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Nogo-A antibodies enhance axonal repair and remyelination in neuro-inflammatory and demyelinating pathology

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Abstract: Two hallmarks of chronic multiple sclerosis lesions are the absence of significant spontaneous remyelination and primary as well as secondary neurodegeneration. Both characteristics may be influenced by the presence of inhibitory factors preventing myelin and neuronal repair. We investigated the potential of antibodies against Nogo-A, a well-known inhibitory protein for neuronal growth and plasticity, to enhance neuronal regeneration and remyelination in two animal models of multiple sclerosis. We induced a targeted experimental autoimmune encephalomyelitis (EAE) lesion in the dorsal funiculus of the cervical spinal cord of adult rats resulting in a large drop of skilled forelimb motor functions. We subsequently observed improved recovery of forelimb function after anti-Nogo-A treatment. Anterograde tracing of the corticospinal tract revealed enhanced axonal sprouting and arborisation within the spinal cord gray matter preferentially targeting pre-motor and motor spinal cord laminae on lesion level and above in the anti-Nogo-A-treated animals. An important additional effect of Nogo-A-neutralization was enhanced remyelination observed after lysolecithin-induced demyelination of spinal tracts. Whereas remyelinated fiber numbers in the lesion site were increased several fold, no effect of Nogo-A-inhibition was observed on oligodendrocyte precursor proliferation, migration, or differentiation. Enhancing remyelination and promoting axonal regeneration and plasticity represent important unmet medical needs in multiple sclerosis. Anti-Nogo-A antibodies hold promise as a potential new therapy for multiple sclerosis, in particular during the chronic phase of the disease when neurodegeneration and remyelination failure determine disability evolution.

Nogo-A-antibodies for progressive multiple sclerosis

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Key points

Solid pre-clinical data suggests Nogo-A-neutralization as a potential therapeutic approach for neuro-inflammatory and demyelinating pathology. Nogo-A-antibodies are now in early clinical development for multiple sclerosis (MS). Their potential to boost axonal regeneration and compensatory fiber growth as well as myelin repair makes them an attractive candidate to treat also progressive MS in which neurodegeneration and chronic demyelination are hallmarks.

Abstract

Most of the current therapies as well as many of the clinical trials for Multiple Sclerosis (MS) target the inflammatory autoimmune processes, but less than 20% of all clinical trials investigate potential therapies for the chronic progressive disease stage of MS. The latter is responsible for the steadily increasing disability in many patients, and there is an urgent need for novel therapies that protect nervous system tissue and enhance axonal growth and/or remyelination. As outlined in this review, solid pre-clinical data suggest neutralization of the neurite outgrowth inhibitor Nogo-A as a potential new way to achieve both, axonal and myelin repair. Several phase I clinical studies with anti-Nogo-A antibodies have been conducted in different disease paradigms including MS and spinal cord injury. Data from spinal cord injury and amyotrophic lateral sclerosis (ALS) trials accredit a good safety profile of high doses of anti-Nogo-A-antibodies intravenously or intrathecally. An antibody against a Nogo receptor subunit, LINGO-1, was recently shown to improve outcome in acute optic neuritis in a phase II study. Nogo-A suppressing antibodies could be novel drug candidates for the relapsing as well as the progressive MS disease stage. In this review, we summarize the available pre-clinical and clinical evidence on Nogo-A and elucidate its potential use as a therapy for progressive MS.

1. Introduction

Multiple sclerosis (MS) is the leading cause of non-traumatic neurological disability in young adults in Europe and America [1]. It is an inflammatory demyelinating disease of the central nervous system (CNS) [2]. The causes of MS are not yet fully understood, but a complex genetic trait and several environmental risk factors have been identified, and the prevailing view suggests an autoimmune etiology [3].

In 85% of cases, the disease begins with a relapsing remitting course (RRMS), during which bouts of inflammatory disease activity with transient blood brain-barrier (BBB) opening are observed. In most patients, RRMS is followed by a progressive phase. This phase is defined by a disappearance of relapses and a gradually increase in disability without remission; finally, many patients become wheelchair bound. This form is referred to as secondary progressive MS (SPMS). In 10% of cases, patients suffer from a primary progressive disease course with no superimposed neuro-inflammatory bouts, referred to as primary progressive MS (PPMS) [4]. Thus, approximately 95% of patients are affected by extensive neurodegeneration sooner or later in the disease course.

However, no therapies are currently available that simultaneously dampen the inflammatory reactions and also protect the axons from degeneration [5]. Over the last 20 years, several compounds have been well-established in the treatment regimen of MS patients: interferon beta, glatiramer acetate, teriflunomide, dimethyl fumarate, natalizumab, fingolimod, alemtuzumab and mitoxantrone [6]. All of these have mainly immunomodulatory or immunosuppressive properties and are therefore more effective for the treatment of early MS disease stages where acute inflammation is predominant [7, 8]. Although interferon beta and mitoxantrone were investigated in progressive MS, final conclusions about their effectiveness in treating this chronic disease stage are still unclear and the effect is at best moderate [9, 10]. Another compound was approved in 2016: daclizumab, a humanized IgG against interleukin-2 receptor alpha chain on T cells [11, 12]. Ocrelizumab, a humanized anti-CD20 IgG [13, 14], is at the edge to clinical approval. Ocrelizumab is notably the first treatment option for PPMS (reviewed in [15]).

This is a breakthrough, in particular with regard to disappointing trials during the last 25 years assessing the effectiveness of drug candidates in progressive MS stages [16]. Important reasons for failure of these trials include that they only targeted the adaptive immune mechanisms. Besides that, the design of these studies was not suited to identify effects in patients suffering from progressive MS [17]. The difficulties in testing potential therapies for progressive MS are as well reflected by the fact that only a small amount of all current clinical MS trials investigates interventions for the progressive stage. This is shown by a systematic search on clinicaltrials.gov detecting as few as 17.3% of all studies investigating such interventions (126 of 727 studies, search string: "Multiple Sclerosis OR MS", last search date: 01st of March, 2016). A further complication at the preclinical level is that no proper animal model is available for progressive MS [18]. However, many lessons were learned from preclinical [18] and clinical trials [16]. Additionally, promising new drug candidates are currently in development (reviewed in [19]).

One such candidate, which is in early clinical development for progressive (and relapsing) MS, is an antibody against the protein Nogo-A. In this review, we give a brief overview on this myelin-associated, nerve fiber growth inhibitory protein and its receptors and we summarize its relation to demyelinating, neuro-inflammatory and neurodegenerative pathology from clinical and preclinical evidence.

2. Multiple sclerosis leads to extensive neurodegeneration

2.1. The pathogenic cascade leading to neurodegeneration
Axonal and neuronal injury is a common phenomenon in acute, relapsing MS lesions as shown by histological analysis of MS tissue [20, 21], MRI [22] and magnetic resonance spectroscopy [23]. The prevailing view is that infiltration of immune cells and subsequent demyelination are responsible for this axonal loss [24].

The early pathological processes trigger a cascade of events which often lead to chronic demyelination and neurodegeneration [25], the hallmarks of the secondary progressive disease stage. The chronic inflammation results in microglia and macrophage activation producing reactive oxygen and nitrogen species (ROS/NOS) which can lead to mitochondrial damage [26, 27] (reviewed in [28]), metabolic stress, protein misfolding and deceased axonal transport [29, 30]. Accumulating evidence suggests genuine hypoxia as another mechanism contributing to the tissue injury in acute as well as chronic MS lesions [31-33].

2.2. Chronic demyelination amplifies neurodegeneration
The demyelinated tissue often fails to successfully repair the myelin leading to chronically denuded axons [34, 35]. This is in part due to failure of oligodendrocyte precursor differentiation and myelination [35]. This chronic demyelination can lead to a lack of support function from the oligodendrocytes/myelin sheaths which ultimately results in axonal decline [36, 37]. Ion channel redistribution is yet another sequel of chronic demyelination: different neuronal ion channels show redistribution along axons to compensate ionic imbalance, e.g. voltage-gated sodium channels (Nav1.2 and Nav1.6) [38], acid-sensing ion channel 1 (ASIC1) [39, 40], and transient receptor potential cation channel subfamily M member 4 (TRPM4) [41] (reviewed in [42]). This channel redistribution results in ionic imbalance further perpetuating tissue damage [42].

All above-mentioned destructive processes can be amplified by pre-existing brain injuries and factors associated with brain-ageing [25]. Resulting lesions show slow expansion leading to general CNS tissue atrophy [43]. This can result in extensive axonal injury which can be as high as 70% of axons being destroyed in chronic white matter lesions of severely disabled MS patients [44, 45]. In fact, the progressive neuronal degeneration closely correlates with the progressive neurological decline [46].

2.3. Therapeutic opportunities
This cascade of pathogenic events culminating in extensive axonal decline offers therapeutic opportunities. One of them is the enhancement of neuronal plasticity and regeneration of neurons and axons. The promotion of remyelination by recruitment of the oligodendrocyte precursor pool is yet another one. Important for therapeutic approaches is that successful remyelination was shown to stop the progressive axonal loss [47, 48].

3. The Nogo-A protein: its distribution and receptors
One of the drug candidates in early clinical development which would be able to induce axonal regeneration and remyelination is an antibody against the neurite outgrowth inhibitory protein Nogo-A [49]. The Nogo-A protein was first characterized as an inhibitory protein for neurite outgrowth in brain and spinal cord myelin *in vitro* in 1988 [50]; its cDNA was cloned in 2000 [51, 52]. It downregulates neuronal growth, stabilizes the CNS wiring and restricts plasticity in the adult CNS [53]. Nogo-A is a member of the reticulon family and is highly conserved across species. Three isoforms are generated by alternative splicing; A, B and C, but only Nogo-A has been shown to have

inhibitory effects in the CNS. Nogo-A is present in various cell types of the CNS, including oligodendrocytes and neurons [54]. It is localized at the cell surface and in the endoplasmatic reticulum (ER) and can have different membrane topologies with regard to a cytoplasmic versus extracellular position of the N terminus [55].

Two inhibitory regions have been identified in this 1200 amino acid long protein, Nogo-A-Δ20 (amino acids 544–725 in rat Nogo-A) and Nogo-66 (amino acids 1055-1079 in rat Nogo-A, links the two long hydrophobic stretches which span the cell membrane) [49]. The Nogo receptor 1 (NgR1) has been described as a receptor for Nogo-66 [56]. Upon Nogo-A-binding, NgR1 associates with the transmembrane proteins leucine rich repeat and Immunoglobin-like domain-containing protein 1 (LINGO-1) and p75 or tumour necrosis factor-α (TNFα) receptor superfamily member 19 (TROY) to form a multisubunit receptor complex [49, 57]. The sphingosine-1-phosphate receptor 2 (S1PR2) is a receptor for Nogo-A-Δ20 [58] **(Fig. 1a)**.

Although Nogo-66 and Nogo-A-Δ20 bind to distinct receptors, it was demonstrated that they modulate the same intracellular signaling pathway: ras homolog gene family member A (RhoA) and rho-associated protein kinase (ROCK) [57] (**Fig. 1d**). The intracellular changes induced by the signaling cascade include reduced growth of actin filaments, collapse of the growth cone, destabilization of microtubules, and downregulation of growth genes in the neuronal cell body [57, 59]. Paired Immunoglobulin-like receptor B (PirB) is an alternative receptor for Nogo-66. This receptor is expressed in many parts of the CNS at low levels but its relevance for signaling might be restricted to neural development or after ischemia [60].

LINGO-1 is a co-receptor for NgR1 upon Nogo-A binding **(Fig. 1a)**. It is expressed exclusively on oligodendrocytes and neurons and it is upregulated upon CNS injury across various animal models and human CNS diseases [61]. Antagonization of LINGO-1 has been shown to be beneficial in various animal models in terms of axonal regeneration and remyelination [61]. However, as LINGO-1 is distributed more widely in the CNS than NgR1 [62], other ligands might exist besides Nogo-A and NgR1. Comparison of the effects of LINGO-1 neutralization to those of Nogo-A neutralization is therefore of great interest (**Table 1**). Moreover, Nogo-A, like LINGO-1, is largely expressed in the CNS representing a mostly CNS-specific drug target.

Besides Nogo-A, the myelin proteins myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (OMgp) are also ligands for NgR1 and were also reported to have neurite growth inhibitory activity *in vitro*. Not much is known about their *in vivo* functions in the context of neurite growth [63]. Nogo-A is by far the best studied one, and its extensive characterization suggests an important role during different neurodegenerative disorders [64, 65].

4. Nogo-A is an inhibitor of nerve fiber growth and regeneration

In neuronal development, Nogo-A acts as a regulator of axonal growth, branching and fasciculation [66]. It also regulates the internode length and Ranvier node formation during myelin formation [67].Nogo-A is involved in the termination of the critical plastic period in several regions of the postnatal CNS [49, 53]. In the adult CNS, Nogo-A takes part in the stabilization of the structure and wiring of the CNS by restricting neurite growth to short distances. However, this growth restricting function of Nogo-A also inhibits regenerative and longer-range plastic events following injury or disease [53]. Suppressing the actions of Nogo-A by different means enhances plasticity and axonal regeneration, e.g. in rodent and monkey models of spinal cord injury and stroke; genetic Nogo-

deletion, Nogo-neutralizing antibodies, auto-immunization with Nogo-A, a blocking Nogo receptor fusion protein or a Nogo receptor blocking peptide have been used in these animal models (reviewed in [53]).

Throughout these studies, Nogo-A-neutralization enhanced regrowth and compensatory sprouting of functionally important fiber systems such as the corticospinal tract as well as anatomical plasticity on different levels. At the same time, animals consistently showed significant functional recovery, e.g. in locomotion, skilled stepping over irregular ladders, balance or fine forepaw movements during grasping [53]. These beneficial effects appeared within 2 – 4 weeks after the treatment and, at least in spinal cord injury, seemed to be more efficient in acute settings [68]. However, blocking Nogo-A with NgR1-Fc fusion protein enhanced anatomical and functional recovery also when administered several months after spinal cord trauma [69]. In stroke, even treatment in chronic stages after large ischemic cortical strokes led to improved functional recovery and increased axonal sprouting [70]. Furthermore, the antibody treatment also had positive effects on stroke recovery in aged rats [71].

Of note, transient pharmacogenetic blockade of the newly grown midline-crossing corticospinal fibers after stroke abrogated the recovered grasping function [72]. This shows that newly grown fibers are indeed responsible for improved recovery after anti-Nogo-A antibody treatment. Especially important regarding future human use of these antibodies are the observations that neurological adverse effects like enhanced pain, spasticity or epileptic attacks have never been observed in these studies [73]. Several antibodies with different epitope specificities for the rat and human Nogo-A have been used in preclinical and clinical research. **Table 2** summarizes these antibodies, their epitopes, and types.

Another function of Nogo-A, potentially contributing to its ability to enhance recovery from CNS injury is its involvement in synaptic plasticity. These processes are mainly dependent on neuronal Nogo-A. Dendritic as well as synaptic spine architecture was shown to be influenced by Nogo-A and NgR1 signaling in the hippocampus [74]. Long term potentiation of hippocampal and cortical synapses was increased when Nogo-A or NgR1 were inactivated [75-77]. Furthermore, genetic deletion of the Nogo receptor family NgR1, NgR2, and NgR3 revealed an important role of Nogo-A as a brake on synapse formation in development [78].

5. The role of Nogo-A in neuro-inflammatory and demyelinating conditions

In recent years, the idea has been presented that Nogo-A may play a regulatory role in animal models of neuroinflammatory diseases and may thus be considered a potential therapeutic target for MS. Its abundant presence in myelin and myelin debris around acute and chronic MS lesions could restrict axonal regrowth as well as efficient myelin repair (**Fig. 1**). This hypothesis is supported by a considerable amount of studies from different laboratories using different animal models of MS and strategies for Nogo-A-neutralization. Moreover, there are supportive data on Nogo-A in MS patients. For a comprehensive molecular review on Nogo-A and MS, see Lee and Petratos [79].

Pioneering work by Karnezis and colleagues showed an important role of Nogo-A in the mouse model of autoimmune inflammatory CNS disease experimental autoimmune encephalomyelitis (EAE) [80]. They suppressed Nogo-A either by vaccination against Nogo-A or gene deletion. They found lower clinical scores and slower disease progression as well as reduced histological inflammation, demyelination and axonal damage. The reduced axonal pathology correlated well with the reduction

in the severity of clinical disability and EAE progression. At the immunological level, vaccination with Nogo-A induced a shift in the production from pro-inflammatory Th1 to anti-inflammatory Th2 cells. The latter observation has not been reproduced so far, however.

The immune response to vaccination of EAE susceptible mice with Nogo-A peptides, representing different epitopes of the extracellular Nogo-A domain Nogo-66, revealed that some epitopes were encephalitogenic while others ameliorated established EAE [81]. These effects appeared to be mediated by Nogo-66-specific T cells.

Fontoura and colleagues [82] found that intravenous application of anti-Nogo-A IgG after EAE induction with MOG led to the prevention of disease onset for up to 12 days after the treatment was stopped. Compared to PBS or control IgG injections, the anti-Nogo-A IgG treatment decreased the incidence and severity of EAE and also blocked the progression of the disease.

Blocking Nogo-A by means of neutralizing antibodies has also been shown to enhance recovery in established neuro-inflammation [83]: An improved clinical as well as anatomical outcome, i.e. reduced levels of axonal degeneration shown with the marker P-CRMP-2, was shown after therapeutic administration of anti-Nogo-A antibodies during the course of EAE in mice.

Better clinical outcome in EAE was also observed by using a different way of Nogo-A suppression, namely by genetic knock down with small interfering RNA (siRNA) [84]. *In vitro* and *in vivo*, Nogo-A knock down led to higher levels of the growth-associated protein 43 (GAP43) in neurons [85]. This indicates that axonal repair was enhanced by suppressing Nogo-A. Interestingly, using this gene-knock-out approach, the proliferation and cytokine production of myelin-specific T cells were unaltered suggesting that enhanced axonal regeneration could be the mechanism for the improved clinical recovery. This could be in line with observations in spinal cord injury and stroke models in which Nogo-A-suppression led to enhanced fiber growth and plasticity. Axonal regeneration could, at least in part, be responsible for functional recovery at an early MS disease stage, e.g. in the remission phases [86]. These plastic compensatory strategies of the CNS, e.g. by sprouting of surviving axons, formation of compensatory circuits or recruitment of reserve pathways, have been shown in animal models of stroke [72], spinal cord injury [87], as well as neuro-inflammatory pathology [88].

Interestingly, Nogo-A mRNA and protein levels were observed to be inversely correlated with those of the axonal growth marker GAP43 at different stages of the course of EAE [89]. This work showed that Nogo-A mRNA expression is reduced at preclinical and acute phases, which is followed by upregulation of mRNA and protein during the chronic EAE stage. In contrast, GAP43 was upregulated during acute EAE and downregulated in chronic EAE, often in close spatial proximity to Nogo-A mRNA/protein. The reduction of Nogo-A expression within an active inflammatory lesion therefore could facilitate axonal sprouting, whereas the higher levels of Nogo-A in the chronic lesions could impede neurite growth.

The pre-clinical assessment of potential therapies for progressive MS is hampered by the fact that no animal models are available for mimicking the progressive MS disease course [18]. Most models fail to reproduce the complexity of the processes which define progressive MS, e.g. widespread microglia activation, chronic oxidative injury, subpial demyelination and cortical pathology at the same time [18]. Moreover, some models that claim to present a progressive phenotype, e.g. immunizing Biozzi ABH mice with spinal cord homogenate and complete Freund's adjuvant [90], reflect stable disease on a high level of disability rather than true progression.

Chronic demyelination is an important hallmark of progressive MS. Therefore, toxin-induced demyelination models are increasingly used to define therapies aiming at enhancing remyelination, e.g. lysolecithin, ethidium bromide, or cuprizone [91]. Results from such studies suggest that Nogo-A may also be involved in myelin repair in addition to possible immune modulatory and regeneration-enhancing effects. Studies on myelin development *in vitro* and *in vivo* [67] showed that, by acting as a repulsive cue, Nogo-A influences and regulates the periodic, non-overlapping arrangement of myelin internodes along axons as well as their number and internodal distances [67]. By genetically deleting Nogo-A in oligodendrocytes, the relevance of this hypothesis was tested *in vivo* in spinal lysolecithin-induced demyelinating lesions. Interestingly, Nogo-A-deficient mice showed enhanced myelin repair compared to control mice. The amount of myelinating oligodendrocytes was unchanged within the demyelinated lesion. This strongly supports the hypothesis of an increased myelinogenic potential of the Nogo-A deficient oligodendrocytes, i.e. Nogo-A-deficient oligodendrocytes can form more myelin internodes around axons. Therefore, the neutralization of Nogo-A by antibodies can potentially be exploited to enhance remyelination. The involvement of Nogo-A and its receptors during myelin formation is supported by several other developmental *in vitro and in vivo* studies [92-94].

In a clinically relevant approach using antibody mediated Nogo-A-neutralization, subsequent to lysolecithin lesions in adult rat spinal cords, our lab obtained evidence for enhanced myelin repair as a consequence of this treatment (unpublished observations). Of note, the antibody mediated neutralization of LINGO-1, a component of the NgR1-Nogo-A receptor complex, also led to enhanced oligodendrocyte precursor cell differentiation during primary myelination as well as during myelin repair in different *in vivo* MS animal models [95-97]. In contrast, over-expression of LINGO-1 impeded this precursor differentiation and abolishing myelin repair [98]. These (re-)myelination promoting effects are potentially also mediated by RhoA downstream signaling [95], but other signaling cascades were as well shown to be involved [98] (**Table 1**).

The potential of antibodies directed against myelin antigens to promote remyelination has already been shown (reviewed in [99]). Murine as well as human anti-myelin antibodies were able to boost remyelination in rodent MS models. The proposed mechanisms were either involving removal of myelin debris after injury or direct effects on oligodendrocyte precursor cells.

6. The role of Nogo-A in multiple sclerosis

In addition to these preclinical studies, Nogo-A has also been examined in MS. Nogo-A was found to be upregulated in surviving oligodendrocytes in chronic active demyelinating lesions of MS patients [100]. This is consistent with findings in EAE mice [89]. Elevated serum and cerebrospinal fluid (CSF) autoantibody levels against the large N-terminal domain of Nogo-A have been found in blood samples from MS patients [101], but anti-Nogo-A antibodies exist also in healthy controls. The levels of these antibodies were reported to be increased in RRMS patients compared to chronic-progressive MS patients. Additionally, these anti-Nogo antibodies were more frequent in younger MS patients compared to older ones [101]. The role(s) of these Nogo-A-antibodies is currently unclear; they could reflect the ongoing auto-inflammatory processes against myelin including Nogo-A. Whether some of these antibodies could participate in reparative functions myelin or axons remains to be determined.

7. Nogo-A-antibodies in clinical trials

The experimental data from studies using different MS animal models and the exploratory data from MS patients which have been summarized above suggest Nogo-A-antibodies as a potential

therapeutic agent for the treatment of RRMS and/or progressive forms of MS. Two phase 1 studies for Nogo-A-antibodies were recently conducted in patients with RRMS (ClinicalTrials.gov, NCT01424423 and NCT01435993). Both studies, with a very limited number of patients, have been terminated, but their results are not fully published yet. In its online clinical study register, GlaxoSmithKline states that the reasons for termination were unrelated to safety issues of the antibody treatment.

Two phase 1 studies to assess acute safety, tolerability and pharmacokinetics of anti-Nogo-A antibodies were successfully completed in two other CNS diseases: one after intrathecal administration of anti-Nogo-A antibodies over 30 days in patients with acute spinal cord injury (NCT00406016; Kucher et al., in preparation), and another trial using very high doses of intravenously infused antibodies in patients with amyotrophic lateral sclerosis (ALS, NCT00875446) [102]. Both of these trials showed excellent safety and tolerance of the Nogo-A-antibody treatment. A phase 2 study including more than 300 patients with ALS who were randomised to either anti-Nogo-A antibodies or placebo further showed that there was no apparent imbalance between treatment groups in adverse events (NCT01753076). Placebo-controlled multicentric phase 2 proof-of-principle studies are currently in preparation for spinal cord injury.

An antibody against a Nogo-66 receptor associated component, LINGO-1 (opicinumab), has been tested in phase 1 and 2 studies in patients with RR-/progressive MS or with optic neuritis. High doses of i.v. antibodies were well tolerated [103] . In a phase 2 study in acute optic neuritis patients treated with anti-LINGO-1, vision did not improve after 8 months, but the velocity of nerve conduction, as measured by visually evoked potentials (VEP), was up to 41% less reduced compared to placebo treatment [104]. Since the latency of the VEP primarily reflects intact myelin sheaths, these data are interpreted as a sign of preserved myelin sheaths or remyelination. The failure to meet the secondary outcome of attenuation of the loss of retinal nerve fibers by optical coherence tomography indicates that retinal neurons and/or axons were lost despite of the anti-LINGO-1 treatment. However, these interpretations are preliminary and await further confirmation.

A very recent press release from a phase 2 study using monthly i.v. anti-LINGO-1 for relapsing and progressive MS did not meet its primary endpoint, a multicomponent analysis evaluating motor and cognitive function as well as disability (http://media.biogen.com/press-release/investor-relations/biogen-reports-top-line-results-phase-2-study-opicinumab-anti-lingo). The opicinumab infusions were combined with a concurrent interferon beta add-on therapy. A potential reason for this failure despite promising pre-clinical results could be the ambitious and complex trial design. It used a complex multicomponent readout as primary endpoint including the Timed 25-Foot Walk (T25FW), the 9-Hole Peg Test (9HPT), and the Expanded Disability Status Scale (EDSS). The latter two tests have received criticism for being insensitive outcome measures for progressive MS trials [16]. EDSS is an ordinal scale, therefore, the statistical comparison between contiguous scores is insensitive. Moreover, scores can show high differences between different raters [105]. The 9HPT has floor and ceiling effects limiting its use for progressive MS trials. The patient collective was also very heterogeneous consisting of RRMS and SPMS patients further complicating final interpretations of the preliminary published study results. Full study results including subgroup analysis of RRMS vs. SPMS patients will be available in the next few months and will bring further clarification.

8. Clinical aspects of a Nogo-A-antibody therapy

8.1. Requirements for novel MS therapies

As summarized above, a number of trials to achieve a therapeutic effect in progressive MS are currently ongoing. Experience from previous trials points to different requirements which need to be fulfilled by new therapies [25]: first, on-going or new inflammatory activity needs to be blocked, and, if possible, the compartmentalized intra-CNS chronic activation of microglia should be inhibited as well. Second, neuro- and/or myelin protection should be achieved to inhibit the above mentioned mechanisms that contribute to neuronal decline in the progressive disease stage. Third, given the presumable intrathecal compartmentalization of inflammation in the progressive stage, a potential drug needs to reach the CNS tissue in spite of a mostly intact BBB [25, 106], e.g. by intrathecal application [107], or delivery into the CSF space via a shuttle system. Intrathecal application leads to locally maximized drug concentrations with low systemic exposure and can therefore be expected to have minimal systemic side effects [107]. Fourth, besides immunomodulation and neuro-/myelin protection, the regenerative effects of treatments like anti-Nogo-A should foster repair as much as possible in this phase of disease. Fifth, the different pathogenic mechanisms in the progressive disease may require combinatorial therapies tackling the different targets of the degenerative process as well as various repair processes like axonal sprouting and myelin repair [16]. Sixth, and critical for testing the effects of such single or combination therapies, the most informative patient subgroups should be identified, and suitable trial designs including imaging readouts and biomarkers for degeneration and repair need to be applied.

Under these assumptions, neutralization of Nogo-A seems a feasible and promising therapeutic strategy for the progressive disease stage of MS. As summarized above, a number of pre-clinical studies, mainly in the rodent EAE and lysolecithin model, and different ways of inhibiting Nogo-A showed functional as well as anatomical recovery in terms of axonal sparing, sprouting and myelin repair [67, 80, 83, 84]. One study also showed a beneficial immunomodulatory function of Nogo-A-inhibition shifting the cytokine profile of T cells from a pro- to an anti-inflammatory one [80]. An anti-Nogo-A therapy could therefore tackle two or even three of the major pathogenic hallmarks of MS (**Fig. 1b, c, and e**). NgR1/Lingo-1 inhibition has also been proposed as an approach to abolish Nogo-A-signaling in MS [79]. Disappointing results of clinical trials investigating growth factors such as BDNF, NGF, CNTF, PDGF, or GDNF suggest that targets should be tissue- and cell type-specific. None of the other MS drugs, either still in development or already clinically approved, have CNS-specific targets. Therefore, both Nogo-A-antibodies and LINGO-1-antibodies are promising agents due to their high CNS target-specificity. In addition, it seems plausible that Nogo-A is present in the myelin debris in MS plaques prior to macrophage clearance thereby limiting the potential of these plaques for axonal repair. This myelin debris-associated Nogo-A could in fact be an important target of the Nogo-A-antibodies to achieve axonal repair in MS. Moreover, the engulfment of myelin debris, potentially ameliorated through opsonization by Nogo-A-antibodies, could enhance remyelination [79].

8.2. Pharmacological aspects of Nogo-A-antibody therapy

Perhaps the greatest concern of the Nogo-A-antibody therapy is its potential limited access to the CNS through the BBB, especially in progressive MS in which the disease process is compartmentalized behind a mainly intact BBB [25].

Preclinical data from a rat stroke model in our laboratory revealed that about 0.1% of a blood infused anti-Nogo-A antibody reached the CSF 7 days after i.v. bolus infusion (unpublished observations).

This is consistent with trials in mouse EAE using an IgG [108] and in naïve rats using an anti-LINGO-1-IgG [109].

Data from clinical trials support this notion. A study investigating the CSF concentration of rituximab after i.v. application in two MS patients (one with progressive disease) revealed 0.1 – 0.25% of this antibody being present in the CSF 24 weeks after application [110]. More evidence comes from clinical trials with LINGO-1-IgG [111] and rHIgM22 antibody [112]. Data from a pharmacokinetic study with rHIgM22 show that it was found in the brain of 14/14 patients after 2 days and in 5/12 patients after 29 days following intravenous infusion of a single dose. This IgM antibody is 5 times larger than IgG such as the LINGO-1- or Nogo-A-antibody. In the phase 1 study with i.v. anti-LINGO-1 in 72 healthy volunteers or in 47 patients with either relapsing or progressive MS, doses of ≥ 10 mg/kg resulted in CSF concentrations of this antibody which were associated with 90% of the maximum remyelination effect in rat remyelination studies [103]. It seems therefore plausible that sufficient amounts of antibodies can cross the BBB when very high i.v. doses are administered.

8.3. Potential "Off-target" effects of Nogo-A-antibodies

Both, animal models and human observations have linked Nogo-A and NgR1 to schizophrenia (reviewed in [113]). Genetic association studies have identified Nogo-A and NgR as risk polymorphisms for neuropsychiatric disorders such as schizophrenia [114]. Besides this, mice lacking Nogo-A or NgR1 demonstrate behavioral phenotypes reflecting aspects of schizophrenia, e.g. deficits in prepulse inhibition (PPI) [115, 116]. Neurodevelopmental mechanisms seem to be responsible for this. In line with this, schizophrenic phenotypes have never been observed in rodents [115, 117] or primates [73] during or after therapeutic Nogo-A-antibody application.

Nogo-A restricts neuronal plasticity and fiber growth in the adult CNS. Inhibition of this protein by antibodies could therefore potentially lead to aberrant fiber growth and wiring causing e.g. chronic pain or dyskinesia. These pathologies were, however, not observed in the trials for ALS [102] and spinal cord injury (NCT00406016; Kucher et al., in preparation) or in adult spinal cord injured rats [68] or primates [73] under Nogo-A-antibody therapy.

9. Conclusions

Axonal degeneration and chronic demyelination are hallmarks of chronic, slowly progressing MS. Preclinical studies in rodent EAE models showed that suppression of the growth inhibitory CNS protein Nogo-A, e.g. by neutralizing anti-Nogo-A-antibodies, enhance axonal sprouting and regeneration as well as the formation of new, functional circuits. Also myelin repair was enhanced after genetic deletion of Nogo-A in the lysolecithin myelin lesion model. Based on these data, anti-Nogo-A antibodies could be a promising new therapy for relapsing as well as progressive MS. Phase 1 and 2 studies with anti-Nogo-A-antibodies are currently under way for other neurological indications. The time seems ripe to test these antagonists of the Nogo-A – Nogo receptor pathway as novel therapeutic agents for relapsing and in particular progressive MS.

Tables

Table 1 Differences between the Nogo-A-antibody and the LINGO-1-antibody.

	Anti-Nogo-A-antibodies	**Anti-LINGO-1-antibodies**
Target	Nogo-A	LINGO-1
Expression	Neurons and oligodendrocytes, oligodendrocyte precursor cells (OPCs)	Neurons and oligodendrocytes, OPCs
Receptors for ligands	Sphingosine-1-phosphate receptor 2 (Nogo-A-Δ20), NgR1-LINGO-1-p75 receptor complex (Nogo-66), Paired Immunoglobulin-like receptor B (PirB)	Nogo-A, oligodendrocyte-myelin glycoprotein (together with p75 and NgR1), other unknown ligands
Downstream signaling	Ras homolog gene family member A (RhoA)	RhoA, protein kinase B (Akt), other downstream signaling unknown
Beneficial effects in pre-clinical animal models	Promoting axonal sprouting and regeneration Enhancing remyelination Modulating immune response in neuro-inflammation	Enhancing remyelination by improving oligodendrocyte differentiation. Promoting axonal repair.
Other animal models in which the therapy showed beneficial effects	Stroke, spinal cord injury, traumatic brain injury, optic nerve injury, amyotrophic lateral sclerosis (ALS)	Glaucoma, Parkinson's disease, spinal cord injury
Presumable indication spectrum in MS	Relapsing and progressive MS	Relapsing and progressive MS
Current clinical phase in MS	Phase 1	Phase 2 completed (optic neuritis)

Table 2 Target regions and antibody types of monoclonal Nogo-A-antibodies in preclinical and clinical research. The protein Nogo-A has two inhibitory domains, Nogo-A-Δ20 (amino acids 544–725 in rat Nogo-A) and Nogo-66 (amino acids 1055-1079 in rat Nogo-A, links the two long hydrophobic stretches which span the cell membrane).

Antibody	**Target amino acids of Nogo-A**	**Target region**	**Antibody type**
11C7 [118]	623-640 (rat)	Nogo-A-Δ20	Mouse IgG1
11A8 [118]	~209-233 (rat)	N-terminal extracellular part	Mouse IgG1
3D11 [118]	~910-920 (rat)	N-terminal extracellular part	Mouse IgG1
7B12 [118]	~763-820 (rat)	N-terminal extracellular part	Mouse IgG1
IN1 [119]	Not specified	-	Mouse IgM
ATI-355 (NCT00406016)	345-354 (human)	N-terminal extracellular part	Human IgG4
Ozanezumab [102]	610-621 (human)	Nogo-A-Δ20	Humanized IgG1

Figure legends

Fig. 1 Receptors and downstream signaling of Nogo-A and potential mechanism(s) of Nogo-A-antibody therapy in multiple sclerosis (MS).

(a) The myelin-associated protein Nogo-A has two inhibitory regions, Nogo-66 and Nogo-A-Δ20. Nogo-66 binds to Nogo-receptor 1 (NgR1) which forms a complex with LINGO-1, p75, and/or TROY. Sphingosine-1-phosphate receptor 2 (S1PR2) has recently been identified as a Nogo-A-Δ20 receptor [58].

(b) In acute MS lesions, the attack of the myelin sheath by immune cells leads to demyelination. The myelin-associated protein Nogo-A is then present in the myelin debris potentially limiting axonal/myelin repair in MS plaques.

(c) In acute and chronic MS lesions, neuronal/axonal degeneration can be very prominent. Nogo-A, present in the myelin debris and surrounding myelin sheaths, limits regeneration and compensatory sprouting of these damaged axons. Blocking Nogo-A with antibodies potentially leads to regeneration of axons and restoration of compensatory neuronal circuits.

(d) Signaling cascade upon binding of Nogo-A to its cognate receptors: neuronal S1PR2 as well as the NgR1-LINGO-1-p75/TROY receptor complex activates RhoA and RhoA-Associated Kinase (ROCK). Different downstream targets including the phosphorylation of collapsin response mediator protein 2 (CRMP-2) [83] lead to a disassembly of the actin and microtubule system with subsequent detrimental collapse of the growth cone.

(e) After demyelination, oligodendrocyte precursor cells are recruited and differentiate into oligodendrocytes to remyelinate the naked axon. As suggested by recent evidence [67], Nogo-A seems to limit the myelinogenic potential of oligodendrocytes: oligodendrocytes from Nogo-A-deficient mice can form an increased amount of myelin internodes. Nogo-A mediated repulsive interactions would lead to spatial segregation at Ranvier node formation. Remyelinated myelin sheaths are characteristically thinner than original myelin sheaths.

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Compliance with Ethical Standards

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Conflicts of interest

MES is a founder and board member of the University of Zurich spin-off company NovaGo Therapeutics Inc., seeking to develop Nogo-A-antibody-based therapeutics. Benjamin V. Ineichen, Patricia S. Plattner, Nicolas Good, Roland Martin, and Michael Linnebank declare no conflict of interest.

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