Title: Cladribine decreases CD95 expressing CD4+ and CD8+ cells in lymphoid organs in naïve marmosets (*Callithrix jacchus*)

Authors: Yolanda Kap (main author)[1], Ursula Boschert[2], Bert t'Hart[1,3]

[1]Biomedical Primate Research Center, Department of Immunobiology, Rijswijk, The Netherlands; [2]EMD Serono Research & Development Institute Inc., Billerica, MA, United States; [3]University Groningen, University Medical Center, Department of Neuroscience, Groningen, The Netherlands

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

Background: Treatment of multiple sclerosis (MS) with cladribine tablets is based on reducing active lymphocytes, where cladribine has high selectivity. CD95/FAS cell surface receptors are impaired in active lymphocytes of MS patients and immune cell reduction in lymphoid organs is unknown. Marmosets have been used for pharmacodynamic (PD) studies in naïve or experimental autoimmune encephalomyelitis models to test peripheral lymphocyte-targeting MS drugs with no rodent species cross-reactivity. This PD analysis assessed lymphocyte subtype reduction in blood and lymphoid organs of marmosets after cladribine treatment.

Methods: Twin marmosets were randomised to four groups (n=2 each). Three doses of subcutaneous cladribine (0.2,0.4,0.8mg/kg/day for 5 consecutive days in 2 weekly periods) were assessed; control group received 0.9% NaCl. Blood samples were taken weekly, 2 weeks before, and up to 12 weeks after first dose of cladribine for analysis of serum chemistry and immune monitoring. Animals were sacrificed in week 12; blood and lymphoid organs were sampled and immunophenotyped using flow cytometry and immunohistochemistry.

Results: At necropsy, no differences in serum chemistry were observed between animals at different cladribine doses. Blood samples showed no profound reduction of absolute T- or B-lymphocytes or their subsets over time following cladribine treatment. In lymphoid organs, the percentage of CD95-expressing $CD4^+$ or $CD8^+$ T-cells in the $CD3^+$ population were lower in cladribine-treated animals than in controls. This difference was statistically significant when cladribine-treated animals were pooled.

Conclusions: Following cladribine administration**,** no profound reduction of total lymphocytes, T- or B-cells was observed. CD95-expressing $CD4^+$ or $CD8^+$ cells were reduced in lymphoid organs, potentially identifying a novel *in vivo* mechanism of action of cladribine involving the CD95/CD95L pathway. The limited effect on other immune cells is consistent with observations in normal lymphoid organs that the CD95/CD95L pathway is only expressed in scattered lymphocytes and is dysregulated in MS patients.

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