Cladribine controls multiple sclerosis by memory B cell depletion

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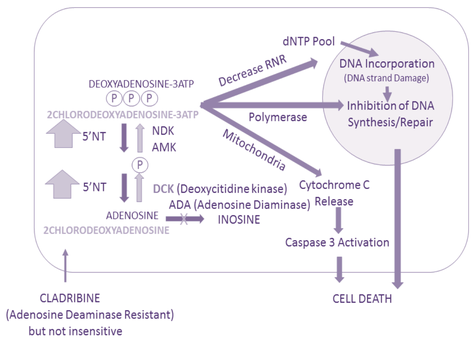
SUMMARY

- **Cladribine induces long-term depletion of memory B cells in MS and exploits the sensitivity of B cells towards the drug and the slow repopulation characteristics of this cell subset**
- **This further emphasizes that highly effective disease modifying therapies deplete memory B cell subsets in MS**
- **It remains to be established whether monitoring CD19⁺,CD27⁺ B cells can be used as a biomarker of subclinical disease activity, prognosis and retreatment requirements as shown in other neurological and non-neurological conditions.**

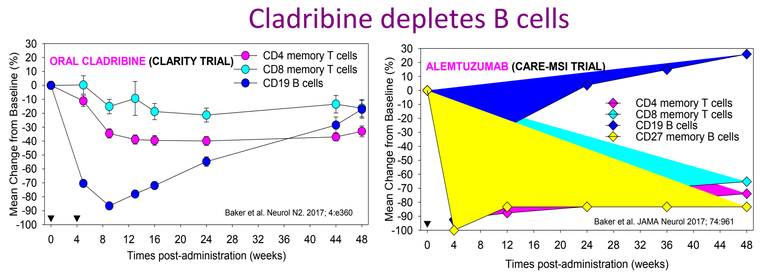
BACKGROUND

- Oral cladribine has recently been licenced for the treatment of relapsing MS, but the mechanism of action in MS is unknown.
- Cladribine is a purine analogue that is resistant to but not insensitive to degradation of adenosine deaminase (ADA).
- This creates a substrate for phosphorylation by deoxycytidine kinase (DCK).
- Cladribine triphosphate is cytotoxic for dividing and non-dividing lymphoid cells and cancers.



- The action of DCK is countered by 5'nucleotidases (5'NT) which dephosphorylates cladribine monophosphate.

Cladribine depletes B cells



- Although it has been suggested that oral cladribine is a T cell immuno-suppressive agent, previous studies show minor T cell depletion.
- CD19⁺ B cell depletion is more marked. Although alemtuzumab depletes CD4⁺ and CD8⁺ T cells, the transient CD19⁺ B cell-depletion response masked the marked and long-term depletion of CD19⁺,CD27⁺ memory B cells.
- It was found that all active disease modifying therapies (DMT) could inhibit relapsing multiple sclerosis (MS) to a greater or lesser extent (Baker et al. EBioMed 2017 16:41).
- As cladribine is a highly efficacious compound it was hypothesised that the drug would deplete memory B cells as a central mechanism of action

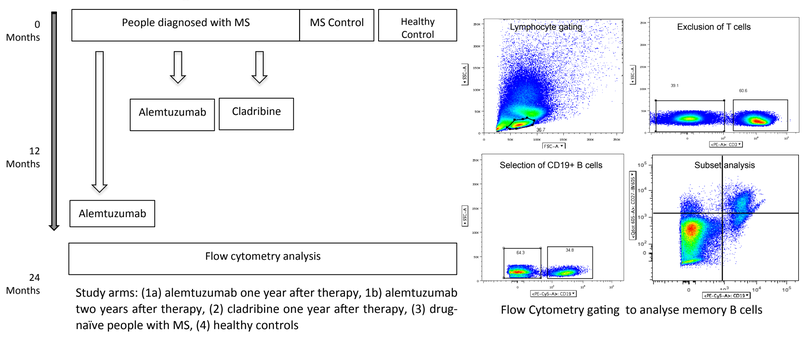
METHODS

- 40 subjects were recruited to a cross-sectional study to examine the depletion effect of subcutaneous cladribine (100% bioavailable compared to 42% for oral cladribine) as part of compassionate use programme, developed prior to the relicensing of oral cladribine prodrug.
- Ethics approval was obtained from Health & Social Care Research Ethics Committee B

Group	Sex (M/F)	Age (Y)	Disease duration(Y)	WebEDSS Median (Range)
Healthy controls	5/4	36.1 ± 9.9	N/A	N/A
MS controls	2/6	36.7 ± 8.2	3.9 ± 4.2	3.0 (0.0-5.0)
Cladribine	2/6	46.8 ± 11.4	6.3 ± 3.1	5.25 (0.0-6.5)
Alemtuzumab Y1	2/6	38.0 ± 10.6	4.6 ± 2.4	3.0 (0.0-6.5)
Alemtuzumab Y2	3/5	40.3 ± 8.2	5.8 ± 3.9	3.0 (0.0-5.0)

Protocol of drug administration and monitoring

- Subcutaneous cladribine (3-4 x 10mg) was dose-adjusted to weight and 4 weeks later to lymphocyte numbers to limit severe lymphopenia. This was compared with alemtuzumab administered during routine care.
- As memory B cells showed long lasting depletion, blood samples were collected at the end of the year blood sampling prior to the next treatment cycle or 12 months after the last one.
- Cell numbers and proportions of CD3⁺ T cells and B cell subsets were assessed using flow cytometry



Study arms: (1a) alemtuzumab one year after therapy, 1b) alemtuzumab two years after therapy, (2) cladribine one year after therapy, (3) drug-naïve people with MS, (4) healthy controls

Flow Cytometry gating to analyse memory B cells

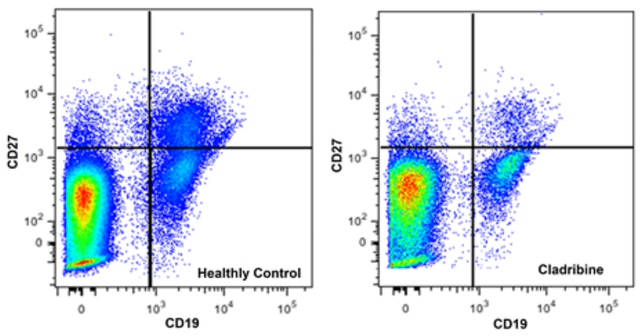
RESULTS

Dose-adaption of cladribine limits lymphopenia

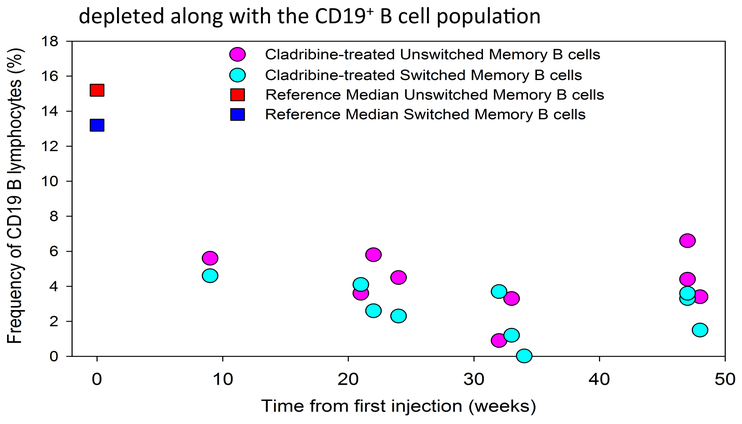
Degree of Lymphopenia (NCI CTCAE V 4.0)	Cladribine Baseline/Litak™	Alemtuzumab Baseline/ Lemtrada™
Total	57 (100%)	126 (100%)
Grade 0 (≥Lower Limit of Normal)	(98.2%) 56/31 (54.4%)	(96.8%) 122/1 (0.8%)
Grade 1 (<1.0-0.8 x 10^9/L)	(0.0%) 0/9 (15.8%)	(2.4%) 3/2 (1.6%)
Grade 2 (<0.8-0.5 x 10^9/L)	(0.0%) 0/16 (28.1%)	(0.8%) 1/17 (13.5%)
Grade 3 (<0.5-0.2 x 10^9/L)	(0.0%) 0/1 (1.8%)	(0.0%) 0/64 (50.8%)
Grade 4 (<0.2 x 10^9/L)	(1.8%) 1/0 (0.0%)	(0.0%) 0/42 (33.3%)
Frequency grade 3/4 lymphopenia	1/57 (1.8%)	106/126 (84.1%)

Lymphopenia before (baseline) and after treatment (percentage of total)

Cladribine depletes memory B cells

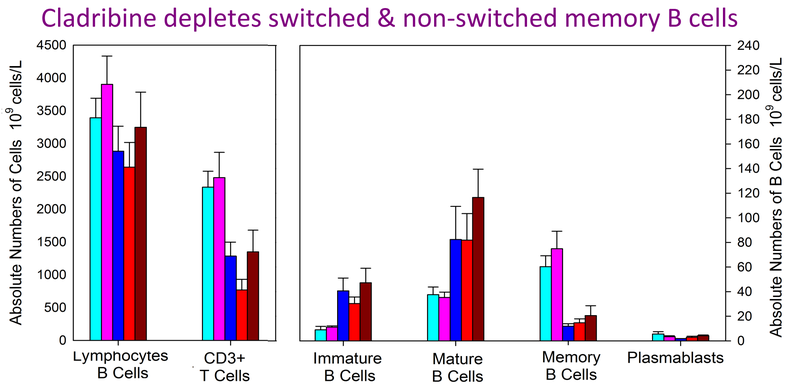


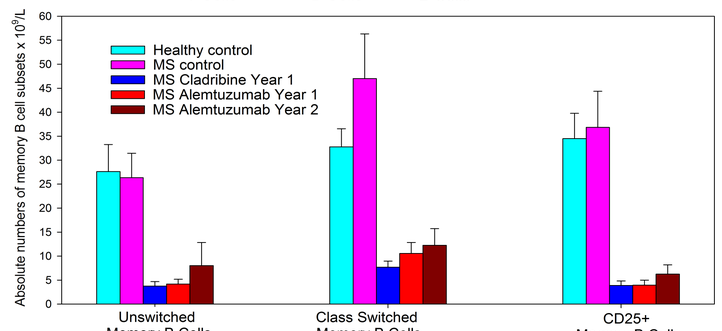
- Following cladribine treatment CD27⁺ memory B cells were depleted along with the CD19⁺ B cell population

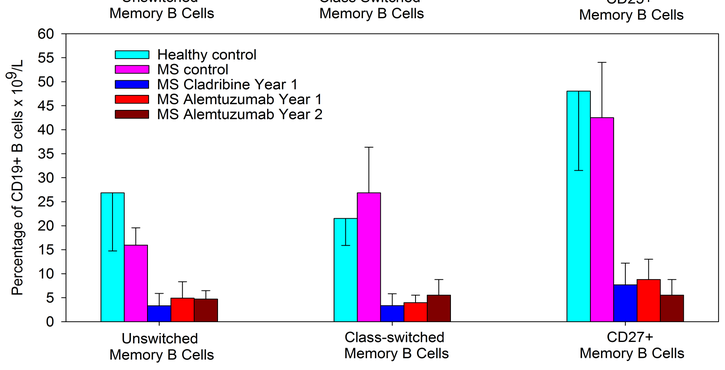


- The proportion of unswitched (CD19⁺,CD27⁺,IgD⁺) and class-switched (CD19⁺,CD27⁺,IgD⁻) memory B cells were below reference levels across 12 months from treatment onset (n=11).

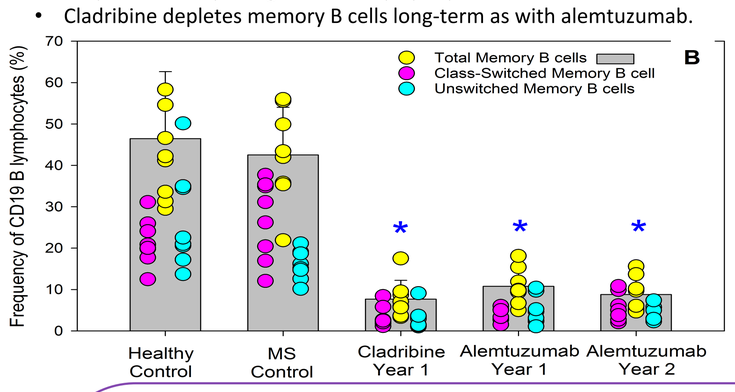
Cladribine depletes switched & non-switched memory B cells





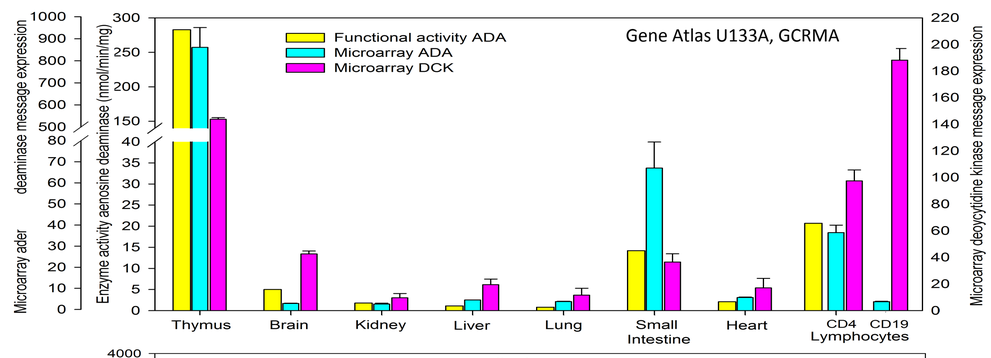


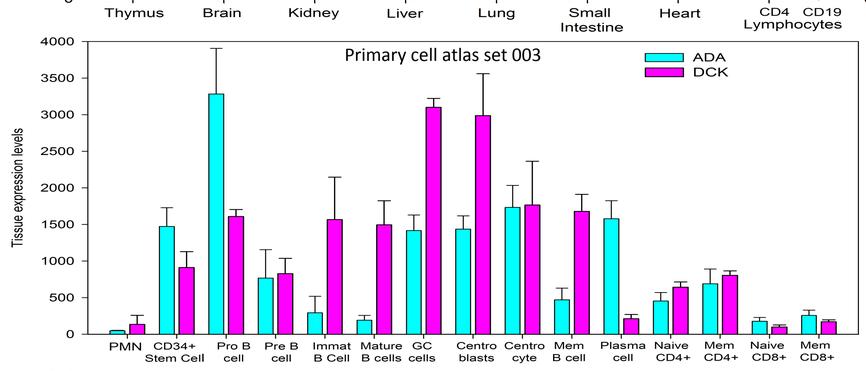
- Unswitched, class-switched and CD25+ memory B cells were markedly depleted to levels found in people with MS treated with alemtuzumab, despite relatively low generalised lymphopenia.
- Cladribine depletes memory B cells long-term as with alemtuzumab.

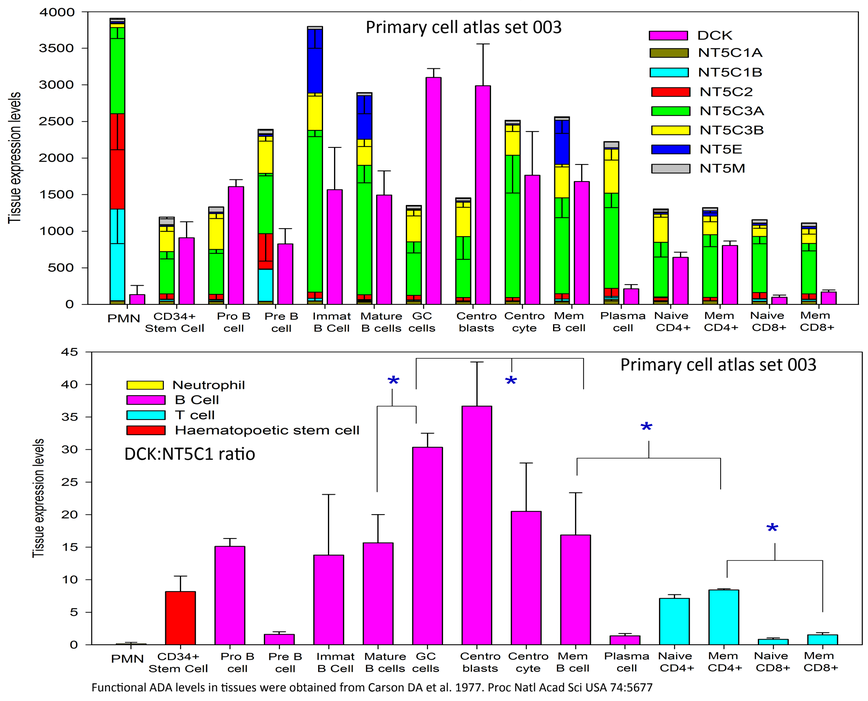


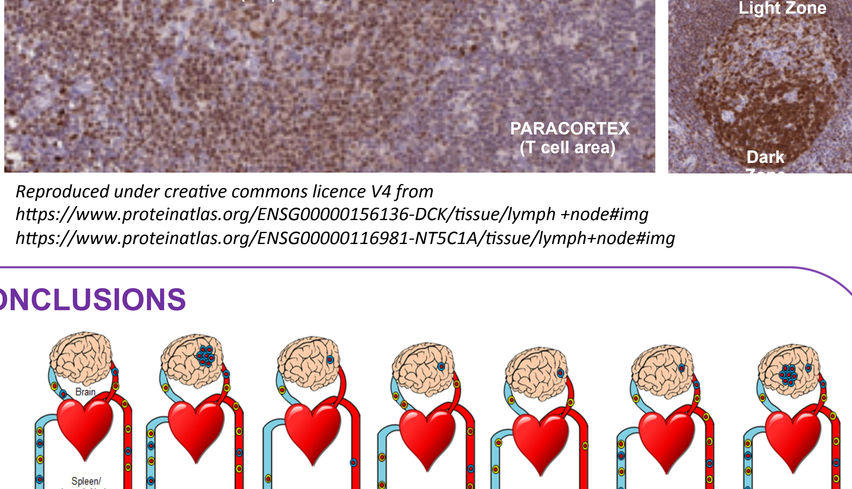
Microarray expression of purine salvage pathways genes

- The purine salvage pathway genes were examined in public microarray gene expression data bases (www.biogps.org), to examine the mechanism of action. B cells expressed less ADA and often more DCK than T cells.
- Cytosolic 5'NTs that inhibit adenosine monophosphate (NT5C1A & NT5C1B) were limited in lymphocytes.
- DCK and DCK:NT5C1 ratio demonstrated variability across B cell subsets and correlated with protein expression







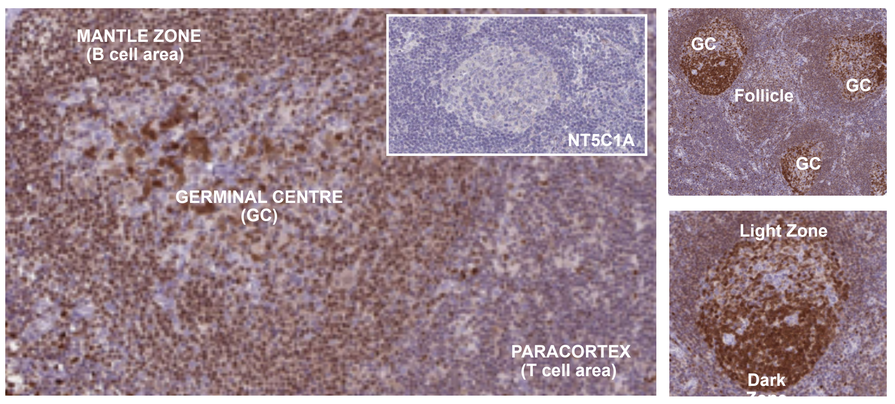


Functional ADA levels in tissues were obtained from Carson DA et al. 1977. Proc Natl Acad Sci USA 74:5677

- Microarray suggests that CD4 T cells are more likely to be targeted than CD8 T cells and that B cells express more DCK than T cells. Immature, mature and memory B cells express similar levels of DCK, where as plasma cells express significantly less DCK. Germinal centre (GC) cells and centroblasts express high levels of DCK in microarray.

Expression of deoxycytidine kinase in lymph nodes

- Microarray expression is consistent with protein expression in lymph nodes.
- There was lower expression in the paracortex (T cell areas) than in mantle zone. There was high expression in the dark zone of the secondary follicles, containing GC B cells and centroblasts. NT5C1A exhibited essentially no staining of lymph node tissue, but was highly expressed in myocytes consistent with microarray analysis.



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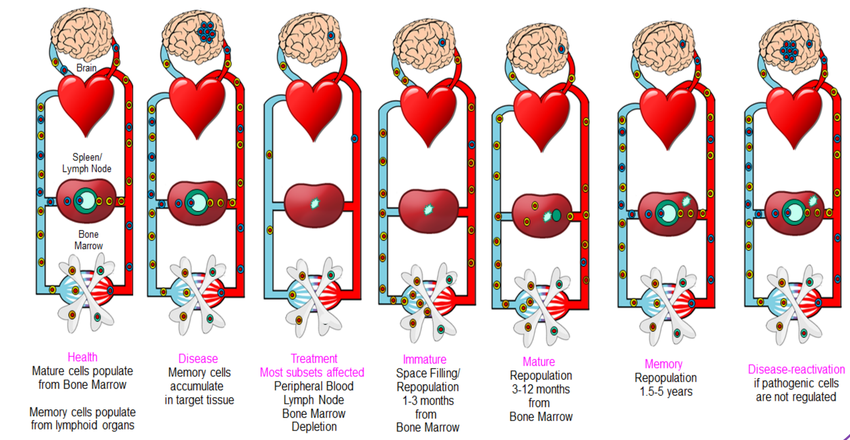
https://www.proteinatlas.org/ENSG00000156136-DCK/tissue/lymph +node#img
https://www.proteinatlas.org/ENSG00000116981-NT5C1A/tissue/lymph+node#img

CONCLUSIONS

- Memory B cells are depleted by cladribine, comparable to alemtuzumab, although it induces limited lymphopenia.
- Memory B cells are sensitive to the effects of cladribine.
- Peripheral blood (immature, mature, memory cells) express comparable levels of DCK and will be sensitive to the action of cladribine
- Immature and mature cells repopulate the blood following depletion from bone marrow.
- Memory B cells repopulate from lymphoid tissue slowly, and based on development and response to rituximab repopulation often takes 18months-5 years .
- This study provides further evidence that the memory B cell is central to the therapeutic activity in MS.



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