Three drug discovery approaches for multiple sclerosis

Abstract

Discovering and developing effective therapeutic treatments for multiple sclerosis (MS), an autoimmune disease, are critically needed due to the lack of therapeutic solutions. Three MS drug discovery techniques and technologies using dendritic cells, oligodendrocytes, and Th17 cells are reviewed.

Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease that affects the central nervous system, including the brain and spinal cord. Myelin sheath damage on nerve cell axons is known to be the primary cause of the disease, and this slows the nerve system's transmission of messages between brain and body (Figure 1).

Symptoms include weakness, tingling, numbness, visual disturbances, muscle stiffness, and memory loss. There are currently treatments to dampen the symptoms and delay disease progression, but no known cure for multiple sclerosis has been found to date. The disease is provoked by auto-aggressive immune cells that cause devastating damage to the central nervous system.

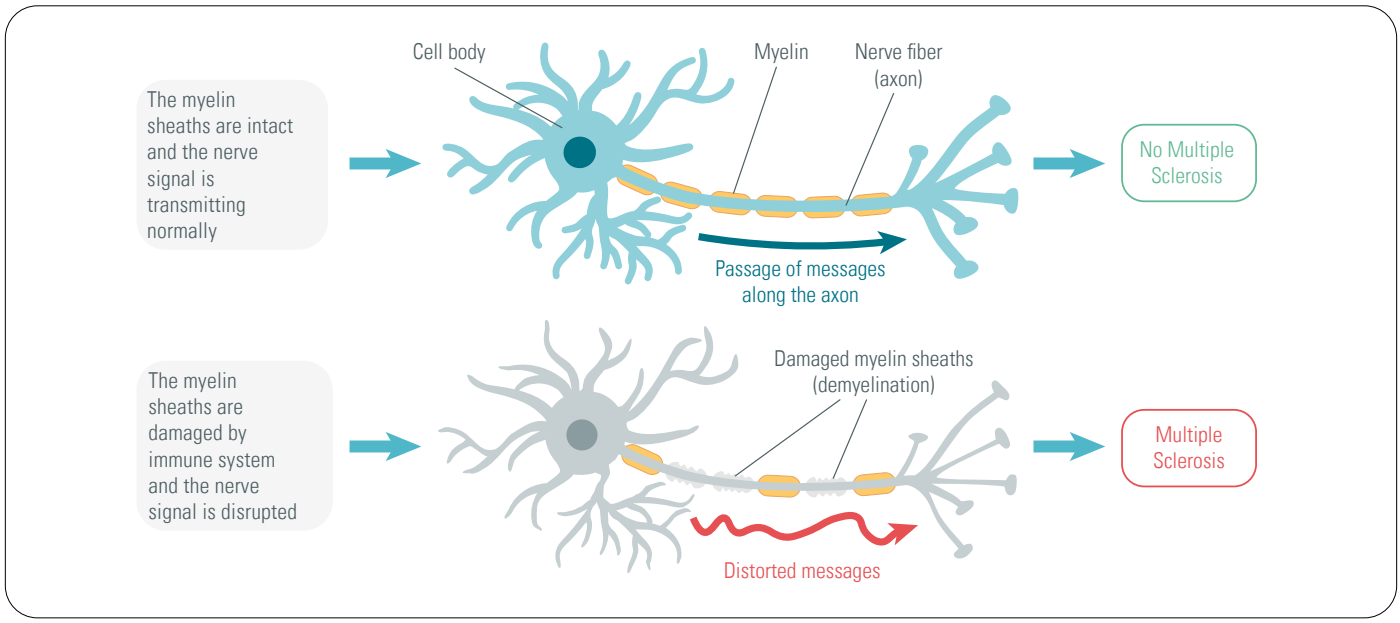

Figure 1: Normal healthy neuron and demyelinated neuron in multiple sclerosis.

MS exacerbation is instigated when circulating T cells become mis-programmed to target and attack myelin. The T cells release cytokines to recruit similar T cells, B cells, and macrophages to mount the attack. The inflammatory cells in MS can cross the blood brain barrier which normally separates the immune cells in the periphery from the central nervous system. Once inside the brain, the more aggressive T cells release more cytokines (for example interleukins) to activate B cells, macrophages, and microglia to propagate a cycle of inflammation which will attack the myelin sheath, axons, and oligodendrocytes, leading to cell death. Thus reducing IL-6 secretion by dendritic cells, protecting oligodendrocytes (myelin producing cells), and inhibiting cytokine producing pathways are essential to minimize the progression of MS.

Targeting dendritic cell cytokine production

Chen *et al.* have identified a new potential drug against MS that reduces the production of IL-6 by dendritic cells. This compound was discovered by running a high throughput screen on a focused library. To screen large numbers of compounds, the strategy was to produce a larger quantity of mature dendritic cells (mDC) by collecting GFP-expressing bone marrow cells that were first activated with a GM-CSF and then stimulated with LPS (Figure 2). Dendritic cells were used to screen 1280 compounds along with positive and negative controls. Modulations of dendritic cell IL-6 secretions in cell supernatants were determined by HTRF® Mouse IL-6 assay kit (Revvity) as a screen assay end point, and the IL-6 assay signal intensities were measured using an EnVision multimode plate reader (Revvity). The HTRF mouse IL-6 assay kit was chosen for the compound screen validations. The benefits of using HTRF (new technology, cost-effective, no wash, simple steps, and stable signal) are described in the first paragraph of discussion by the authors (Chen *et al.*)

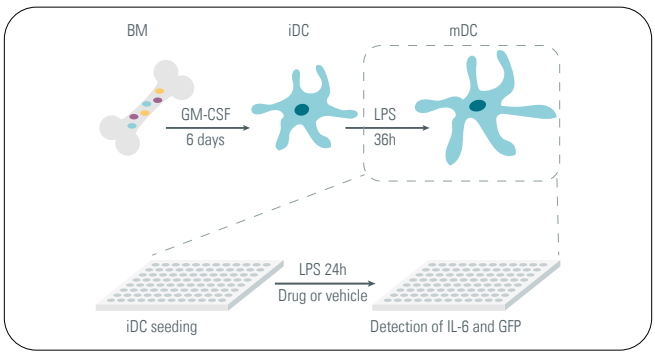

Figure 2: Scheme of mature dendritic cell (mDC) production and screening of drug candidates for MS.

Chen, S., Zhou, J., Cai, Y. *et al.* Discovery of BVDU as a promising drug for autoimmune disease therapy by Dendritic-cell-based functional screening. Sci Rep 7, 43820 (2017). https://doi.org/10.1038/srep43820

LPS treatment potentiates the release of IL-6 in a dose dependent manner, and the amount of IL-6 produced by the cells is dependent on the cell density (Figure 3A and Figure 3B). Based on the validation data, they were able to set up defined screening conditions: LPS stimulation was 10 ng/ml, cell number was 10,000 cells per well, DMSO concentration was 0.1% and incubation time was 24 hours for the further screening of IL-6 modulators in 96-well plates.

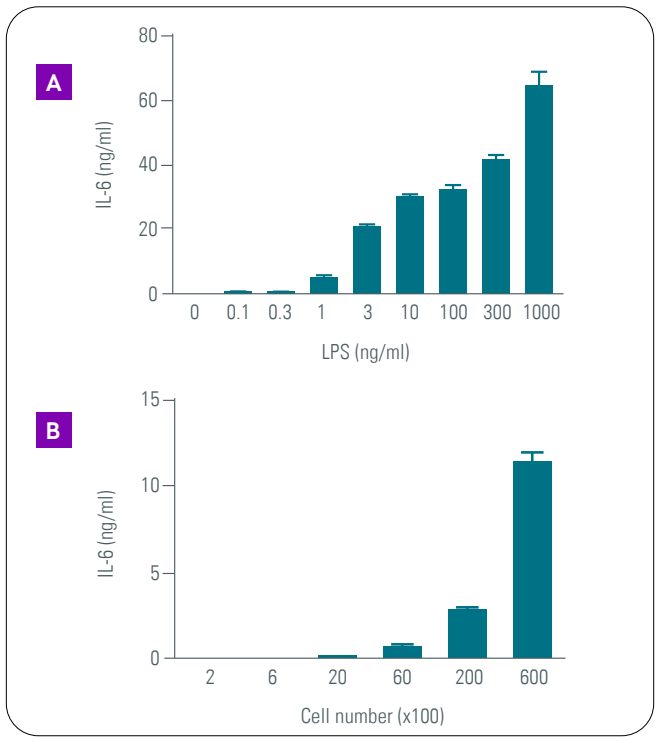

Figure 3: Dose Dependent Stimulation of LPS on IL-6 secretion in DC cultures (Figure 3A). Increasing number of cells with greater production of IL-6 in cultures (Figure 3B).

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The screening was very robust, with a Z′ value higher than 0.9 enabling the identification of 12 compounds that activate the IL-6 secretion and 9 compounds that reduce IL-6 production (Figure 4). Of the 9 selected compounds, BVDU was the most potent in reducing LPS-stimulated IL-6 secretion (Figure 5).

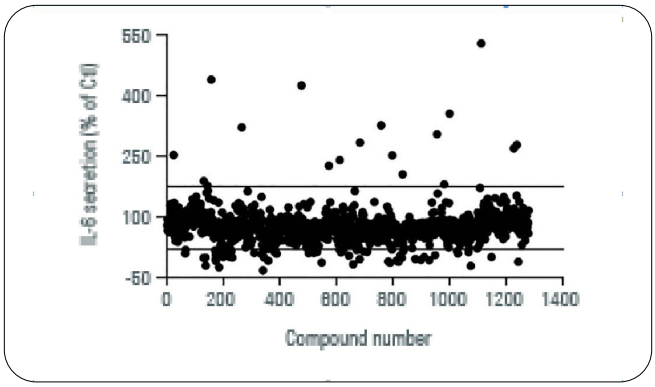

Figure 4: The results of screening 1280 compounds were shown by the secretion of IL-6 by DCs. The IL-6 secretions were expressed as % of the control.

Chen, S., Zhou, J., Cai, Y. *et al.* Discovery of BVDU as a promising drug for autoimmune disease therapy by Dendritic-cell-based functional screening. Sci Rep 7, 43820 (2017). https://doi.org/10.1038/srep43820

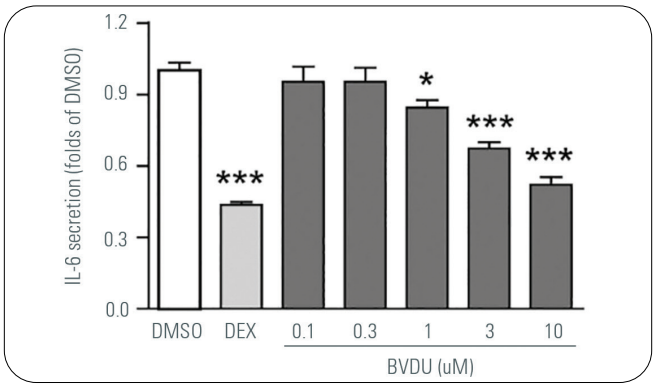

Figure 5: Reduction of IL-6 secretion by BVDU in a dose dependent manner.

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Further studies indicated that BVDU inhibited the differentiation of T cell to Th17 cells when naive T cells were co-cultured with DCs in presence of BVDU. It is well known that pro-inflammatory cytokine IL-6 secreted from DCs can stimulate naive T cells to become T helper cells (Th1 and Th17). Activated T helper cells are involved in the pathogenesis of MS diseases. BVDU alone appeared to act as an anti-inflammatory agent. Treatment of DCs with this compound significantly decreased both CD11c and CD80 positive cells and reduced IL-6, IL-12, IL-23, and IL-1β mRNA expression, and also increased the expression of anti-inflammatory cytokine IL-10. Additional cell validations suggested that BVDU maintains the DCs in an immature state (iDC) by inhibiting LPS-induced expression of CD80, CD86, and CD11c, and reducing the production of both IL-12p70 and IL-1β. The mechanism of action has been further delineated by demonstrating that BVDU suppressed MAPK and NF-κB signaling pathways in LPS stimulated DCs. Interestingly, BVDU seems to alleviate the demyelination process in the Experimental Autoimmune Encephalomyelitis (EAE) animal model (the most commonly used MS mouse model) by lowering leucocyte infiltration.

In summary, HTRF based IL-6 assays with DCs were successfully implemented to screen a library in order to identify numerous compounds that stimulate or inhibit LPS-stimulated IL-6 secretion. BVDU as an inhibitor of IL-6 secretion modulates other cytokines and their pathways that are critical for the pathogenesis of MS. It also reduces the severity of the EAE mouse model (a well-known animal model of MS) as it induces decreased neuroinflammation and demyelination, indicating the potential of BVDU as an active drug candidate to treat multiple sclerosis.

Triggering oligodendrocyte differentiation to promote myelin production

Another potential therapeutic approach is the repurposing of an existing drug candidate, HAMI3379, as shown by Merten *et al.* HAMI3379 is a known antagonist of the cysteinyl-leukotriene CysLT2 receptor, and was originally developed to address cardiovascular and inflammatory disorders. In this study, HAMI3379 was assessed for its ability to promote oligodendrocyte differentiation via the orphan GPR17 receptor,for the potential treatment of MS.

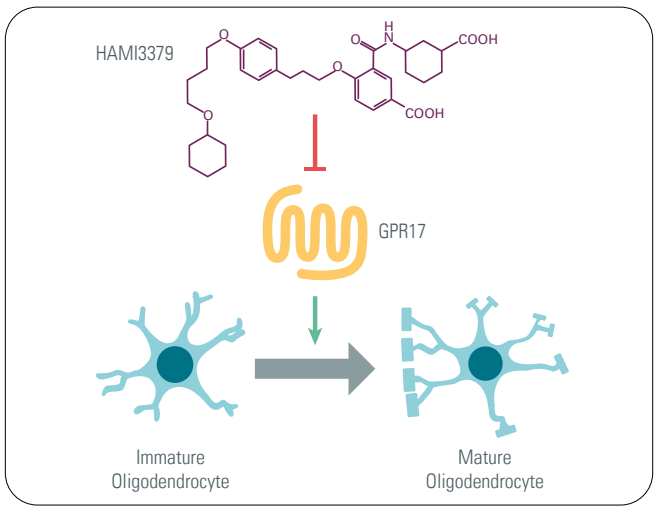
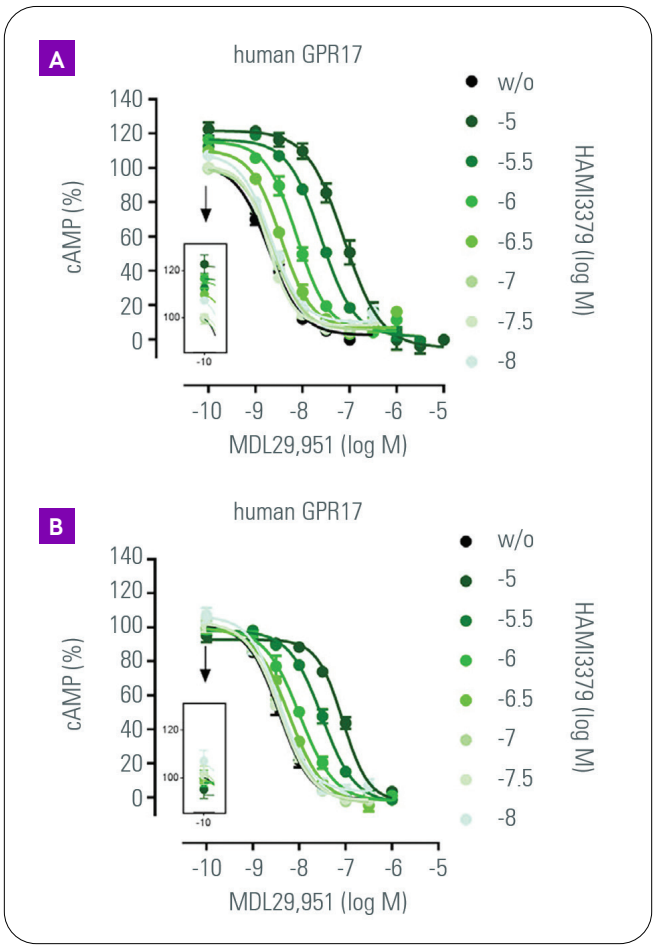


Figure 6: Structure of HAMI3379 and its possible mechanism in oligodendrocyte maturation.Mention of copyright rights: "Republished with permission of Cell Chemical Biology journal, from Merten, *et al* (2018). Repurposing HAMI3379 to Block GPR17 and Promote Rodent and Human Oligodendrocyte Differentiation; permission conveyed through Copyright Clearance Center, Inc."

The orphan GPR17 receptor, a GPCR receptor, seemed particularly interesting since GPR17 knockout is associated with precocious myelination at the neonatal stage.
By utilizing specific GPR17 ligands (MDL29.951), the study addressed the role of this receptor for the survival and differentiation of human oligodendrocytes derived from human induced pluripotent stem cells. The CysLT2 receptor antagonist HAMI3379 blocked human GPR17 Signaling.
Changes of ligand potency are known to occur among species orthologs of GPCRs, precluding direct conversion of ligand pharmacology from human to rodent receptors and animal models. The *in vitro* potencies of HAMI3379 were represented by the right shifts of intracellular cAMP accumulation curves (Figure 7), suggesting that HAMI3379 and MDL29.951 may bind to the same GPR17 site, and that HAMI3379 antagonized the actions of MDL29.951. Subsequently, HAMI3379 may promote oligodendrocyte maturation and trigger the myelination process in MS.
To measure intracellular cAMP, an HTRF-based cAMP accumulation assay was used (HTRF-cAMP dynamic 2 kit; Revvity), and the assay signals were measured with a Revvity EnVision multilabel plate reader. The HTRF-cAMP dynamic 2 kit results are shown in Figures 7A, 7B, and 7C.



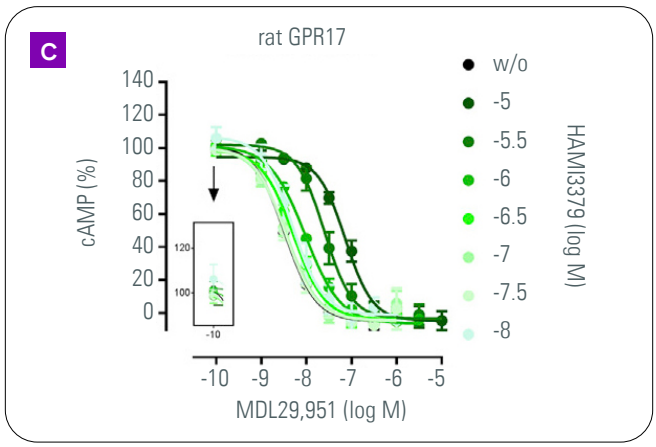


Figure 7: HAMI3379 inhibits human (Figure 7A), mouse (Figure 7B), and rat (Figure 7C) GPR17-mediated signaling, as shown by the right shift of intracellular cAMP accumulation curves.Mention of copyright rights: "Republished with permission of Cell Chemical Biology journal, from Merten, *et al* (2018). Repurposing HAMI3379 to Block GPR17 and Promote Rodent and Human Oligodendrocyte Differentiation; permission conveyed through Copyright Clearance Center, Inc."

In summary, the study revealed that HAMI3379, as an experimental drug that favors differentiation of rodent and human oligodendrocytes, efficiently blocks human and rodent GPR17 signaling in recombinant mammalian expression systems. It may qualify as a pharmacological tool to investigate the GPR17 function in rodent and human oligodendrocyte differentiation to promote the myelination process in MS.

Targeting rorγ on Th17 cells as a potential treatment for MS

Amaudrut *et al.* have identified a potent and selective RORγ inverse agonist which is active in a mouse experimental autoimmune encephalomyelitis (EAE) model, a preclinical model for multiple sclerosis. RORγt is highly expressed in immune cells such as Th17 and γδT cells, and is a key player in the IL-17 pathway. It is involved in the differentiation of naive T cells into Th17 cells, where both RORα and RORγ are highly expressed. RORγt is also involved in the synthesis of proinflammatory cytokines such as IL-17A, IL-17F, and IL-22.

The Gal4/ RORγ-LBD luciferase reporter gene assay was used for an HTS campaign. The initial hit compounds were modified (SAR) to obtain potent and selective RORγ inverse agonists that inhibited IL-17A production in a cellular assay. The selectivity vs the other ROR isoforms was evaluated in a coactivator recruitment assay using AlphaScreen® technology, in which the binding of N-biotin co-activator

PGC1g peptide and His-RORγt (amino acid T259-K518) was inhibited by the ROR isoforms (RORα, RORβ, or ROYγ (Figure 8). Assays testing the inhibition of IL-17A resulted in the selection of a potent and selective RORγ inverse agonist (Compound 29).

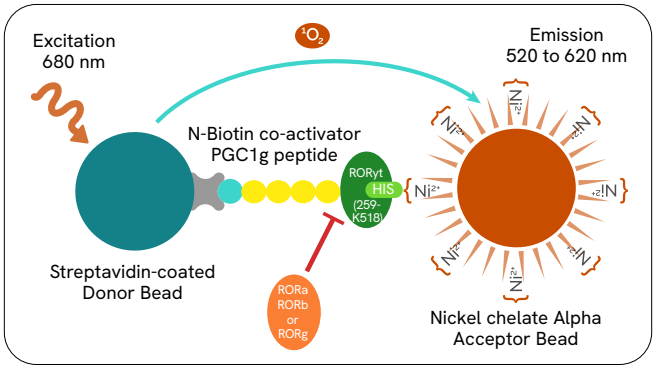


Figure 8: The principle of AlphaScreen® Assay Technology. AlphaScreen® Technology (Amplified Luminescent Proximity Homogeneous Assay) was used to determine the compound dependent interaction of the RORγt ligand-binding domain (His-RORγ (amino acid T259-K518)) to the co-activator PGC1g (LXD1) peptide (N-biotin-QEAEEPSLLKKLLLAPANTQL-COOH).

Following the binding of a biotinylated co-activator peptide to His-Tag RORγt protein, the biotin on the co-activator binds to the streptavidin-coated donor beads and the His-Tag of RORγt protein interacts with Ni-chelate alpha acceptor beads, bringing the beads into close proximity. The excitation of the donor beads provokes the release of singlet oxygen molecules, which triggers a cascade of energy transfer in the Acceptor beads, and results in a light emission at 520-620 nm that can be detected by an Envision plate reader.

Compound 29 was first evaluated in an *in vivo* mechanistic model where mice were first immunized with a mixture of mouse MOG35-55 peptide (Myelin Oligodendrocyte Glycoprotein) and PTX (pertussis toxin). After 5 days, the test compound was administered 45 min before a challenge with anti-CD3. Two hours later, circulating cytokines in serum were measured. As expected for a RORγ inverse agonist, Compound 29 had a significant dose-dependent effect on Th17 cytokines (IL-17A, IL-17F, and IL-22 to a lesser extent) starting from the dose of 30 mg/kg (Figure 9). Compound 29 was then evaluated in an EAE model in mice (b.i.d. at 60 and 100 mg/kg p.o.), and inhibited disease onset by 81 and 76%, respectively, indicating that the compound was active in an *in vivo* animal model of MS.

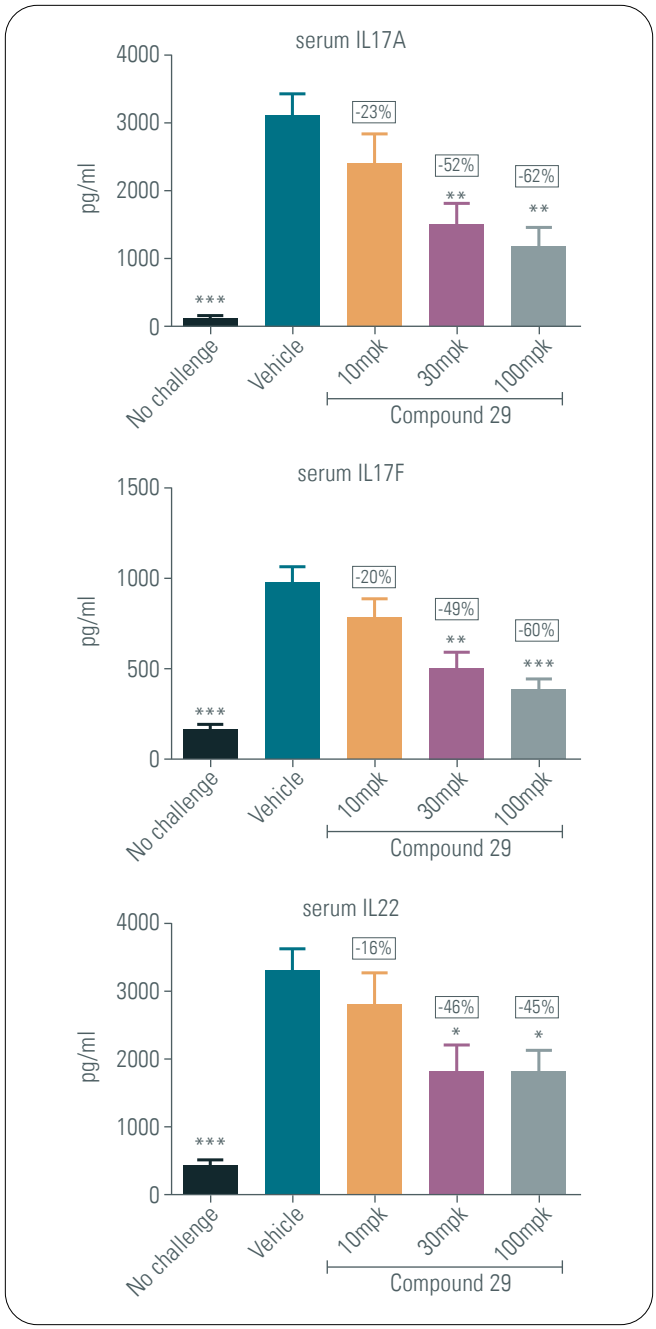


Figure 9: Effect of Compound 29 on anti-CD3 induced cytokine production in mice. Amaudrut, J. et al. Discovery of novel quinoline sulphonamide derivatives as potent, selective, and orally active RORγ inverse agonists. Bioorg. Med. Chem. Lett. 29, 1799–1806 (2019).

In summary, the previously-identified compound 29 was shown to be potent and selective in cell-based assays and active in EAE mouse models, demonstrating the potential use of RORγ inverse agonists for the treatment of Th17-related autoimmune diseases. However, additional optimizations (SAR) are ongoing due to the inhibition of CYP3A4 by compound 29 in an oral PK study.

Conclusion

In this review, we describe three small molecule drug discovery approaches, targeting dendritic cell pro-inflammatory cytokine, promoting oligodendrocyte differentiation, and discovering an RORγ inverse agonist. In each approach, potential drug candidates were identified to treat the pathogenesis of multiple sclerosis. The studies showed that reducing the levels of IL-6 in mature DCs, inhibiting GPR17 on oligodendrocytes, and inhibiting RORγ in Th17 cells may provide treatments for MS.

References

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