measured every six months, and 45.4% (10 out of 22) reported a decrease in titers after six months of treatment.

Conclusion: INEB significantly reduces relapse rates, improves mRS scores in patients with AQP4+ NMOSD. Further prospective and comparative study are needed to reveal the long-term efficacy and safety of INEB.

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P309/1214
Selective, Potent, Brain-Penetrant Kv1.3 Blockade Abrogates Inflammatory Processes Relevant for Multiple Sclerosis in Brain and Blood

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Introduction: The ion channel Kv1.3 is a key regulator of central and peripheral inflammation and autoimmunity. Kv1.3 is expressed on cells belonging to both the innate and adaptive immune system including proinflammatory microglia and chronically activated memory T cells. Several studies suggest that pharmacological blockade of Kv1.3 reduces neuroinflammation and enhances neuroprotection in rodent microglia and further prevents disease manifestations in rodent models of autoimmune diseases. Here we present studies of the role of Kv1.3 in neuroinflammation using the first selective, small molecule Kv1.3 blockers with CNS exposure.

Objectives/Aims: The aim of this work is to characterize the impact of potent, selective, brain penetrant Kv1.3 blockers on T cells and microglia in the context of inflammatory stimulation *in vitro* and *in vivo*.

Methods: Selectivity of Kv1.3 blockers was determined by patch-clamp electrophysiology in heterologous cells. Compounds were tested *in vitro* in human T cells and human IPSC (induced pluripotent stem cell) derived microglia with inflammatory stimuli. Compounds were tested *in vivo* in a human microglia xenograft mouse model, as well as in wildtype rodent models challenged with pro-inflammatory stimuli centrally or peripherally. Assessment of cytokine release and gene expression analyses by qPCR and/or RNA-seq were performed on T cells and microglia from *in vitro* and *in vivo* models.

Results: We identified small molecules with low double-digit nanomolar potency for Kv1.3, high selectivity against other Kv1 family members, and excellent brain pharmacokinetics. These compounds exert anti-inflammatory effects both on T cells and microglia *in vitro* and *in vivo* across several different pro-inflammatory stimuli. Transcriptional analyses of xenografted hIPSC-derived microglia showed that Kv1.3 blockade *in vivo* normalized a pro-inflammatory signature and regulated expression of MS associated microglia specific GWAS genes.

Conclusion: Kv1.3 blockade impacts both T cells and microglia, suggesting synergistic effects in the context of neurodegenerative disorders characterized by both peripheral and central inflammation. These results strongly support Kv1.3 as a novel drug target for the treatment of Multiple Sclerosis and potentially other neurodegenerative disorders.

P310/1649
Longitudinal alteration of gut microbiome composition in subjects with Multiple Sclerosis during Dimethyl Fumarate therapy: a one-year follow-up study

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Introduction: Gut microbiota composition is involved in host immune system dysregulation and can affect onset and progression of multiple sclerosis (MS). Therapies also regulate the immune balance; however, whether they influence the composition of the gut microbiome remains unclear.

Objectives/Aims: This study aims to investigate gut microbiota alteration in subjects with MS during therapy with dimethylfumarate (DMF).

Methods: 30 MS subjects starting treatment with DMF were clinically evaluated. Anamnestic characteristics, diet and lifestyle habits were collected. Stool samples were prospectively collected before (T0), one (T1), and 12 (T12) months after starting DMF treatment. DNA was extracted from stool samples, and shotgun metagenomic sequencing was performed. Reads were aligned with the BWA algorithm against the human genome (hg38 and CHM13) for host contaminant removal. Taxonomic classification was performed with Kraken2 against bacterial NCBI databases. Using the DeSeq2 R package, raw data was normalized and analyzed for differentially abundant species at different time points adjusted through sex and batch composition.

Results: No significant differences in alpha diversity nor distinct groups in beta diversity representation were evident over time points. 56 differentially abundant species (DAS, absolute fold change value >1 and adj p-value < 0.05) were found in common between T1 vs T0 and T12 vs T0, 80% of the total DAS overtime points. Among them, Akkermansia Muciniphila was upregulated, and >20 species belonging to the Prevotellaceae family were downregulated. 14 DAS were found only in the comparison between T1 and T0, indicating a transient modulation of the microbiota, including Lactobacillaceae and Streptococcaceae. Finally, 6 DAS were found only in T12 vs T0 establishing the late gut microbial asset. Among them, species belonging to the families of Streptococcaceae and Bifidobacteriaceae and genera belonging to the class of Clostridia.

Conclusion: Overall, our results suggest that DMF can act through a moderate modulation of gut microbiota that is induced already at one month after the beginning and maintained over one year. Clinical, dietary and lifestyle characteristics will be further analyzed to identify any clinical implications for microbiota alteration during DMF treatment.

P311/294
Lymphocyte subset repopulation after immune reconstitution with alemtuzumab, autologous hematopoietic stem cell transplantation or cladribine: a comparative study

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Introduction: Immune reconstitution therapies (IRTs) can induce long-term drug-free remission in relapsing-remitting multiple sclerosis (RRMS).

Objectives/Aims: Analyze differences in the repopulation of peripheral lymphocytes following alemtuzumab (ALZ)-, autologous hematopoietic stem cell transplantation (AHSCT)-, and cladribine (CLAD)-induced lymphocyte depletion.

Methods: RRMS initiating ALZ (n=51), AHSCT (n=20), and CLAD (n=59) were included. Blood samples were collected at baseline, month (m) 6, 12, and 24. Lymphocytes were subjected to a comprehensive flow cytometry analysis.

Results: 130 RRMS were included. Gender (71% females, p=0.05) and mean age 35.4 (SD 8.3) years (p=0.6) did not differ between groups (grps). Patients (ps) with prior second-line disease modifying treatment were 95% (ASHCT), 75% (ALZ), and 48% (CLAD), while treatment-naïve ps comprised 0% (ASHCT), 14% (ALZ), and 33% (CLAD). Total lymphocytes, CD3+, CD8+ T cells, and CD19+ B cells were within normal range at baseline, while CD4+ T cells were subnormal [mean 0.95 (SD 0.44)], but with no difference between IRT grps (p=0.4). All IRT grps significantly decreased lymphocyte counts at m 6 and 12, compared to baseline. Subnormal levels persisted for ALZ and CLAD during follow-up, while AHSCT had normalized their lymphocyte counts at m 24. Both CD3+ and CD4+ T cells were significantly decreased for all IRT grps. The reduction was most pronounced in the ALZ grp which showed lower CD3+ (m 6 and 12, p<0.001 and p<0.001) and CD4+ (m 12, p<0.001) T cell counts compared to the AHSCT and CLAD grps. CLAD-treated ps had least affected CD4+ T cell count (p<0.001). Ps treated with AHSCT recovered their CD3+ and CD4+ T cell counts faster and had higher cell counts at m 24 than the ALZ and CLAD grps (p<0.001 and p<0.001). CD8+ T cells were significantly reduced in ALZ and CLAD grps, but not in AHSCT-treated ps, and remained low at follow-up. Similar, the number of CD19+ B cells were significantly reduced in the ALZ and CLAD grps, but not in the AHSCT grp. The repopulation of CD19+ B cells was slower for CLAD-treated ps than for ALZ- and AHSCT-treated ps at m 24 (p<0.001).

Conclusion: Lymphocyte depletion and repopulation differed between IRTs. While AHSCT treated ps recovered their lymphocyte subsets most rapidly, depletion of T cell subsets was most pronounced and durable after ALZ treatment, and CLAD-treated ps had the slowest repopulation of CD19+ B cells. The impact from these differences on treatment efficacy and safety require further investigation.

P312/1449
Activity and Manufacturing of KYV-101 Anti-CD19 Chimeric Antigen Receptor T Cells Derived from Patients With Neurologic Autoimmune Diseases

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