One of the most common practical in vivo models used in the study of mechanisms of multiple sclerosis (MS) is the MOG35-55 induced experimental autoimmune encephalomyelitis (EAE) in mice. This technique involves inoculation with MOG35-55 protein, which is emulsified to complete Freund’s adjuvant, to evoke inflammatory responses against proteins. Further, an injection of pertussis toxin (PTX) allows immune cells access to the CNS through the blood-brain barrier (BBB) causing T cell related demyelination. Fingolimod (FTY-720), an immunomodulating drug approved by FDA for treatment of MS, is commonly used as a positive control in MOG35-55 induced EAE in mice. FTY-720 is a sphingosine-1-phosphate receptor modulator, which can isolate the lymphocytes in lymph nodes to prohibit immune cells from reacting against tissues.

### MATERIALS AND METHODS

#### Experimental set-up

Animals were maintained and cared for according to the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals, and approved by the National Animal Experiment Board. All animals (female C57BL/6 mice, 8-10 weeks and purchased from Charles River Germany) were used in experiments. Animals were housed at a standard temperature (22 ± 1°C) and a light-controlled environment (lights on from 7 am to 8 pm) with ad-libitum access to food and water.

Animals were grouped as follows:
- Sham inoculated group: treated with vehicle on days 0-35
- MOG35-55 inoculated group treated with vehicle on days 0-35
- MOG35-55 inoculated group treated with PTX (4 µg/ml) on days 0-35
- FTY-720 treated group with MOG35-55 inoculated on days 0-35

EAE induction

Mice were inoculated using commercially available ready to use inoculum (SR-110, Noble Laboratories, USA) containing 100 µg of MOG35-55, 350 µg heat-inactivated of Mycobacterium tuberculosis in mineral oil in 100 µl of saline. Inoculation was done by using 20 g of intraperitoneal injections at 0, 500 µl each, on day 2. In this study, 100 µl of pertussis toxin (PTX, 100 µg/ml) was also administered as a prophylactic treatment.

Clinical score and body weight follow-ups

Body weight and clinical status of mice were monitored daily. Clinical signs were scored as follows:

- **0.0**  Complete tail paralysis
- **0.5**  Partial paralysis which also causes muscle atrophy and loss of body weight
- **1.0**  Complete tail paralysis
- **1.5**  Flaccid tail and abnormal gait
- **2.0**  Partial paralysis which also causes muscle atrophy and loss of body weight. After the period of 35 days, IHC analyses of spinal cord cervical segment reveals demyelination, microglia activation and inflammation level. At the end of experiment (day 35), animals were sacrificed and cervical spinal cord was collected and used for stainings.

### RESULTS

#### Immunohistochemical analysis

In immunohistochemical analysis, FTY-720 treated and MOG35-55 inoculated group showed higher body weights from day 0 to 11 and decreased body weights from day 11 to 15 compared to the sham group treated with vehicle (Fig 1A). And therapeutic group showed decreased body weight from day 11 to 15 and increased body weights from 15 to 20 and decreased clinical scores from 11 to 20 compared to prophylactic group treated with vehicle (Fig 1C).

Microglia activation and inflammation level were observed in this group compared to the sham group treated with vehicle (Fig 1B). And therapeutic group showed statistically significant increase in body weights (Fig 1A) and also statistically decreased daily clinical scores and decreased cumulative disease index compared to vehicle treated group (Fig 1C).

In the light of the model phenotype, MOG35-55 EAE in mice is a useful model to study novel compounds against MS. In addition to models’ disease related behavioral and tissue pathological outcomes and their relationships, model is also useful to examine molecular mechanisms and therapeutic targets in detail to understand human disease better and to develop more effective therapies against MS.