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Selective $S1P_1$/$S1P_5$ Modulation Impacts Neurologic Architecture/Function by Improving Kinematic Gait and Protecting From Neuronal Breaks in a Demyelinating Mouse Model

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BACKGROUND

- Ozanimod (RPC1063), an oral, once-daily immunomodulator selectively targeting the sphingosine 1-phosphate 1 ($S1P_1$) and $S1P_5$ receptors, has shown therapeutic benefit in clinical trials of relapsing multiple sclerosis (MS) and ulcerative colitis[1,2]
- Ozanimod down-regulates $S1P_1$, resulting in the retention of T and B cells in peripheral lymphoid tissues
- Ozanimod penetrates the blood–brain barrier and binds $S1P_1$ and $S1P_5$ on neural cells, which may confer preservation of central nervous system (CNS) tissue[3]

OBJECTIVE

- To assess the effects of ozanimod on motor function using the cuprizone-induced mouse model of demyelination

METHODS

- Efficacy of ozanimod or an ozanimod surrogate (RP-101074) was examined in the cuprizone-induced mouse model of demyelination
- C57BL/6 mice were treated over 6 weeks with cuprizone (0.3% weight/weight orally) and rapamycin (10 mg/kg intraperitoneally daily) to demyelinate axons in the brain and concurrently treated with ozanimod (0.1, 0.3, or 1 mg/kg) or RP-101074 (1 mg/kg) by daily oral gavage
- Analyses of individual parameters of kinematic gait, as well as overall kinematic gait score, were used to assess the impact of ozanimod on function
  - Overall kinematic gait score was composed of:
    - General spatio-temporal parameters (stride distance, duration, speed, stance time, swing time)
    - Inter-limb coordination (diagonal cadence, left/right alternation rhythm)
    - Swing phase and paw trajectory (swing speed, smoothness, trajectory shape, abnormality)
    - Body posture and joint angles (hip height, tail, head, limb function)
    - Gait variability and deviations
- Preservation of CNS tissue by RP-101074 was assessed through examination of axonal pathology in the corpus callosum using SMI-32 staining

RESULTS

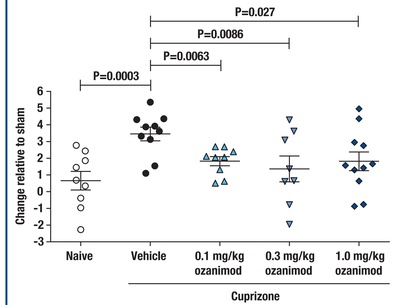
Ozanimod Treatment Improved Multiple Parameters of Kinematic Gait

- Ozanimod (1 mg/kg) improved kinematic gait in cuprizone-induced demyelinated mice compared with vehicle, demonstrating improved functional outcomes with ozanimod treatment
- The altered gait parameters in vehicle-treated mice that significantly improved in ozanimod-treated mice included tail tip height, altered hip orientation, knee/ankle function, and lower forelimb paw trajectory (Figure 1; scan QR code to access video content)

Overall Kinematic Gait Score Improved With Ozanimod Treatment

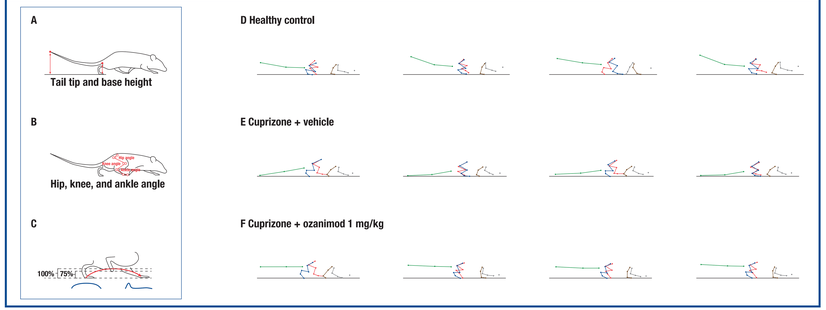
- The addition of ozanimod (0.1, 0.3, or 1 mg/kg) was associated with significant improvements in overall kinematic gait score vs vehicle in cuprizone-treated mice (Figure 2)

Figure 2. Effect of Ozanimod on Overall Kinematic Gait Score in Cuprizone-Treated Mice



Statistical comparisons are based on unpaired t-tests of vehicle vs naive or treatment vs vehicle.

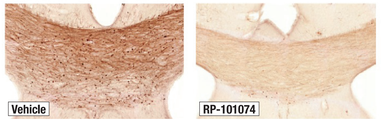
Figure 1. Effect of Ozanimod on Kinematic Gait. Most Improved Parameters Were (A) Tail Tip Height, (B) Hind Limb Jerk, and (C) Forelimb Trajectory. Kinematic Gait Tracings for (D) Healthy Naive Control, (E) Cuprizone + Vehicle-Treated, and (F) Cuprizone + Ozanimod-Treated Mice.

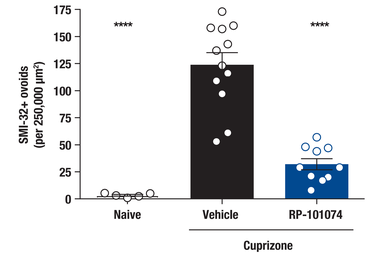


The Ozanimod Surrogate RP-101074 Reduced the Number of Swollen and Transected Axons

- To elucidate how $S1P_1$/$S1P_5$ modulation impacted kinematic gait, the effects of treatment with an ozanimod surrogate, RP-101074, were examined in cuprizone-treated mice
- Histological analysis demonstrated that mice treated with RP-101074 (1 mg/kg) showed reduced neuronal breaks and fewer neuronal ovoids vs vehicle (32 vs 124 SMI-32–positive ovoids per 250,000 μm²; P<0.0001), suggesting that RP-101074 preserved neuronal axons in the cuprizone-induced mouse model (Figure 3)

Figure 3. Effect of RP-101074 on the Number of Swollen and Transected Axons





****P<0.0001 for treatment vs vehicle. Statistical comparisons are based on one-way analysis of variance.

SMI-32, antineurofilament H non-phosphorylated mouse monoclonal antibody.

CONCLUSIONS

- Ozanimod treatment mitigated the negative effects of cuprizone-induced demyelination on kinematic gait in mice
- Axonal breaks in cuprizone-treated mice were reduced with the ozanimod surrogate RP-101074
- These data suggest that modulation of $S1P_1$/$S1P_5$ may potentially directly preserve CNS integrity and improve gait, as indicated by improved tail strength and hind-limb and forelimb fluidity in a mouse model of demyelination

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DISCLOSURES

Kristen R. Taylor Meadows, Gregory J. Opiteck, and Fiona L. Scott were employees of Celgene Corporation at the time these analyses were undertaken. Kevin C. Dines is an employee of and shareholder in Celgene Corporation.

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