Innovative Comparative Study Assessing the Effect of Siponimod on Reactive Microglia/Astrocytes in Patients With Secondary Progressive Multiple Sclerosis: Study Design

Robert Zivadinov¹,²,³, Bianca Weinstock-Guttman², Ralph Benedict², Michael G. Dwyer¹,²,³, Ferdinand Schweser¹,²,³, Dejan Jakimovski¹, Niels Bergsland¹,², Daniela Piani-Meier⁴, Harald Kropshofer⁴

¹Buffalo Neuroimaging Analysis Center, Buffalo, NY, USA, ²Department of Neurology, University at Buffalo, Buffalo, NY, USA, ³Center for Biomedical Imaging, Clinical and Translational Science Institute, University at Buffalo, Buffalo, NY, USA, ⁴Novartis Pharma AG, Basel, Switzerland

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Niels Bergsland has no disclosure of interest.

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Introduction

- TSPO is considered a PET imaging marker of brain-resident micro-/astroglia and myeloid cells that have invaded the brain. Over 95% of TSPO+ cells in PET scans are macrophages and microglia. Both homeostatic and pro-inflammatory, denoted as “reactive,” microglia cells express the same levels of TSPO.
- The apparent signal increase in TSPO+ cells in PET of MS as compared to healthy brains is due to formation of clusters of reactive microglial cells and macrophages in chronic active paramagnetic lesion rims, slowly evolving lesions, and in NAWM and NAGM. Thus, TSPO-PET elucidates the ultrastructural hallmarks of progressive MS.
- USPIO* i.v. can be potentially used to differentiate between TSPO+ microglia/macrophages: it is phagocytosed by myeloid cells in the periphery and serves as a biomarker of invaded TSPO+ macrophages or resident TSPO+ microglia.
- Astroglia contributes weakly (<5%) to TSPO+ cells and is mainly found in inactive lesions and in the center of active lesions, both considered less relevant for progression.
- In the SPMS phase III (EXPAND) study, siponimod effects on GM atrophy (neurodegeneration) and magnetization transfer ratio (myelin content) have been shown.

*This is ultrasmall paramagnetic iron which is taken up by monocytes in the periphery and later shows up in the brain and can be detected in iron-enriched lesion rims, overlapping, in part, with the location of the TSPO PET signal in active SPMS patients.

GM, gray matter; i.v, intravenous; MS, multiple sclerosis; NAGM, normal-appearing gray matter; NAWM, normal-appearing white matter; PET, positron emission tomography; SPMS, secondary progressive multiple sclerosis; TSPO, translocator protein; USPIO, ultrasmall superparamagnetic iron oxide particles


Figure: (a) 7T MRI scan with a paramagnetic rim lesion in progressive MS. Red arrows highlight the area where TSPO+ cells are expected to localize in corresponding PET images. (b) Differential localization of microglia, astrocytes, myeloid cells and lymphocytes in the periplaque area, core and edge of chronic active (CA) and chronic inactive (CI) lesions.
### Objective & Study Design

**An open-label, single-blind (MRI analysis), comparative, prospective, 36-month adaptive study**

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<tr>
<th>Timelines</th>
<th>Baseline</th>
<th>6 Month</th>
<th>12 Month</th>
<th>24 months</th>
<th>36 months</th>
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<td><strong>MRI (Gd+ &amp; USPIO+)</strong></td>
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Number of patients: 60 (30- Siponimod; 30- Ocrelizumab)

To assess the effect of siponimod, compared to ocrelizumab, on reactive microglia/astrocytes using PET, MRI and serum biomarkers NfL and GFAP in patients with active SPMS.

- **Zivadinov R, et al.**
- **BICAMS, Brief International Cognitive Assessment for MS; EDSS, Expanded Disability Status Scale; EOT, end of treatment; Gd, gadolinium; GFAP, glial fibrillary acidic protein; MRI, magnetic resonance imaging; NfL, neurofilament light; PBR, Peripheral benzodiazepine receptor; PET, positron emission tomography; SDMT, Symbol Digit Modalities Test; SPMS, secondary progressive multiple sclerosis; T25FW, Timed 25-foot Walk; USPIO, ultrasmall superparamagnetic iron oxide particles.**

**End points**
- Timelines: Baseline, 6 Month, 12 Month, 24 months, 36 months, EOT
- **Number of patients:** 60 (30- Siponimod; 30- Ocrelizumab)
- **Objective & Study Design:** An open-label, single-blind (MRI analysis), comparative, prospective, 36-month adaptive study
- **Preliminary Results**
- **Conclusions**
Eligibility Criteria

**Key inclusion criteria**
- Diagnosed with aSPMS (Lublin criteria\(^1\))
- Age 18 to 60 years
- EDSS 3.0 to 6.5
- Treatment naïve to both siponimod and to ocrelizumab
- Not using S1P modulators or B-cell therapies for the last 9 months
- Creatinine clearance >59
- No known hypersensitivity reactions to contrast agent

**Key exclusion criteria**
- Treatment within 30 days prior to enrollment with steroids or any other concomitant immunomodulatory therapies
- LAB for the DNA single nucleotide polymorphism of the TSPO gene on chromosome 22q13.2, using a TaqMan assay
- A CYP2C9*3/*3 genotype
- Presence of Mobitz type II second-degree, third-degree AV block, or sick sinus syndrome
- Conditions associated with iron overload
- Women who are pregnant, lactating or of childbearing age

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## Endpoints

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<th><strong>Primary</strong></th>
<th><strong>Secondary</strong></th>
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<td>▪ Change from baseline in PET activation of PBR06 (when 100% of the patients have reached Month 12) in</td>
<td>▪ Change in PET activation of PBR06 between siponimod and ocrelizumab at 6, 12, 24, and 36 months from baseline in</td>
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<td>▪ Lesional and peri-lesional chronic WM areas in the brain, identified as gadolinium non-enhancing hyperintense</td>
<td>▪ Non-lesional normal-appearing white and gray matter</td>
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<td>T2 and hypointense T1 PRL+ and PRL- on QSM</td>
<td>▪ Cumulative number of USPIO lesions, Gd+ active lesions on T1-weighted images</td>
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<td>▪ Slowly expanding lesions</td>
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<td>▪ Association between PET 18F-PBR06 in lesional and non-lesional NAWM and NAGM and in the periplaque area of chronic lesions at 12, 24 and 36 months and</td>
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<td>▪ Cumulative number of new USPIO+ active lesions</td>
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<td>▪ Cumulative number of new Gd+ active lesions</td>
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<td>▪ PBVC, PCVC, and PTVC</td>
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<td>▪ QSM changes in the thalamus and periplaque area of chronic lesions in the brain</td>
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<td>▪ Association between imaging and clinical outcomes including the composite EDSS+SDMT</td>
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<tr>
<td>▪ Association of sNfL, GFAP, OCT and LCLA with TSPO PET and clinical outcomes</td>
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CE, contrast enhancing; GFAP, glial fibrillary acidic protein; LCLA, low contrast letter acuity; NAGM, normal appearing gray matter; NAWM, normal appearing white matter; OCT, optical coherence tomography; PBVC, percent brain volume change; PCVC, percent cortical volume change; PET, positron emission tomography; PRL, paramagnetic rim lesion; PTVC, percent thalamic volume change; QSM, quantitative susceptibility mapping; USPIO, ultrasmall superparamagnetic iron oxide particles.
Preliminary Results

- The study started enrollment in November 2021
- We are expecting completion of enrollment by June 2023 and completion of follow-up by June 2026
- A first interim analysis will be conducted after 50% of the patients have reached Month 12
- A second interim analysis will be conducted after 100% of the patients have reached Month 12 and 50% of the patients have reached 24 month

Figure. Baseline MRI T2-FLAIR image (left) and TSPO PBR06 PET SUV map (right) for a representative subject (50-year-old female with active SPMS). The PET tracer map reveals heterogeneity in lesion microglial concentration in lesions that appear otherwise similar on T2-FLAIR MRI. Some lesions (arrows) show substantial TSPO activity, while others (arrowheads) do not.
- **This is the first study evaluating the effect of siponimod on microglia/astrocyte activation as compared with ocrelizumab in patients with aSPMS**

- **This is also one of the largest and longest, prospective PET MRI study, which will potentially allow to bridge the clinical outcomes (physical and cognitive disability) with a composite of classical and innovative imaging markers (TSPO; USPIO) and fluid biomarkers (NfL + GFAP + Somascan), thereby contributing to advancement in the understanding of prognostic biosignatures for progression in MS**

- **This study will give key insights into how DMTs (with different mechanisms of action and different brain penetration) affect microglial activation**