Inhibition of Bruton’s Tyrosine Kinase Selectively Prevents Antigen-Activation of B cells and Ameliorates B-Cell-Mediated Experimental Autoimmune Encephalomyelitis
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Objective: Evaluation of Bruton’s tyrosine kinase inhibitor (BTKi) evobrutinib (M2951) in a mouse model of experimental autoimmune encephalomyelitis (EAE).

Background: B cells are key mediators of inflammatory processes in multiple sclerosis, a notion substantiated by the success of pan B-cell depletion by anti-CD20 monoclonal antibodies; however, these can affect regulatory B-cell properties, as well as targeting pathogenic B cells. BTK is centrally involved in B-cell receptor (BCR) signaling, and subsequent B-cell activation and differentiation. BTKi could therefore control pathogenic functions such as antigen presentation and cytokine release, without affecting regulatory B-cell properties.

Design/Methods: C57Bl/6 mice received daily oral evobrutinib 1, 3 or 10mg/kg, or vehicle from 7 days prior to immunization with conformational MOG 1-117 protein (a B-cell-mediated model of EAE). EAE severity was assessed daily using a standard scale (0–5). Histopathology was performed at Day 60. Flow cytometry of activation markers on B cells, T cells and myeloid cells, and analysis of B-cell phenotype was performed at Day 12. Intra-cellular calcium flux analysis was performed in vitro, or after 3 days of BTKi treatment ex vivo, using Fluo-3 and Fura Red dyes and anti-IgM BCR stimulation.

Results: Intermediate and highest doses (3 and 10mg/kg) of BTKi showed an amelioration of EAE severity throughout the 60-day observation period. BTKi reduced CNS inflammation and demyelination, and led to an accumulation of naïve B cells, with a corresponding reduction of antigen-activated B cells. Expression of activation markers CD80, CD86, CD69 and MHCII on B cells was significantly reduced. BCR stimulation led to reduced calcium influx in BTKi-treated B cells in vitro and ex vivo.

Conclusions: BTKi reduced excitatory calcium influx in B cells upon BCR stimulation, preventing their activation and conversion from naïve to antigen-activated B cells. This translates into reduced CNS inflammation and clinical amelioration in a B-cell-mediated EAE model.