Supplementary material

Facing the urgency of therapies for progressive MS - A Progressive MS Alliance proposal

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Supplementary Table 1. Markers of biological efficacy (apart from NfL, so far there is no established correlation with disability progression for the majority of these markers)

Oligodendrocyte	**Notes and references**
MBP	Part of theCSF biomarker panel in the Svenningsson intrathecal rituximab trial. No changes after treatment[1]
NAA	Could reflect either oligodendroglial or neuronal damage[2,3]
CHIT1	Astrocytes and microglia in proinflammatory conditions. Correlates with MBP and possible CSF biomarker of demyelination[4]
BDNF	Positive effects on myelination upon glatiramer acetate treatment[5]
tau protein	May affect myelin repair[6,7]
Neuron and plasticity	
NfH	Relation with clinical scores of disability (MSFC and EDSS) and MRI measures of atrophy and disease burden (MRI central cerebral volume and MTR) during lamotrigine treatment[8]
NfL	Present in the lamotrigine trial panel[8] as well as in the two intrathecal rituximab trials, in CSF for the Svenningsson trial[1] and in the peripheral blood in the Bielekowa trial[9]
BDNF	Present in the lamotrigine trial panel with some relation with treatment effects[8]
NGF	Present in the lamotrigine trial panel with some relation with treatment effects[8]
NOx	Present in the lamotrigine trial panel with some relation with treatment effects[8]
sNCAM	Putative CSF biomarker of neuroplasticity, increased during relapses and following steroids and other DMT[10]. Present in the lamotrigine trial panel[8]
GAP-43	Putative biomarker of plasticity[11]. Part of lamotrigine trial panel with some relation with treatment effects[8]
NAA	Putative biomarker of damage[12]. Part of lamotrigine trial panel with some relation with treatment effects[8].
FABP3	Non-specific marker of neuronal damage[13,14]
14-3-3	Expressed in neurons and other cell types[15]
Microtubules (actin and tubulin)	Cytoskeletal components of neurons. Putative CSF biomarkers of damage[16,17]
Nogo-A	Inhibitor of neurite outgrowth. Some controversy about usefulness and MS-specificity[18,19]
Amyloid	Impairment of plasticity. Correlates with cognitive impairment[20]
VEGF	Possibly increased in CSF[21] and down-regulated expression in peripheral blood mononuclear cells[22]
GDF15	Neuronal loss and gliosis possibly related to mitochondrial dysfunction[23]

Astrocyte	
GFAP	GFAP increase in CSF in SPMS compared with RRMS. Correlates with disability[24-26]
VEGF	Astrocyte secretion, influenced by estradiol[27]
Galectin-9	Astrocyte-produced regulator of T-cell differentiation, present in the biomarker panel of the Svenningsson trial[1]
NOx	Fingolimod may support neuroprotection by blocking astrocyte NOx[28]
miR-142-3p	Responsible for IL1-mediated downregulation of the glial glutamate-aspartate transporter that leads to excitotoxic synaptopathy[29]
Chitinases	Also expressed by astrocytes[4]
IL-1b, IL-1ra, TNFa	In CSF. Controversial but possibly of paramount importance in the astrocyte/microglia-mediated neurotoxicity. Possibly related also to mood disturbances[30]
GDF15	Neuronal loss and gliosis possibly related to mitochondrial dysfunction[27]
Microglia	
Chitinases and chitinase-like proteins	Also expressed by microglia[4]
IL-1b, IL-1ra, TNFa	In CSF. Controversial but possibly of paramount importance in the astrocyte/microglia-mediated neurotoxicity. Possibly related also to mood disturbances[30]

Supplementary Table 2. Markers of paraclinical efficacy: evoked potentials and OCT

Technique[31]	Outcome measure: (definition of variable)	Biological substrate:	Target population:	Time to detect a change:	Limitations/ Comments	Clinical measure for correlation	PRO for correlation:
Full-field VEPs[32,33]	Latency, absolute or as change.	Remyelination in the visual pathway / optic nerve.	Acute or chronic optic nerve involvement (optic neuritis or VEP latency delay in the inclusion criteria).	1-6 months	Amplitude variability, poor correlation with axonal loss. Requires patient's cooperation (visual fixation	Visual acuity, low contrast letter acuity;	The 25-Item National Eye Institute Visual Functioning Questionnaire (NEI-VFQ-25).
Multifocal VEPs[34]	Latency as above, possibly with amplitude	as above	as above	as above	Requires patient's cooperation (visual fixation) Not widely available for routine testing	as above	as above
Somatosensory EPs[35]	Central conduction time, absolute or as change.	Remyelination in the somatosensory pathway.	Acute or chronic somatosensory involvement (delay in the inclusion criteria).	1-6 months	Amplitude variability, poor correlation with axonal loss	Vibration threshold, Sensory Functional System.	
Motor EPs[36]	Central conduction time (absolute or	Remyelination in the motor pathway (central conduction time);		1-6 months for latencies; minutes/years to amplitude	Amplitude variability, correlations with axonal	EDSS, motor Functional system, gait speed (25-	MS-spasticity scale, MS-walking scale.

	as change); amplitude ratio of response to peripheral stimulation (absolute or change).	corticospinal excitability /plasticity (amplitude ratio).		(according to biological substrate of intervention).	loss to be established. Some safety limitations (e.g. epilepsy, intracranial metallic implants, pacemakers)	foot walk), spasticity (Ashworth or modified Ashworth scale).	
Optical coherence Tomography[37]	Thickness of Retinal Nerve Fibre Layer-RNFL; thickness of Ganglion Cell-Inner Plexiform Layer-GCL-IPL.	Neuroprotection.	Acute or chronic optic neuritis (optic neuritis history or VEP latency delay in the inclusion criteria); cohorts for long-term monitoring (2 years or more).	1-6 months for acute Optic Neuritis; 2 years or more to detect neurodegeneration not associated with optic neuritis.	Lack of disease specificity. Measures neuroaxonal degeneration that is irreversible even upon remyelination of surviving axons. Requires patients' cooperation (visual fixation)	Visual acuity, low contrast letter acuity	The 25-Item National Eye Institute Visual Functioning Questionnaire (NEI-VFQ-25).

Supplementary Table 3. Markers of paraclinical efficacy: MRI

- Oligodendrocytes

MRI techniques	MRI measures	Specificity for myelin loss	Limitations/comments
Magnetization Transfer Imaging[38]	Magnetization Transfer Ratio (MTR)	Good	Axonal loss also contributes to MTR changes; changes in water content should be considered; MTR quantification depends on acquisition parameters
Diffusion Tensor Imaging[38]	Radial Diffusivity (RD)	Good	Axonal loss, inflammation and water shift contribute to RD changes; difficult to interpret the effect of pathology on individual diffusivity measures
Q-space Imaging[39]	Measures of diffusivity perpendicular to the main direction of fibers (e.g., axis of the cord) (ADCxy, FWHMxy, P0xy)	Good	The use of smaller gradients and longer gradient pulses, which are needed to perform QSI on a clinical scanner, may affect the QSI measures; it may be more specific for axonal loss than RD
Quantitative Susceptibility Mapping[40]	Increase in QSM	Moderate	QSM is sensitive to the demyelination process[34,35]

- Neurons

MRI techniques	MRI measures	Specificity for axonal loss	Limitations/comments
Volumetric imaging[41,42]	Volume loss (or atrophy)	Moderate	Changes in water content should be considered; age-related changes in brain volume also occur; spinal cord atrophy may be happening at a higher rate than brain atrophy, but is technically more difficult to quantify

Diffusion Tensor Imaging[38]	Axial diffusivity (AD), Fractional Anisotropy (FA)	Good	Limited reliability when crossing fibers are present within a voxel; difficult to interpret the effect of pathology on individual diffusivity measures; need to standardised scanning parameters in multicentre studies
Q-space Imaging (QSI)[39]	Measures of diffusivity parallel to the main axis of the cord) (ADCz, FWHMz, P0z)	Good	The use of smaller gradients and longer gradient pulses, which are needed to perform QSI on a clinical scanner, may affect the QSI measures; it may be more specific for axonal loss than FA and AD
NODDI (Neurite orientation and dispersion density imaging)[43]	Orientation dispersion index (ODI), neurite density index (NDI)	Good	Higher specificity for structural markers of neurites (dendrites and axons) than standard DTI measures; axonal loss, inflammation, demyelination may contribute to ODI and NDI changes in vivo; particularly useful in regions where intra-voxel fiber coherence is low; high gradients and advanced MRI encoding schemes are needed for clinical scanners
MS Spectroscopy[44]	N-acetyl-aspartate (NAA)	Excellent	It reflects both neuronal metabolic (or energetic) dysfunction and integrity; its functions are not completely understood; changes in water content and T2 effects should be considered when measuring [NAA]; consider variations in B0 and B1 fields; low spatial resolution
Sodium Imaging[44]	Total sodium concentration	Moderate	It reflects both neuronal dysfunction and loss; the contribution of the intracellular versus extracellular component to the total sodium levels measurements is undefined

- Astrocytes

MRI techniques	MRI measures	Specificity for gliosis	Limitations/comments
MS Spectroscopy[43]	Myo-inositol	Moderate	Low spatial resolution; it reflects astrocytic proliferation and activation
Diffusion Tensor Imaging[38]	Mean Diffusivity (MD)	Low	Increased MD also reflects axonal loss and demyelination

- Microglia

MRI techniques	MRI measures/feature	Specificity for microglia activation	Limitations/comments
Various imaging techniques: phase, Susceptibility-weighted MRI, multi-echo gradient echo R2*, Quantitative Susceptibility Mapping (QSM)	Rim at the edge of the lesion	Moderate	Reflecting iron-laden microglia/macrophages with altered morphology at the edge of the lesion
Diffusion tensor imaging (DTI)[38]	Fractional anisotropy (FA)	Low	Specificity for microglia remains unproven; increased FA in the cortex has been hypothesised to be the result of the local activation of microglia cells characterised by an anisotropic, bipolar-oriented structure[36]

All these MRI biomarkers will need to be evaluated over at least 2 years if chronic patients are studied.

Supplementary information on candidate PET outcomes for the assessment of specific biological mechanisms underlying neurodegeneration

1. **Remyelination.**

Possible radiotracers: 11C-PIB[45-47]; 18F-florbetapir; 18F Florbetaben (ongoing)[48,49]; preferable to choose a fluorinated tracer, 18F Florbetaben preferred (commercially available; higher myelin binding in a comparative study; quantification doable).

Methodology for quantification: semi-quantitative approach (SUVR, static acquisitions) or non-invasive quantitative approach (DVR) using dynamic acquisitions; quantitative approach preferred

Multicentre application: ongoing, data not yet available; Theoretically feasible if results expressed as proportion of remyelinating voxels in lesion and quantification calibrated in each centre using a small group of healthy volunteers.

Possible sample size: based on [^{11}C]-PIB PET pilot study: with a power of 80% to detect a 20% increase (from 14.15% +/- 4.5 to 17%) in the proportion of voxel that remyelinate in white matter lesions, alpha risk 5%, unilateral test, a minimum of 32 patients analyzed per group is required.

Rationale for using PET to detect remyelination: remyelination is heterogenous depending on brain regions and PET enables to capture myelin dynamics at the whole brain level; patients were shown to have heterogeneous individual profiles of remyelination and a pre-therapeutic run in period with PET profiling could quantify this parameter and assess its influence on the pharmacological effect of drugs.

Comment: to be used as a secondary or exploratory outcome to assess the biological mechanism of disease progression or drug efficacy. Sensitivity in patients with a pure progressive course not known.

Time to detect changes: 4-6 months

2. **Innate immune cells activation**

Possible radiotracers: TSPO tracers at available to date (emerging tracers with potentially more specificity, but not yet available for large clinical application): 11C-PK11195 (1st generation TSPO tracer); 11C-PBR28; 18F-PBR111; 18F-DPA714 and others second generation TSPO tracers. preferable to choose a fluorinated tracer, 18F-DPA714 has a good brain entrance[50].

Methodology for quantification: semi-quantitative approach (SUVR, static acquisitions) or non-invasive quantitative approach (DVR) using dynamic acquisitions; quantitative approach preferred, validated for 11C-PK11195 and 18F-DPA714[51-53].

Multicentre application: ongoing, data not yet available; Theoretically feasible if results expressed as proportion of inflamed voxels in tissues and quantification calibrated in each centre using a small group of healthy volunteers.

Possible sample size. Preliminary estimation based on a pilot study applying 18F-DPA714 PET. In order to detect a reduction of 30% of the extent of activation (percentage of inflamed voxels) with a power of 90% and an unilateral type I error rate of 5%, the sample size needed would be 55 subjects per group[54].

Rationale for using PET to detect neuroinflammation: biological mechanisms not detected by MRI; relevance to study innate cell activation linked to lesions (chronic active lesions) and normal appearing tissue[54-56].

Comment: to be used as a secondary or exploratory outcome to assess the biological mechanism of disease progression or drug efficacy. Sensitive method that could be applied both to patients with a relapsing MS or with a pure progressive MS.

Time to detect changes: 12-24 months

Considerations for study design

General:

- A firm biological rationale for moving a drug product candidate into a phase 2 human trial must be provided.
- Phase 2 trials evaluate optimal dosing, improve understanding of pharmacokinetics, help determine biological and target effects, and monitor tolerability and safety. Although clinical efficacy is not the main goal of phase 2 studies, the clinical outcomes can serve as proof of concept that informs progression into the phase 3 (efficacy demonstration) clinical development stage. For this reason, it is important that the primary endpoint is clinically meaningful and reasonably powered to inform phase 3 decisions.
- It is mandatory that drugs used in phase 2 trials have already undergone the proper pre-clinical efficacy and safety experiments and phase 1 (human clinical safety) testing. This requirement is especially critical for drugs that have never been tested in humans. Drugs that are considered new chemical entities (NCE) or new biologics demand extensive and thorough chemistry, manufacturing and controls (CMC)[57,58] pharmacology (absorption, bioavailability, distribution, metabolism, excretion[59,60]) and toxicology testing[61,62] which requires specialized laboratories and teams of experts fully dedicated to these tasks, and not usually found in academic laboratories. For some drugs that are being repurposed, previous approval of the drug in humans for another indication (e.g., oncology use) may not be sufficient to justify the trial in another, as some drugs have different effects on different populations.
- Applicants must certify good standing of clinic participants with Good clinical practices (GCP) and ethical principles (Declaration of Helsinki) training, to ensure the protection of patients, proper monitoring, and quality of study results.
- Follow wording conventions for protocol writing and common terminology use[63], including minimization of redundancies, using cross-references properly, utilize abbreviations (which should be defined when first used), using bullets instead of lengthy text when possible, structure all tables clearly, avoid extensive footnotes for tables or graphics, and use appendices for information deemed too lengthy for the body of the protocol. Have special consideration for local confidentiality and privacy laws that protect personal information or company (sponsor) confidential information. All studies are to be posted on a registry (e.g., EudraCT or ClinicalTrials.gov) along with submission of the protocol and statistical analysis plan (SAP), where applicable. Therefore, all applicable sections of the protocol must follow

the technical and content requirements for that registry. It is important to include in the protocol, statements on background and rationale, as well as considerations of benefit/risk for any compound to be tested.

- Lifestyle considerations, meals and dietary restrictions, use of caffeine, alcohol and tobacco, environmental considerations and listing of prohibited medications should be clearly spelled out. Permitted medicines should be clearly justified.
- Plans for preparation, handling, storage and accountability of investigational product should be clearly described. Randomization method and measures taken to minimize bias should be clarified.
- A firm explanation of dose selection rationale, and anticipation of emergency unblinding scenarios and methods, discontinuation of study drug parameters, plans for drug re-challenge (when applicable) should be included. Plans for handling and reporting of AEs, pregnancy and contraception measures should also be explained in detail. A schedule of assessments chart is recommended to help clarify order and frequency of visits, testing and procedures. Statistical handling of dropouts, application of covariate adjustments, should be pre-specified.
- Investigators are strongly encouraged to collect DNA, serum or PBMCs (which could be stored) for later analyses, that could be informative for data interpretation and for the design of future trials. The use of standardized, high quality methods for collection and storage/banking of biosamples to optimize future comparability and quality of results across trials, is encouraged.
- Use of data registries or historical cohorts for comparison purposes should be described a priori in detail.

Specific for progressive MS:

- Inclusion and exclusion criteria should be carefully considered and justified, to ensure study population homogeneity and minimize confounder characteristics. Tight inclusion criteria that restrict or delay recruitment should be avoided, and an effort to reflect clinical characteristics of a real-world population of primary and/or secondary progressive MS is encouraged.
- It is recommended to include patients 18-65 years old; however, for different ranges, proper justification should be provided. Population homogeneity is important because

some drugs may be more active in patients predisposed to early inflammation followed by late neurodegeneration than in those patients with early degeneration followed by chronic inflammation, or vice versa. It is recommended to avoid stratification by too many variables, as this can compromise the ability to discern subgroup differences in small trials. If stratification is used, the factors considered should be selected based on their relevance to the main outcome measures of the trial.

- Patients with focal inflammation by MRI can be included in PMS trials; however, for evaluation of presumed neuroprotective/repair compounds in these patients, it is recommended that a background combination treatment with immunosuppressants is given, to help sort out effect of presumed protective/repair drug on arresting neurodegeneration.
- In the absence of current effective markers to measure disability in small trials of progressive MS, it is expected that Phase 2 study designs of shorter duration will provide statistical trends suggesting clinical meaningfulness, but not necessarily statistically significant results for clinical outcome measures.
- Shorter phase 2 trials are needed in progressive MS; however, for compounds with presumed neuroprotective mechanisms of action, considerations for longer duration designs should be entertained.

- Considerations for the presumed mechanism of action of the drug impact the planned study duration. For example, a drug thought to directly induce remyelination could be tested with electrophysiological measures over several months in a phase 2 study[64], without the need for more prolonged testing or follow up.
- Progressive MS patients without discernible (i.e., focal) inflammation by MRI, are suspected of harboring silent (NAWM) inflammation, and are not to be considered totally free of inflammation, for design purposes. Focal inflammation may resurface in these patients during the trial. Anti-inflammatory/immunosuppressive drugs should be added in the background when testing neuroprotective compounds; a rationale for not doing so should be provided in the application (for instance, when testing compounds in subsets of very advanced SPMS, elderly patients with no evidence of ongoing inflammatory activity). Considerations must be taken for the clinical phenotypes as patients with highly active inflammation may have a worse prognosis (faster progression) during the trial duration. For designs that combine anti-inflammatory with neuroprotective agents, it is

recommended that biologic measures relevant to each mechanism are included in the analyses, to help evaluate the relative contribution of each compound to the outcomes.

- For eligibility, consider the relevance of disability score as it relates to time since disease onset, and their impact on patient population homogeneity. A patient with an EDSS score of 2 and 20 years since onset has a milder course than a patient with an EDSS of 5 and 5 years since disease onset.
- When planning for study duration, consider if the number of years will be sufficient to detect changes in disability score, especially if a population with less active disease is being studied.
- The impact of ageing on physical strength, gait, hand coordination, and cognition must be factored in, when selecting quality of life or disability measures that reflect changes across these functional domains.
- Patients with advanced disability (i.e., wheelchair-bound) will require more precise methods to measure progression of disability, such as cognition[64] and hand function[65] measures.
- Consideration for the use of predictive laboratory markers (when available) of progression will require a better understanding of the clinical stage where neurodegeneration clearly predominates over inflammation. A marker of worsening inflammation, if used too late in the disease, most likely will not aid in predicting severity of neurodegeneration.
- Serum neurofilaments[66] and brain atrophy estimation by MRI[67] are minimal requirements for paraclinical outcomes to be included, because of their potential to reflect tissue damage, with the understanding that much needs to be learned about their predictive and prognostic capacity in progressive MS trials.
- The selection of quality of life instruments will need to take into account real-life situations that reflect patients' daily activities and preferences[68]. For a young patient with early-onset progressive MS, independence and ability to maintain employment, can be extremely important. For patients who are bedridden but with preserved hand function and cognition, the ability to type or use electronic equipment to communicate are of utmost importance.
- Patient reported outcome measures (ie, quality of life, physical function, cognition or fatigue), healthcare resource utilization questionnaires and patient diaries, which may help reveal novel attributes of drugs in the study population, are strongly encouraged for

inclusion[67]. It is strongly recommended to use measures that have been properly validated and widely recognized as useful in the field of progressive MS.

- Agents of primary interest for the field of progressive MS should target neurodegeneration (via neuroprotective or repair mechanisms); however, agents that target residential compartmentalized inflammation (lymphoid follicles, innate immune cells), thought to play a key role in the degenerative phase, are also acceptable.
- The judicious use of proper composite clinical outcome measures may help to increase power, allowing the capture of diverse functional deficits that could be missed by a single measure (ie, EDSS alone), especially for a primary endpoint. Ancillary testing with electrophysiologic methods, technology-assisted measures (for mobility or tremors) or other (OCT) techniques should be considered, especially as secondary or exploratory endpoints.
- Methods for handling intercurrent events that could confound the results (ie, a drug that causes fatigue as an AE can have an impact on the Expanded Disability Status Scale (EDSS) scoring due to poor cooperation; patients who worsen in one study arm but receive more rehabilitation assistance could show unexpected improvement during the trial), should be carefully considered and specified a priori.
- Seeking progressive MS patient feedback in the study design elements (including elements to be included in patient diaries) and planned execution is strongly encouraged.
- Investigators funded by the Alliance must commit to publish results (whether positive or negative) in peer-reviewed journals. The trials should be optimized to yield information about the biological mechanisms, even in the absence of statistically significant results on clinical measures.
- Work funded by the Alliance is required to present results in open access format; the larger community of investigators will benefit from these results[37,38], and this will help avoid unnecessary duplication of efforts.

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