# ASH41020, A Novel Hydroxyl Dendrimer CSF1R Tyrosine Kinase Inhibitor 'Dendranib' Nanomedicine, Polarizes Macrophages Toward an Anti-Inflammatory Phenotype and Improves Disease Severity in a Mouse Model of Multiple Sclerosis

Naze G Avci1, Tony Wu2, Nina Woodworth3, Stanley Bode1, Xiaoyan Li1, Jiazhong Zhang1, Jeffrey L Cleland1, Stephen M Maricich1, Natacha Le Moan1
1Ashvattha Therapeutics, Redwood City, United States, 2Ashvattha Therapeutics, Baltimore, United States, 3Redoxis AB, Lund, Sweden

## Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). Accumulating evidence suggests that MS pathogenesis is related to peripheral macrophage and CNS resident microglia activation that leads to production of inflammatory molecules, causing axonal damage and cell death (Fatoba et al., 2020). Polarization of these cells toward an M1 phenotype is associated with relapse-independent disease progression (RIDP), whereas M2-polarized cells are involved in remyelination processes (Poppell et al., 2023). Therefore, re-programming macrophages and microglia toward an M2 phenotype could be a viable therapeutic option.

Colony stimulating factor 1 receptor (CSF1R), a type III receptor tyrosine kinase (RTK), is critical for survival and proliferation of CNS microglia, peripheral tissue macrophages and blood myeloid cells. Increased expression of CSF1 in the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE) (Gushchina et al., 2018) and in the brain and CSF of MS patients (Hwang et al., 2022) correlate with microglial activation and disease progression. Targeting CSF1R with selective tyrosine kinase inhibitors such as dasatinib and masitinib lowers EAE severity and delay disease onset (Azizi et al., 2015, Vermersch et al., 2022).

ASH41020 is a new class of tyrosine kinase inhibitors referred to as "dendranibs", which are metabolically stable and eliminated intact through the kidneys. ASH41020 selectively inhibits CSF1R tyrosine kinase only in activated microglia and macrophages. In this study, 1) effects of ASH41020 on macrophage phenotypes were studied *in vitro* using differentiated human primary monocytes, and 2) effects of ASH41020 on macrophage phenotype and clinical symptoms were studied in a mouse EAE model of MS. Symptomatic animals (EAE mean score of ~1.5) were treated daily for 14 days at 20, 60 or 200 mg/kg ASH41020 (IP) and compared to fingolimod (3 mg/kg, oral (PO) daily) as a positive control. Clinical scores were monitored daily, and spinal cords were analyzed for M1/M2 macrophage markers using fluorescence-activated single cell (FACS) analysis.

## Methods

### In Vitro Effect of ASH41020 on Macrophage Phenotypes

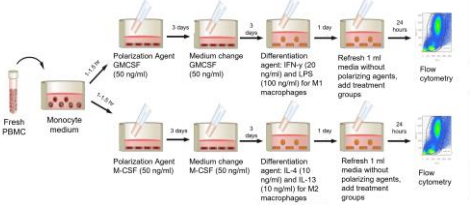


**Figure 1**. **Study schematic** for *in vitro* screening of ASH41020 on macrophage phenotype switch using human peripheral blood mononuclear cells (PBMC).

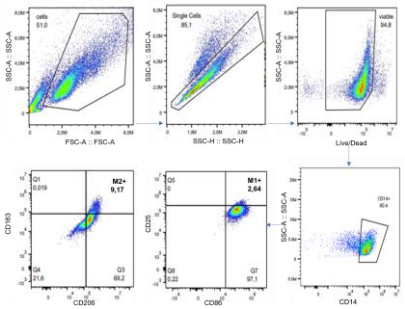


**Figure 2. FACS gating strategy.** Primary monocytes were differentiated from M0 macrophages using GMCSF or M-CSF (50 ng/ml) and treated with ASH41020, dasatinib or masitinib for 24 hours. On day 8, macrophage phenotyping was performed using FACS analysis. Live cells were first gated for CD14+ then CD25+ CD86+ cells were classified as M1 phenotype, CD163+ CD206+ cells as M2 phenotype.

### Efficacy of ASH41020 in Mouse EAE Model of MS

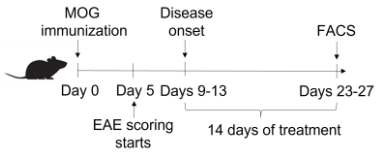


**Figure 3**. **Study schematic** for *in vivo* screening of ASH41020 in EAE-induced C57BL/6 mice.

**Table 1.** FACS panel for in vivo screening.

| Cell Population | Phenotypic Markers | Quantitation |
|---|---|---|
| **M1 Macrophage** | CD86+CD80+ | % CD45+CD11b+ |
| **M2 Macrophage** | CD163+CD206+ | % CD45+CD11b+ |

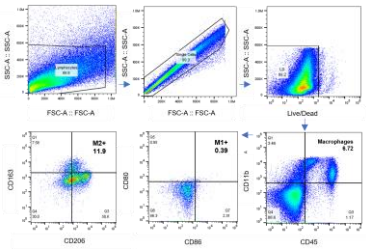


**Figure 4. FACS gating strategy.** Spinal cords were collected after 14 days of treatment at study termination. Tissues were dissociated and cells stained with corresponding antibodies for FACS analysis. Live cells were first gated for CD45+ CD11b+ cells then CD80+ CD86+ cells were classified as M1 phenotype, CD163+ CD206+ cells as M2 phenotype.

## Results

### *In Vitro* Effect of ASH41020 on Macrophage Phenotype

ASH41020 decreased M1 and increased M2 macrophages in the M1 polarizing environment in a dose dependent manner, while dasatinib and masitinib did not (Figure 5A). In the M2 polarizing environment, ASH41020 treatment did not affect M1 macrophage proportion but showed a similar trend with dasatinib in increasing M2 macrophage proportion. Dasatinib and masitinib both increased the proportion of M1 macrophages in the M2 polarizing environment (Figure 5B).

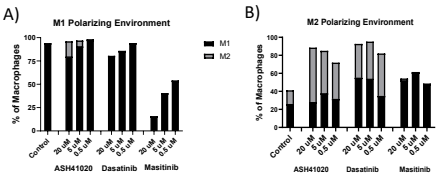


**Figure 5. Macrophage phenotype switch.** PBMCs differentiated into A) M1 or B) M2 macrophages were treated with 0.5, 5 and 20 μM of ASH41020, dasatinib or masitinib for 24 hours then analyzed by FACS.

### Efficacy of ASH41020 in EAE Mouse Model of MS

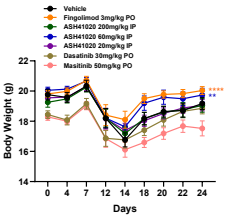


**Figure 6. Body weights in EAE mouse model.** EAE-induced mice were treated with ASH41020 at 20, 60 or 200 mg/kg daily (IP); dasatinib at 30 mg/kg daily (PO), masitinib at 50 mg/kg daily (PO); Fingolimod at 3 mg/kg daily (PO) for 14 days after mean clinical scores reached 1.5 on day 11. Body weights were measured starting from day 0. n=15/group. Data are mean +/- SEM, analyzed by 2-way ANOVA, treatment vs vehicle, ** p<0.01, **** p<0.0001.

Mice treated with 60 mg/kg ASH41020 recovered more body weight than other treatment groups and ended with weights similar to fingolimod treated animals (Figure 6).

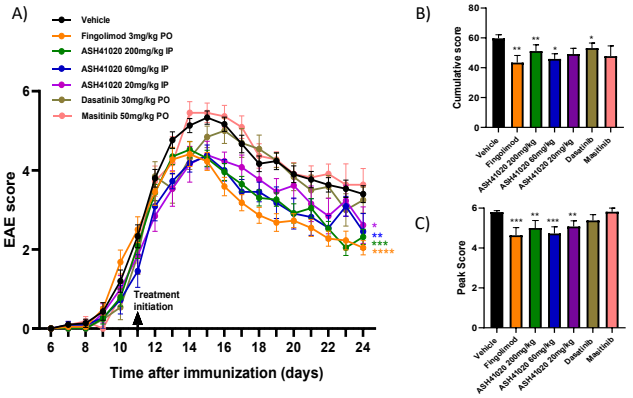


**Figure 7. Effect of ASH41020 on EAE clinical scores.** EAE-induced mice were treated for 14 days after mean clinical scores reached 1.5 on Day 11. (A) EAE scores for ASH41020 daily at 20, 60 or 200 mg/kg; dasatinib at 30 mg/kg and masitinib at 50 mg/kg daily; Fingolimod at 3 mg/kg daily. B) Cumulative scores over the course of 14 days of treatment. C) Peak scores over the course of 14 days of treatment. n=15/group; Data are mean +/- SEM, analyzed by 2-way ANOVA or two-tailed t-test, treatment vs vehicle, *p<0.05, **p<0.01, ***p<0.001, ****<0.0001.

ASH41020 administration significantly decreased EAE disease severity in a dose dependent manner compared to vehicle. To benchmark ASH41020 against similar compounds, dasatinib and masitinib were administered daily after disease onset at 30 and 50 mg/kg, respectively. However, they did not show significant efficacy (Figure 7A). Cumulative scores followed a similar trend with significant effects after ASH41020 administration (Figure 7B). EAE animals scored from 0 (asymptomatic) – 8 (premorbid or dead). Peak scores showed that control animals peaked at 5.8, indicative of a tail paralysis and paralysis of a pair of limbs, while animals treated with ASH41020 at 20, 60 and 200 mg/kg had mean peak scores of 5, 4.7 and 5, respectively. These scores were indicative of only a tail paralysis and paralysis of one limb (Figure 7C). The magnitude of this effect was similar to that of fingolimod with a mean peak score of 4.6, indicating improved well-being.

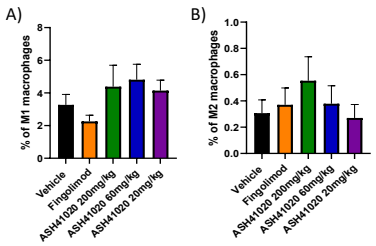


**Figure 8. Effect of ASH41020 on macrophage phenotypes in the EAE model.** After 14 days of ASH41020 treatment, spinal cords were collected for FACS analysis. A) Percentage of CD86+CD80+ M1 macrophages. B) Percentage of CD206+CD163+ M2 macrophages. n=15/group. Data are mean +/- SEM, analyzed by One-way ANOVA, treatment vs vehicle.

ASH41020 daily administration increased the percentage of M1 and M2 macrophages, showing a trend toward dose dependency for M2 macrophages (Figure 8).

## Conclusions

Tyrosine kinase inhibitor, dasatinib, showed efficacy in the mouse EAE model of MS when treatment started before disease onset (Azizi et al., 2015) and masitinib demonstrated efficacy in a Phase 3 study of progressive MS (Vermersch et al., 2022). However, whether they can modulate macrophage phenotype has not been investigated. Dendranib ASH41020 is a potent tyrosine kinase inhibitor and a macrophage switching nanomedicine technology. In this study, ASH41020 significantly directed macrophage polarization toward the anti-inflammatory M2 phenotype, which in turn ameliorated symptoms in the mouse EAE model of MS after disease onset. These preclinical studies suggest that ASH41020 could be a potent anti-inflammatory and immunomodulatory agent that warrants further development as a promising treatment for MS patients.

## Disclosures

Ashvattha Therapeutics funded the research.
Redoxis was contracted by Ashvattha Therapeutics to perform the animal studies.