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Evaluating the Effect of Cladribine on Marmoset B- and T-cell Proliferation and Survival

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INTRODUCTION

- Translational experimental autoimmune encephalomyelitis (EAE) marmoset (*Callithrix jacchus*) models have been used to assess peripheral and central activities of B- and T-cell-targeting multiple sclerosis (MS) drugs, without rodent species cross-reactivity.[1]
- Marmoset genetic and immunological profiles are comparable to those of humans which makes the EAE marmoset model particularly useful for research into the immunopathogenic mechanisms of new therapies for MS.[2,3]
- Cladribine is phosphorylated to the active compound by the enzyme deoxycytidine kinase. High levels of 2'-deoxycytidine (2dC) levels can compete with cladribine and may reduce the *in vivo* efficacy. Variations in plasma 2dC levels are observed in mammals.[4]
- Here we present the feasibility of using an EAE marmoset model to assess selective depletion of lymphocyte subsets in blood, lymphoid organs and the central nervous system following cladribine exposure.

OBJECTIVES

- The aim of this study was to assess the effect of cladribine on marmoset lymphocyte survival and proliferation *in vitro*.
  - Activated and non-activated B- and T-cells, myelin oligodendrocyte glycoprotein (MOG) peptide or MOG protein re-stimulated lymph node cells from EAE marmosets and Epstein-Barr virus (EBV) transfected B-cells were assessed.
- Additionally, this study aimed to assess 2dC levels in marmoset blood.

METHODS

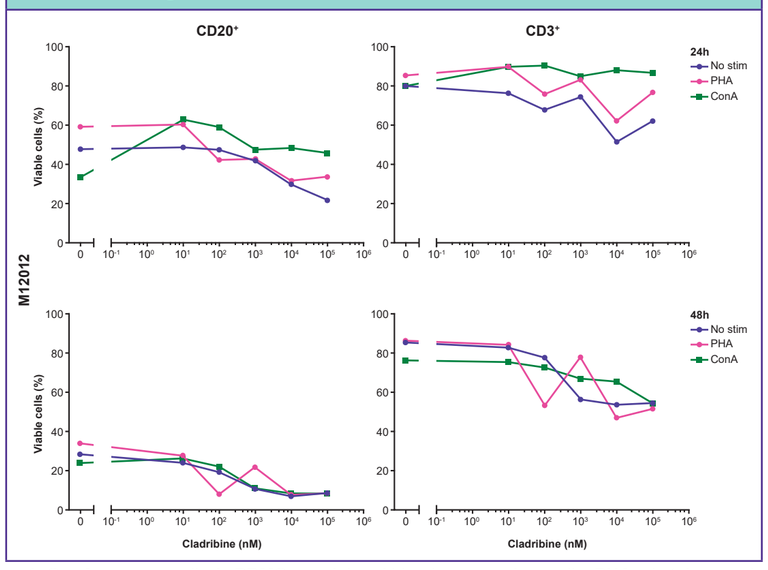
- Following exposure to cladribine (1 nM–100 µM), *in vitro* proliferation and survival of naïve and activated (concanavalin A [ConA], phytohemagglutinin [PHA], MOG34-56, recombinant hMOG) peripheral blood mononuclear cells (PBMC), spleen and lymph node mononuclear cells and EBV-infected B-cells were assessed.
- Cells were cultured for 24, 48 or 72 hours before analysis of lymphocyte proliferation and survival.[5]
- For proliferation assays, cells were stimulated for 48 hours followed by incubation for 16 hours with 3H-thymidine to measure proliferation.
  - The results were expressed as stimulation index, calculated by dividing the average counts per minute (cpm) of stimulated cells by the average cpm of unstimulated cells. A stimulation index above 2 is set as cut-off for a positive proliferative response.
- The effect of cladribine on viability was assessed by flow cytometry after staining with the Fixable Viability Dye eFluor 506.
- Marmoset 2dC levels were measured by reverse-phase chromatography.

RESULTS

Survival Assays

- The effect of cladribine on viability of freshly isolated, naïve or mitogen (PHA, ConA) stimulated marmoset PBMCs was studied at 24 and 48h.
- Cladribine reduces $CD20^+$ B-cell and $CD3^+$ T-cell viability, with a more pronounced effect on $CD20^+$ B-cells (**Figure 1**, n=1).

Figure 1. Effect of cladribine on the viability of freshly isolated, naïve or mitogen stimulated marmoset PBMCs



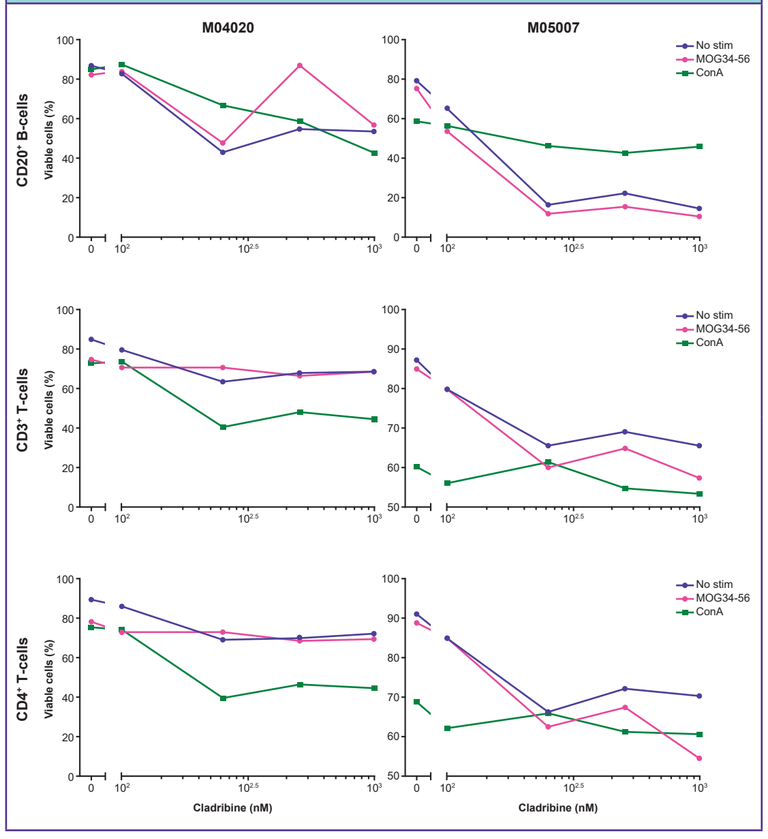
n=1. ConA, concanavalin A; **PBMCs**, peripheral blood mononuclear cells; **PHA**, phytohemagglutinin.

- The effect of cladribine on the viability of marmoset axillary lymph node (ALN) cells, stimulated with mitogen or antigen was studied after 72 hours by flow cytometry. Cells were gated on single cells, lymphocytes, $CD3^+$ versus $CD20^+$ and then for $CD4^+$ within the $CD3^+$ compartment (**Figure 2**, n=2).
- EBV-infected B-cell (B-LCL) survival was unaffected by cladribine at 24, 48 and 72 hours. (**Figure 3**, n=2).

Proliferation Assays

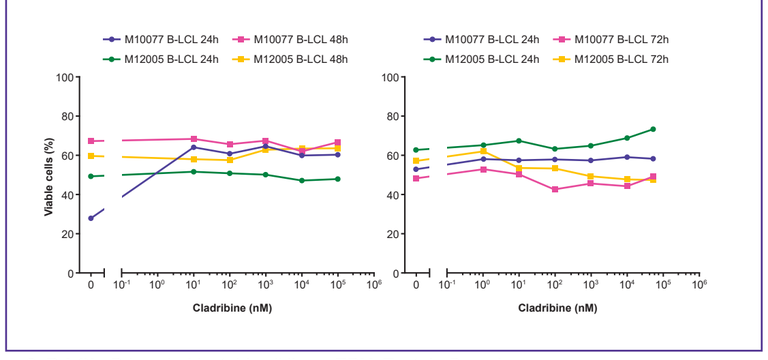
- A dose dependent inhibitory effect of cladribine was seen on proliferation of fresh PBMC stimulated for 72 hours with PHA or ConA. In the last 16 hours, 3H-thymidine was added to the culture to measure proliferation (**Figure 4**, n=2).
- No difference in effect of cladribine was seen between naïve versus stimulated cells.
- A gradual decline of proliferation was observed in the spleen and ALN cells of EAE marmosets re-stimulated with MOG34-56 and rhMOG for 72 hours. Cladribine concentrations between 0–500 nM were used (**Figure 5**, n=4).

Figure 2. Effect of cladribine exposure on the viability of EAE marmoset lymph node cells



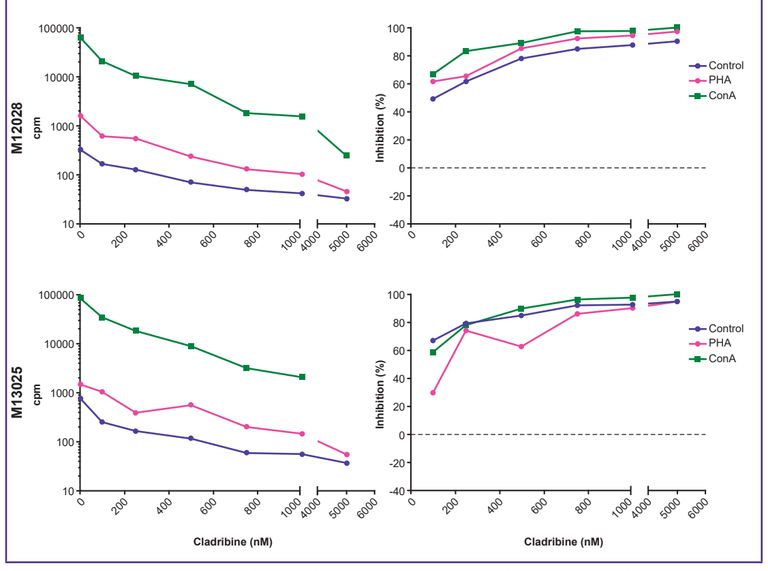
n=2. **ConA**, concanavalin A; **EAE**, experimental autoimmune encephalomyelitis.

Figure 3. EBV-infected B-cell survival



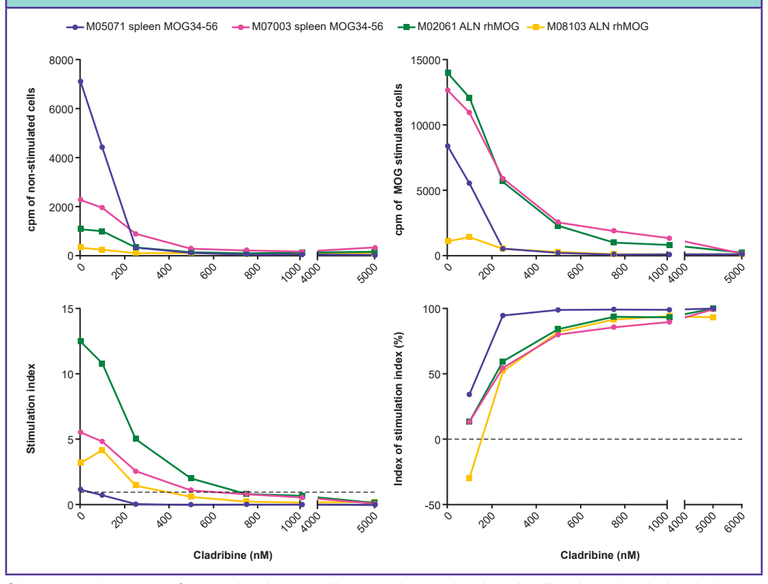
n=2**. B-LCL**, B-lymphoblastoid cell lines; **EBV**, Epstein-Barr virus.

Figure 4. Proliferation of naïve and mitogen-stimulated PBMCs following cladribine exposure



The left graphs show the cpm, the right graphs show the inhibition compared to samples without cladribine. n=2. **ConA**, concanavalin A; **cpm**, counts per minute; **PBMCs**, peripheral blood mononuclear cells; **PHA**, phytohemagglutinin.

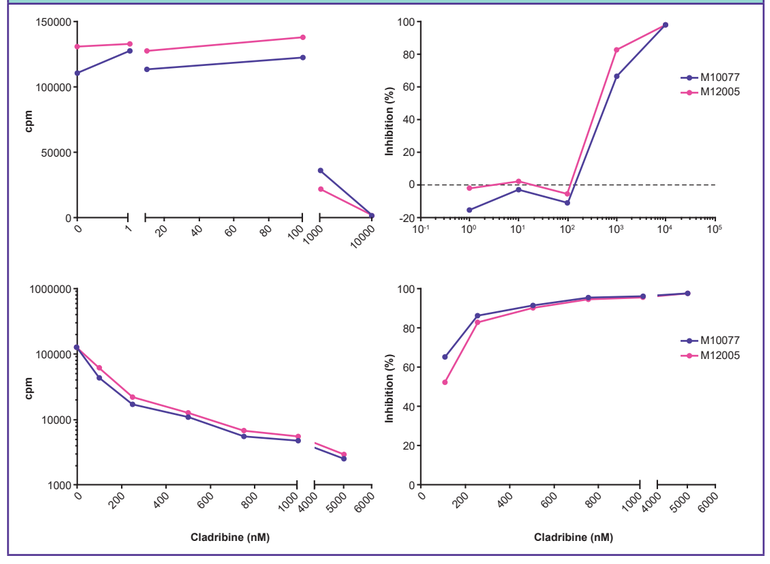
Figure 5. Effect of cladribine on proliferation of spleen and axillary lymph node cells taken from EAE marmosets



Shown are the cpm of non-stimulated cells or antigen stimulated cells, the stimulation index, and the inhibition of the stimulation index compared to samples without cladribine. n=4. **cpm**, counts per minute; **EAE**, experimental autoimmune encephalomyelitis.

- Analysis of MOG34-56 and MOG protein re-stimulated spleen and ALN mononuclear cells from EAE marmosets confirmed a dose-dependent effect of cladribine on proliferation after antigen stimulation (100–500 nM). Cladribine inhibits the proliferation of EBV-infected B-cells (B-LCL) (**Figure 6,** n=2).

Figure 6. Effect of cladribine on proliferation of EBV-infected spleen or axillary lymph node B-cells



Shown are the cpm and the inhibition of proliferation compared to samples without cladribine. n=2. **cpm**, counts per minute; **EBV**, Epstein-Barr virus.

2dC Levels in Marmoset Blood

- Low 2dC concentrations were detectable in marmoset blood (**Table 1**).

Table 1. 2dC levels in marmoset blood

Marmoset #	Sex	2dC (ng/ml)	2dC (µM)
MiE381	Female	46.3*	0.204
MiE577	Female	26.8*	0.118
MiE287	Female	69.5	0.306
M10072	Male	134	0.590
M10110	Male	62.4	0.275
M13030	Male	95.9	0.422

*Below the lower limit of quantitation, but high enough to determine the concentration.
2dC, 2'-deoxycytidine.

CONCLUSIONS

- **This study describes the *in vitro* efficacy of cladribine on marmoset lymphocyte proliferation and survival and the levels of 2dC in marmoset plasma.**
- **Cladribine-induced cell death was more pronounced for B-cells (100 nM to 1 µM) than for than T-cells.**
- **No difference in the effect of cladribine was observed between naïve and mitogen-stimulated PBMC.**
- **Cladribine inhibited proliferation and survival of EAE marmoset spleen and axillary lymph node mononuclear cells after antigen re-stimulation.**
- **Cladribine did not affect survival of EBV infected marmoset cells but inhibited proliferation in a dose dependent manner.**
- **In marmosets the highest blood 2dC value was 0.590 µM, therefore the low level of 2dC suggests that the marmoset model is appropriate for further study for PK/PD evaluation.**

REFERENCES

1. Kap YS, et al. *Drug Discov Today* 2016;21:1200–1205.
2. 't Hart BA, et al. *Trends Mol Med* 2011;17:119–125.
3. 't Hart BA, et al. *Ann Clin Transl Neurol* 2015;2:581–593.
4. Chan TS, et al. *Biochem* 1983;210:367–371.
5. Jagessar SA, et al. *Exp Anim* 2013;62:159–171.

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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

Cladribine Tablets are approved by the European Commission for the treatment of adult patients with highly active relapsing multiple sclerosis (MS) as defined by clinical or imaging features.

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