candidate biomarker should (1) reflect the underlying pathophysiology of MS; (2) differentiate sensitively between the disease phenotypes in a prospective assessment of MS populations; (3) detect disease reactivation; and (4) provide reliable data for a precise prediction of disease severity and prognosis. These criteria are difficult, but achievable, goals.

### Endophenotypes in MS classification

Endophenotypes refer to subclasses present within one clinical diagnosis.<sup>54</sup> They have various manifestations that are linked to underlying genetic and epigenetic differences between individuals.<sup>55</sup> However, their divergence never exceeds the definitions of the background clinical syndrome. Since they are rooted in the genetic variations, endophenotypes are inheritable. Their effects are reflected through a matrix of biochemical molecules that are made or adjusted based on the genetic code. Biomarkers, defined as an objectively measurable feature that differentiates between normal and pathologic states and responds to therapeutic interventions,<sup>56</sup> are suitable candidates for (1) the disease diagnosis and (2) the identification of endophenotypes in disease populations. Such endophenotypes are represented through a meaningful shift in biomarkers compared with other disease subclasses that allows for disease monitoring.

The majority of previous biomarker studies in MS was hypothesis-driven and focused on the associations of MS clinical phenotypes with single biomarkers. In some instances, studies with wider scopes were able to draw conclusions from shifts in patterns of large biomarker groups within individual MS patients or across MS populations.<sup>57</sup>

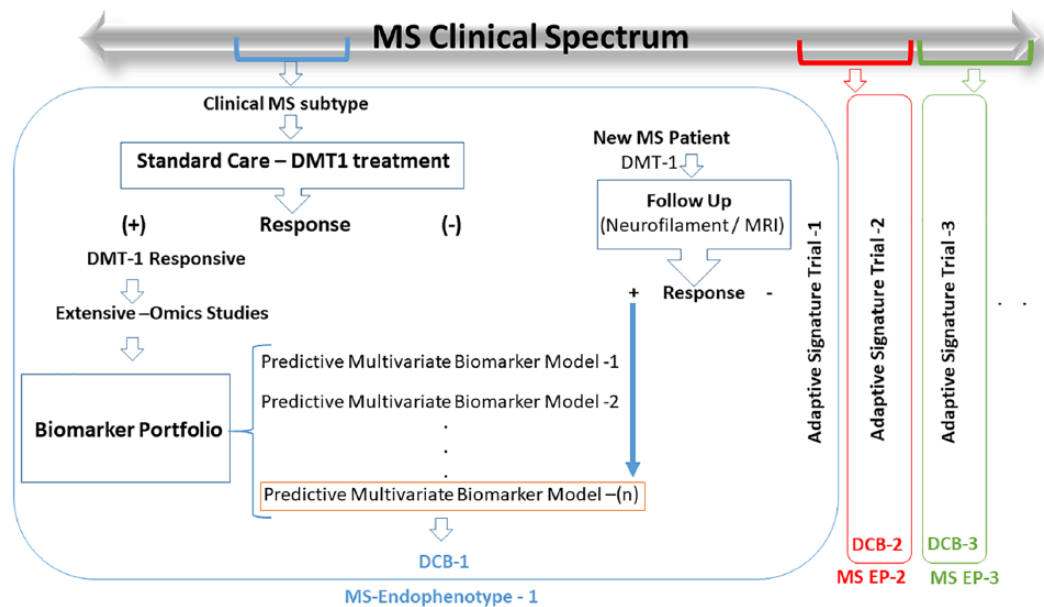
The multifactorial and complex pathogenesis of MS likely causes variable changes across a wide

field of interdependent biomarkers that amount to system-level cascades of biochemical rearrangements. Thus, implementation of a reductionist approach based on genomic and proteomic analysis of biomarkers where type and number of measured variables are limited only by the overall power of the final model should be utilized to define objective MS endophenotypes. In this article, we utilize the term ‘disease classifying biomarkers’ (DCB) as a representation of a multivariable model based on multiple genomic and proteomic measurements of biomarkers in MS biological samples.

Through novel proteomics assays, biomarker-based categorization of MS subclasses suggest (1) stable intraindividual levels of MS-related biomarkers in steady states; (2) meaningful differences in biomarker levels between healthy populations and MS patients; (3) variations in biomarker levels and ratios across different MS subclasses; and (4) an active response to MS progression and therapeutic interventions.<sup>57</sup> These methods are still limited due to the noise that is inherent to spectroscopic prote-omics assessments. In particular, the biomarker discovery phase often suffers from overfitting, meaning small samples on which a high number of tests is being conducted, and often without correction for multiple comparisons. These new strategies should be verified in controlled prospective validation studies.<sup>57,58</sup>

### Prospective cohorts for endophenotype validations

The implementation of DCB-based endophenotype definition in MS classification is the primary requirement for the improvement of future MS trials. A variable disease spectrum is also likely with biomarker-based endophenotypes. For a new DCB-based MS disease spectrum to have any clinical significance, validation studies have to be undertaken to establish treatment response rates of patients within each endophenotype across the whole spectrum. Measuring DCBs through extensive -omics studies in patients with proven response to certain DMTs across the entire range of MS phenotypes determines the distribution of specific DCBs for each part of the disease spectrum. We hypothesize that suitable DCBs yield measurable predictions in terms of prognosis and response to their corresponding treatments. Such predictions will require validation through demonstrable



**Figure 1.** The definition of multiple sclerosis (MS) endophenotypes (EPs) will be based on response to disease-modifying therapies (DMTs) in prospective validating cohorts. Disease classifying biomarkers (DCBs) constitute the most reliable multivariate model recognizable from biomarker portfolios in each MS subpopulation. The implementation of new endophenotypes translates the current clinical and paraclinical subtypes of MS into finite biomarker-based MS endophenotypes.

correlations with MRI imaging outcomes and dynamic and sensitive disease markers, such as CSF and blood neurofilament levels in relatively short prospective studies among age- and comorbidity-adjusted patient cohorts. This will be a dynamic process that adds validated DCBs which, once validated, may replace their MRI and clinical assessment predecessors in future trial designs (Figure 1). Furthermore, in prospective cohort studies treatment response data of each DMT can be analyzed against endophenotype subpopulations. By using the already established DCBs, current DMTs with the most effectiveness for each endophenotype will be determined (Figure 2).

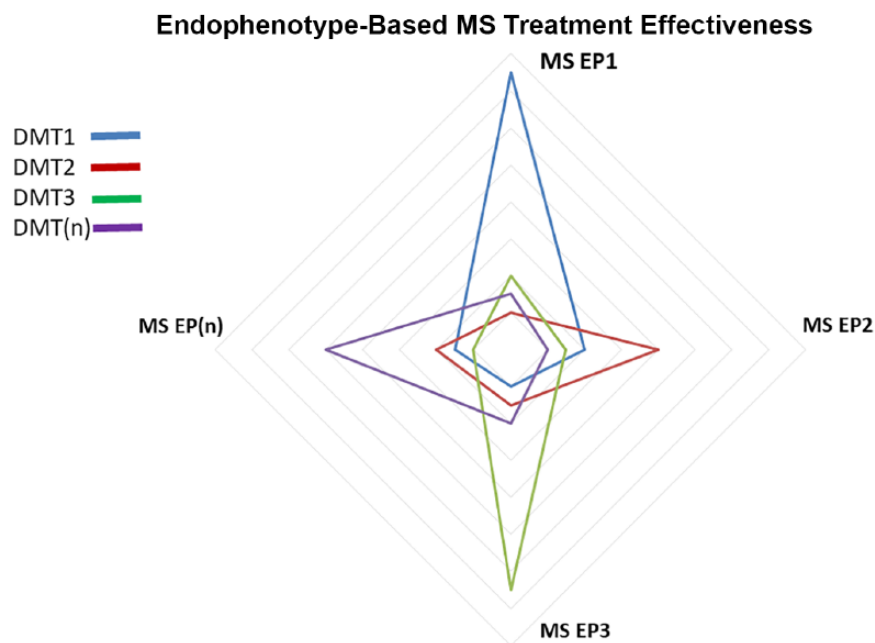
### Novel MS trial design

Prospective validation of DCB-based MS endophenotypes in terms of treatment responses will allow new trial designs. This new concept would be especially useful in phase III trial designs where objective effectiveness benefits of novel therapies are weighed against currently approved DMTs. However, there needs to be some precautions and guidelines to consider in a DCB-based trial design. Simon and Maitournam's discussion of targeted trial design points out that many factors

impact the success for targeted designs, which fall under the rubric of precision medicine.<sup>59</sup> First is the proportion of responsive patients, the accuracy of the assay identifying the targeted group, and the degree to which the mechanism of action is understood. They point out that when the group that tests positive for the biomarker is over 50% of the population, the value of the targeted design is minimal, and the costs of screening and recruitment can outweigh any benefits. Similarly, unless there is marked differences in responsiveness between those with and without the biomarker, the targeted design may not be efficient since the larger sample size can overcome the mixing of the benefits between those with and without the biomarker by eliminating the need to screen and only randomize those with the biomarker.

### Study goals: primary outcome measures

The study goals, as in any other new MS treatment trial, should be the reduction or complete suppression of disease activity and the prevention and/or minimization of neurological deficit accumulation in the shortest possible time span. In comparison with current outcome measures in MS trials, where studies look for relapse rate



**Figure 2.** Endophenotype (EP)-based therapeutic interventions are most effective within their corresponding multiple sclerosis (MS) endophenotype.

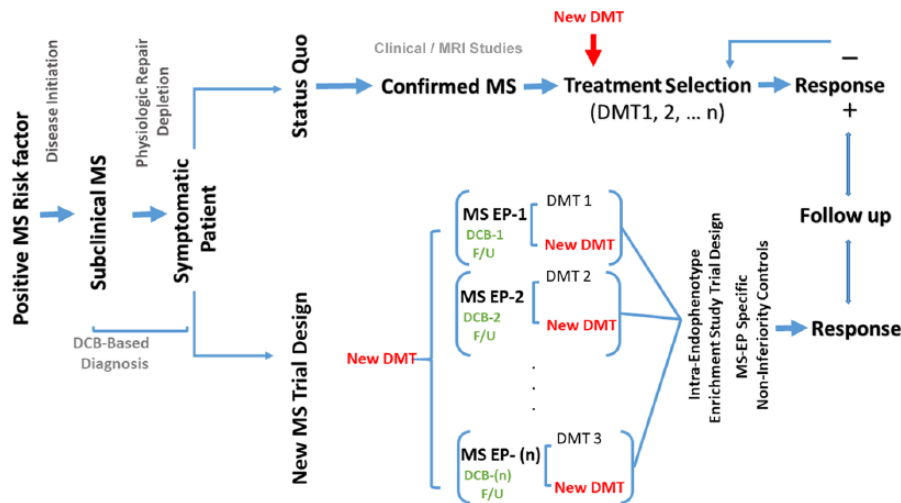
reduction, slowed disability worsening, stabilization, or improvement, and a decrease in the number of brain MRI lesions and global or regional atrophy, biomarker-based outcomes could arrive at the same conclusion faster and with more reproducible results.

DCBs for each endophenotype will be used for establishment of effectiveness in comparison with previously available effective DMTs for the same endophenotype. The nature of direct measurements of biomarkers provides a faster registration of response to treatment, but at the same time the additive power of measuring multiple biomarker variables in DCB models can be harnessed to arrive at a definitive conclusion of study outcomes in significantly less time compared with current designs.

This may afford what are called basket and bucket trials, where one mutation or molecular variation is studied with multiple drugs or one drug is applied to multiple mutations or molecular variations. These trials may allow various approaches to treatment trials where multiple component trials are ongoing with the same infrastructure and same measurements, but the outcomes may differ, and/or the drugs and treatments may differ.

#### *Controls: randomization*

Each new DMT will go through phase I and II trials where the most eligible endophenotypes for the new treatment are determined, before it is assessed in a phase III trial. Recruited control patients, randomized into an active comparator group, will also be selected from the same endophenotypes. An enrichment trial design with noninferiority control model might be used to determine the effectiveness of the experimental treatment against a control population with the same disease endophenotype receiving the best alternative DMT evident by DCB responses (Figure 3). Alternatively, multiple treatments may be compared, dropping inferior treatments as they are identified with comparisons with cohorts of controls in what are called platform trials. Platform trials use a single protocol with multiple treatments evaluated. Adaptive platform trials allow for dropping treatments for futility, superiority, or adding new treatments to be tested. They require extensive planning and cooperation on the part of the treatment providers, especially pharmaceutical companies who may feel they have too much at risk to participate. These approaches are admittedly novel in the field of MS. However, similar designs have been successfully implemented in other areas of clinical medicine, including oncology trials. The



**Figure 3.** New endophenotype (EP)-based multiple sclerosis (MS) trial design versus the status quo. Following an enrichment trial design, new disease-modifying therapies (DMTs) will be assessed against intra-endophenotype patient-DMT controls.

similarities of a DCB-based definition of endophenotypes in MS to mutation-based models of cancer categorization provide plausibility for their potential success in MS. Very likely, DCB-based MS endophenotypes will be inherently different from the strictly genetic basis of cancer subpopulations.<sup>60–62</sup>

#### *Sampling population: sample size*

The lowest number of inclusion criteria will allow more applicable or pragmatic clinical trials and will achieve the greatest generalizability. However, patient enrollment should include individuals with similar endophenotypes who will be identified at a screening DCB evaluation. To avoid confounding by prior treatment, it is ideal to have treatment-naïve patients with the desirable endophenotypes randomly assigned to the experimental treatment group or an active comparator control. Since the DCB-based endophenotypes are reactive to DMT, biomarker-washout process should be validated for each DMT in comparison with treatment-naïve patients prior to study enrollments.

Selecting patients from within one endophenotype homogenizes the samples in the experimental treatment and control groups, reducing the variability among patients and thus allowing for detection of treatment differences with smaller sample sizes.

Furthermore, the shift from clinical outcome measures toward DCB-based assessments changes the framework of the minimum accepted changes for a clinical significance: The new variables will have considerably wider ranges resulting in a significant drop in the required sample size. The combined effects of these two factors are potentially strong enough to decrease the number of enrolled patients in the new trials. In current trial settings for MS, the heterogeneity of the patient population and DMT responses invokes the need for sample sizes of over 1000 patients. The hope with employing an endophenotype-based trial approach is to substantially diminish the number of patients that need to be enrolled to show a beneficial effect of a therapeutic intervention.

Another factor that needs to be considered is the time to events or changes of interest. Even biomarkers that are responsive and predictive may take some time to validate, optimize the timing of assessment, and/or even understand within the context of a statistical design, and thus affect the sample size.

#### *Generalizability of the outcome*

The final results of the study will be generalizable to all MS patients within the same endophenotype subclass that participated in the trial. As was noted above, this may depend on the homogeneity of prior therapy, if any, and other factors that

may coexist with these endophenotypes. However, it will be of critical importance that enrolled patients in the experimental treatment group or controls are of similar age, gender, and preexisting comorbidities, for example, since these factors potentially confound biomarker-based readings through their effects on biochemical properties. The use of randomization produces comparable groups in the long run, but the reduction of sample sizes may also result in greater natural variability. This may lead to matching and/or stratifying and should be taken into account in the analyses.

By using similar endophenotypes, one might expect that the ‘nonresponder effect’ on the results will also be diminished, which provides greater reliability regarding other, similar study populations. DCB-based endophenotypes will likely be dynamic due to the administration of therapeutic agents and their potential effects on biomarkers, and due to the natural progression of the disease with changes in its biology. These changes could potentially move individual patients between endophenotypes. It will likely be necessary that treatment strategies and trial planning be tailored to patients over time and to assess the impact of these changes. A longer duration of clinical trials may increase the chance of such endophenotype changes.

### Conclusion

Despite the progress that has been made in the field of MS therapeutics through the development and clinical testing of DMTs, the already heterogeneous MS population is further diversified through variable responsiveness to individual DMTs. Any novel and more effective DMT requires a significant superiority over previous treatments established through rigorous trials. However, treatments that may have specific effects that are of value over the long term may be missed by an aggregate result that looks like other standard therapies. An objectively universal measure will be required to be incorporated into the future MS trials that categorizes MS patients into homogenous subclasses. The hope of this approach is that statistical and clinical significance of clinical trials will be improved. A biomarker-based model of endophenotyping in MS populations using compound genomics and proteomics patterns should provide a basis for faster and more reliable trial designs in MS populations. Studies for biomarker-based models of endophenotyping in MS and

validation studies required for utilization of such models in research should be considered a priority in MS research.

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### Conflict of interest statement

The authors declare that there is no conflict of interest.

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