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Title: Evaluating the effect of cladribine on marmoset B- and T-cell proliferation and survival

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Short title to be displayed on app: Effect of cladribine on marmoset B- & T-cells

Background: Translational experimental autoimmune encephalomyelitis (EAE) marmoset models have been used to assess peripheral and central activities of B-/T-cell-targeting multiple sclerosis drugs, without rodent species cross-reactivity. Here we present the feasibility of using an EAE marmoset model to assess selective depletion of lymphocyte subsets in blood, lymphoid organs and central nervous system following cladribine exposure. This study assessed cladribine's effect on marmoset lymphocyte survival and proliferation *in vitro* and deoxycytidine (dCTP) levels in marmoset blood.

Methods: Following exposure to cladribine (1nM-100μM), *in vitro* proliferation and survival of naïve and activated (concanavalin A [ConA], phytohemagglutinin, MOG34-56, recombinant hMOG) peripheral blood mononuclear cells (PBMC), spleen/lymph nodes mononuclear cells and Epstein-Barr virus (EBV)-infected B-cells were assessed. Cells were cultured for 24, 48 or 72 hours before lymphocyte proliferation and survival analysis. Marmoset dCTP levels were measured by reverse-phase chromatography.

Results: Naïve and mitogen-stimulated (phytohemagglutinin/ConA) PBMC demonstrated dose-dependent declines in proliferation (~40% for cladribine 1μM/72h; 100% for 10μM/72h). No difference in efficacy was seen between naïve versus stimulated cells. $CD20^+$ B-cells were more sensitive to cladribine than $CD3^+$ T-cells in the survival assay. Analysis of MOG34-56 and MOG protein re-stimulated spleen/lymph node mononuclear cells from EAE marmosets confirmed a dose-dependent effect of cladribine after antigen stimulation (100nM-500nM). EBV-infected B-cells demonstrated reductions in proliferation with cladribine (~60-100% inhibition at 1-5μM/72h). EBV-infected B-cell survival was unaffected by cladribine. Low dCTP concentrations were detectable in marmoset blood.

Conclusions: Cladribine concentrations as low as 1μM can inhibit proliferation of naïve or activated PBMC. B-cells were more sensitive than T-cells to cladribine-induced cell death. Cladribine inhibited proliferation and survival of marmoset EAE spleen/lymph node mononuclear cells after antigen re-stimulation and inhibited proliferation, but not survival, of EBV-infected marmoset B-cells.

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Author disclosures:

YK does not declare any conflicts of interest

UB is an employee of EMD Serono Research & Development Institute Inc., a business of Merck KGaA, Darmstadt, Germany

BtH does not declare any conflicts of interest