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CN045 is a novel small molecule lead for remyelinating therapies in multiple sclerosis

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Introduction: Pathological insults in MS result in loss of oligodendrocytes (OLs), demyelination, axonal degeneration and ultimately irreversible neurological disability. Currently available MS therapies focus solely on modulating the immune system. Remyelination therapies that enhance the generation of new oligodendrocytes from oligodendrocyte progenitor cells (OPCs) would restore salutatory conduction, prevent degeneration of demyelinated axons and reduce disability progression in MS. OPCs are present in demyelinated lesions. Enhancing OPC differentiation into mature OLs is considered a viable therapeutic target for promoting remyelination in MS.
Objective: To develop orally bioavailable, safe, effective and patentable small molecules that enhance remyelination in MS.
Methods: OPCs were generated from neurospheres derived from mouse embryos of transgenic mice that express EGFP driven by the PLP promoter. OPCs were cultured in 96-well plates, treated with a library of 20,000 small molecules and analyzed for PLP-EGFP expressing oligodendrocytes. Positive controls included known OPC differentiation molecules: T3, Clemastine and Beztropine. Dose response curves (EC50s) were performed for positive hits and the lead hit was tested in the cuprizone model of demyelination/remyelination. Sixty-four novel analogs were generated for the lead hit and screened in the OPC assay.
Results: We identified 42 hits in the in vitro screening of 20,000 small molecules and 18 compounds had an EC50 less than 200nM. One candidate, CN045, showed significantly higher OPC differentiation potency compared to T3, Clemastine and Beztropine and enhanced white matter and gray matter remyelination in the cuprizone mouse model of remyelination. CN045 demonstrated low toxicity and good brain penetration. To establish IP and improve pharmacokinetics of CN045, 64 novel CN045 analogs were generated and examine in the in vitro OPC differentiation assay. Eighty percent of the novel analogs retain OPC differentiation potential and several compounds were more potent than CN045.
Conclusion: CN045 is a potent small molecule that enhances OPC differentiation in vitro and remyelination in vivo. We have generated novel and patentable analogs of CN045 that retain OPC differentiation with greater potency than CN045, T3, Clemastine and Beztropine. A remyelination therapy, in combination with the current anti-inflammatory therapies, will promote myelin repair and halt neurological decline in MS patients.

Disclosure
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Dr. S. Medicetty is a co-founder of Cashel Neural, Inc., and he received compensation from Cashel and Cleveland Clinic.
Dr. L. Knutsen received compensation from Cashel. He also works with NMD Pharma and served on the Advisory Board of Charcot-Marie-Tooth Association
Dr. R. Hamlyn received compensation from Cashel.

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Ponesimod, mono-selective sphingosine-1-phosphate receptor 1 modulator enhances oligodendrocyte precursor cell differentiation

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Background: Sphingosine-1-phosphate receptors modulators are used clinically to treat relapsing forms of multiple sclerosis (MS). In the periphery S1P1 modulation (functional antagonism) prevents lymphocytes egress from lymph nodes, hence hampering neuroinflammation in MS. Recent findings on S1P1 modulation of oligodendrocyte precursor cells (OPCs), central nervous system resident cells suggest a potential benefit of S1P1 receptor modulation in neuroprotection.
Objective: As the Giα-coupled S1P1 is the most prominently expressed S1P receptor in OPCs, thus S1P1-monoselective modulator ponesimod may have differential direct effects on OPC compared to others in the class that are nonspecific.
Methods: C57BL/6J mouse pups (0-2 days old) were used to obtain glial cultures. Primary mouse OPCs were harvested via the shake-off method after 14 days of culture and were treated *in vitro*, all at 0.3 μM to 3 μM, with either the S1P1-mono-selective modulator ponesimod, the S1P5-mono-selective modulator A971432, a combination of ponesimod and A971432, or the dual S1P1/S1P5 modulators ozanimod or siponimod. OPC migration was evaluated in the agarose drop migration assay, while differentiation was quantified using a fluorescent immunohistochemical staining for the oligodendrocyte marker O4 and the differentiation marker myelin basic protein.*In vitro* myelination capacity was assessed in the fiber myelination assay.
Results: None of the treatments enhanced OPC migration. Moreover fingolimod-phosphate (3 μM) significantly inhibited cell migration. In terms of OPC differentiation, treatment with

ponesimod significantly increased OPC differentiation capacity compared with S1P5 monoselective modulation by A971432 and Dual S1P1/S1P5 modulation by ozanimod or siponimod which did not have any significant effects, *in vitro*based on O4 immunocytochemistry (0.3 μM and 3 μM, or in combination with A971432 0.3 μM and 1 μM). None of the S1P receptor modulators had direct effects on*in vitro* myelination capacity of primary mouse OPCs.
Conclusion: S1P1 monoselective modulator ponesimod significantly increases OPC differentiation *in vitro* which differentiates it from less specific S1P modulators such as fingolimod, ozanimod and siponimod.

Disclosure
MS, EW ad TV report no competing interest. MAT is an employee of Janssen and may own stock or stock options in Johnson & Johnson.

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Genetic modification of hematopoietic cells accelerates myelin repair

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Introduction: Preventing neurodegeneration-associated disability progression in patients with multiple sclerosis (MS) remains an unmet therapeutic need. As remyelination prevents degeneration of demyelinated axons, promoting this process in patients might halt the development of permanent disability. In demyelinating mouse lesions, local overexpression of Semaphorin 3F (Sema3F), an oligodendrocyte progenitor cell (OPC) attractant, increases OPC recruitment and remyelination. However, molecular targeting to MS lesions is a challenge because these are disseminated in the central nervous system.
Methods: We hypothesized that a clinically-relevant paradigm to deliver Sema3F to demyelinating lesions and increase OPC recruitment may be to use blood-derived macrophages as vehicles. Thus, we chose transplantation of genetically-modified hematopoietic stem cells (HSCs) as means of obtaining circulating monocytes that overexpress Sema3F.
Results: We first demonstrated that the supernatant from Sema3F-lentiviral vector transduced HSCs stimulates OPC migration in Neuropilin 2 (Nrp2, Sema3F receptor)-dependent fashion. We then investigated whether OPCs remain responsive to Sema3F with age. While Sema3F expression in the lesions of middle-aged and old mice was decreased, middle-aged OPCs retained Nrp2 expression and migrated in response to both recombinant Sema3F and Sema3F-transduced cell supernatant in vitro. We then investigated whether blood cells engineered to overexpress Sema3F can target demyelinating CNS lesions and improve remyelination. Thus, we transplanted Sema3F-transduced HSCs and obtained chimeric mice (with Sema3F overexpression in blood cells), in which we induced demyelinating spinal cord lesions. Transgene-carrying cells, predominantly macrophages, quickly infiltrated lesions in both control and Sema3F chimeras. While Sema3F-expressing cells did not alter the inflammatory status of the lesions nor OPC survival, it increased OPC recruitment, which accelerated the onset of remyelination.
Conclusion: Our results provide a proof-of-concept that blood cells, particularly monocyte-derived macrophages, can be used to deliver pro-remyelinating agents "at the right time and place", suggesting novel means for remyelination-promoting strategies.

Disclosure
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Therapy - Long-term treatment monitoring

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Improved clinical outcomes in patients treated with natalizumab for at least 11 years - real-world data from a swedish national post-marketing surveillance study (IMSE 1)

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Introduction: Natalizumab (NTZ) is a highly effective disease modulatory treatment for relapsing-remitting multiple sclerosis (RRMS). Post-marketing surveillance is important for evaluation of long-term safety and effectiveness in a real-world setting. To this end the "Immunomodulation and Multiple Sclerosis Epidemiology Study" (IMSE 1) was initiated upon NTZ launch in Sweden (Aug 2006).
Objectives/Aims: To follow-up the long-term effectiveness and safety of NTZ in a real-world setting.