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Evaluation of the treatment response to cladribine tablets using a transcriptomics and proteomics approach in the cerebrospinal fluid and peripheral blood of people with multiple sclerosis [0037]

Free Communications 3: Treatment 1

19 Sep 2024 09:50 - 10:00 CEST

Prof. Heinz Wiendl University Hospital Münster Department of Neurology Chair

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Background

Proinflammatory immune cells in the CNS drive neuroinflammation and demyelination¹⁻³



1. van Langelaar et al. Front Immunol 2020;11:760; 2. Gross CC et al. Front Immunol 2016;7:606; 3. Bhargava P et al. Brain 2022;145:3363-73

Background **CXCL13 and OCBs may be CSF markers for MS disease progression**



Intrathecal plasma blasts and plasma cells release immunoglobulins, resulting in the presence of OCBs in the CSF²

CSF



CSF, cerebrospinal fluid; CXCL, C-X-C chemokine receptor; OCB, oligoclonal band 1. Alvarez E et al. Mult Scler 2013;19:1204–8; 2. Häusser-Kinzel E, Weber MS. Front Immunol 2019;10:201



Background Cladribine tablets reconstitute the immune system to a less pathogenic state^{a,b,1-4}



^aCladribine tablets have been classified as an IRT³; ^bThe mechanism by which cladribine exerts its therapeutic effects in MS is not fully elucidated but its predominant effect on B and T lymphocytes are thought to interrupt the cascade of immune events central to MS⁵; ^cIL-10+ B cells were increased above baseline levels at Months 3 and 6¹; ^dIL-10+ memory B cells were reduced from baseline levels from Month 3 onward¹; ^eIL-4+ CD4+ and CD8+ T cells were increased above baseline levels from Month 3 onward¹; ^fProinflammatory cytokineproducing B and T cells were reduced compared with baseline levels from Month 3 onward¹. Ig, immunoglobulin; IRT, immune reconstitution therapy; NK, natural killer 1. Wiendl H et al. ACTRIMS 2023 [P008]; 2. De Stefano N et al. CMSC 2024 [DMT01]; 3. Giovannoni G, Mathews J. Neurol Ther 2022;11:571–95; 4. Ruschil C et al. Front Immunol 2023;14:1133967; 5. MAVENCLAD[®] EU SmPC, November 2023



Study design MAGNIFY-MS sub studies investigated the effects of cladribine tablets on neuroinflammation¹⁻⁴

Patient population: highly active RMS, defined as one relapse in the previous year and ≥ 1 T1 Gd+ lesion, or ≥ 9 T2 lesions while on therapy with other DMTs; or ≥ 2 relapses in the previous year whether on DMT or not



^aClinical endpoints measured in the study were MRI lesion count, EDSS/KFS, 9HPT, T25FW, SDMT, relapse count; ^bSafety included TB (active and latent), hepatitis B & C, HIV and pregnancy 9HPT, 9-hole peg test; BL, baseline; CSF, cerebrospinal fluid; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; Gd+, gadolinium-enhancing; HIV, human immunodeficiency virus; KFS, Kurtzke Functional System; M, month; NfL, neurofilament light chain; OCB, oligoclonal band; RMS, relapsing MS; SDMT, symbol digit modality test; T25FW, time 25-foot walk; TB, tuberculosis

1. De Stefano N, et al. Neurol Neuroimmunol Neuroinflamm. 2022;9(4):e1187; 2. Wiendl H, et al. Neurol Neuroimmunol Neuroinflamm. 2022;10(1):e200048; 3. Wiendl H, et al. Neurology 2023;100(17_Supplement_2):3016; 4. De Stefano N et al. CMSC 2024 [DMT01]



Objective and methods **To further elucidate the effect of cladribine tablets on central inflammation, proteomics and transcriptomics were carried out**



^aHighly active RMS was defined as 1 relapse in the previous year and ≥ 1 T1 Gd+ lesion, or ≥ 9 T2 lesions while on therapy with other DMTs; or ≥ 2 relapses in the previous year whether on DMT or not; ^bBack-calculated concentrations of analytes were generated using a 4-Parameter Logistic fit model (Equation 6) with 1/Y2 weighting and analysed with Tukey's multiple comparisons test. All analyses were exploratory. Lower limit of detection was applied; ^cRaw fastq data were aligned to the EnsemblGRCh38 reference genome. Differential gene expression was assessed by DESeq2 methodology. Genes with an absolute value of fold change >1.2 and false discovery rate <0.05 were considered significant. All analyses were exploratory. BAFF, B-cell activating factor; receptor; BB, blood biomarker; BCMA; B-cell maturation antigen; CCL; C-C chemokine ligand; CD, cluster of differentiation; CSF, cerebrospinal fluid; CXCL, C-X-C chemokine ligand; CXCR, C-X-C chemokine receptor; DMT, disease-modifying therapy; FACS, florescence-activated cell sorting; IFN, interferon; IL, interleukin; PB, peripheral blood; RMS, relapsing MS; TACI, transmembrane activator and CAML interactor; TNF, tumour necrosis factor; TNFSF, TNF superfamily



MAGNIFY MS

Results Baseline characteristics



	All MAGNIFY-MS participants N=270	CSF sub study population n=28	Assessed for transcriptomics n=11		
Female, n (%)	180 (66.7)	14 (50.0)	8 (72.7)		
Aged ≤40 years, n (%)	152 (56.3)	17 (60.7)	10 (90.9)		
Time since MS diagnosis in months, mean ± SD	60.9 ± 74.5	38.4 ± 61.0	34.3 ± 53.2		
Number of relapses within 12 months prior to BL, n (%)					
0	3 (1.1)	0 (0)	1 (9.1)		
1	102 (37.8)	8 (28.6)	2 (18.2)		
≥2	165 (61.1)	20 (71.4)	8 (72.7)		
EDSS score >3 at BL, n (%)	66 (24.4)	11 (39.3)	2 (18.2)		
≥2 previous DMTs, n (%)	65 (24.1)	7 (25.0)	7 (63.6)		



BL, baseline; CSF, cerebrospinal fluid; DMT, disease-modifying therapy; RMS, relapsing MS; SD, standard deviation



Results IFNγ protein levels decreased in the CSF at Month 12 after treatment with cladribine tablets



Box plots: x represents mean average, central horizontal line represents median average, upper and lower horizontal lines and solid colour represent the interquartile range BL, baseline; CSF, cerebrospinal fluid; IFN, interferon; M, Month; MRI, magnetic resonance imaging



Results Chemokine CXCL13 protein levels decreased in the CSF at Month 12 and 24 after treatment with cladribine tablets



Box plots: x represents mean average, central horizontal line represents median average, upper and lower horizontal lines and solid colour represent the interquartile range BL, baseline; CSF, cerebrospinal fluid; CXCR, C-X-C chemokine receptor; CXCL, C-X-C chemokine ligand; M, Month



Results CXCL13 and CXCL13 receptor (CXCR5) transcripts in peripheral lymphocytes after treatment with cladribine tablets



• A reduction in CXCL13 transcripts in peripheral B cells was observed at Month 12

• A reduction in CXCL13 receptor (CXCR5) transcripts in peripheral B cells and T cells was observed at Month 12

Raw fastq data were aligned to the EnsemblGRCh38 reference genome. Genes with an absolute value of fold change >1.2 and false discovery rate (FDR) <0.05 were considered significant after correcting for multiple tests on selected genes. All analyses were exploratory.



MAGNIFY MS

Box plots: x represents mean average, central horizontal line represents median average, upper and lower horizontal lines represent the interquartile range



Results Transcriptional changes in peripheral T- and B-cells associated with specific pathways after cladribine tablets treatment



Changes of gene enrichment in biological pathways after cladribine tablets treatment support that cladribine tablets reconstitute the immune system to a less pathogenic state

^aPathway analysis was applied to examine the enrichment of differentially expressed genes on Gene Ontology database. Pathways with FDR threshold <0.05 were considered significant. The 'Upregulated' or 'Downregulated' indicates the enrichment of genes from the specific pathway with upregulated expression or downregulated expression, respectively FDR, false discovery rate, IFN, interferon; Ig, immunoglobulin





Cladribine tablets reduced OCBs in the CSF of patients with MS

The MAGNIFY-MS CSF sub study investigated changes in OCB counts of participants positive for \geq 2 OCBs at BL (n=17)

OCBs were assessed by isoelectric focusing in a central laboratory



Reduction of OCBs in the CSF of most patients supports the effect of cladribine tablets on CNS inflammation

A limitation of the analyses in the CSF sub-study was the small sample size, so these results should be interpreted with caution BL, baseline; CNS, central nervous system; CSF, cerebrospinal fluid; M, month; OCB, oligoclonal band De Stefano N et al. CMSC 2024 [DMT01]



Overview of TEAEs in the MAGNIFY-MS safety analysis set

Number of participants with:	Total N=270 (%)	Participants with ≥1 TEAE	Total N=270 (%)
Any TEAE ^a	227 (84.1)	Headache	87 (32.2)
Mild	114 (42.2)	Nasopharyngitis	57 (21.1)
Moderate	103 (38.1)	Urinary tract infection	32 (11.9)
Severe	10 (3.7)	Fatigue	31 (11.5)
Any study treatment-related TEAE ^a	122 (45.2)	Nausea	31 (11.5)
Mild	71 (26.3)	Back pain	30 (11.1)
Moderate	47 (17.4)	Lymphopenia	28 (10.4)
Any serious TEAE	4 (1.5) 14 (5.2)	Upper respiratory tract infection	27 (10.0)
Any study treatment-related serious TEAE	0 (0.0)	Diarrhoea	26 (9.6)
Any TEAE leading to temporary discontinuation	4 (1.5)	Pain in extremity	22 (8.1)
of study treatment	. (=)	Alopecia	21 (7.8)
of study treatment	1 (0.4)	Dizziness	20 (7.4)

No new safety signals were observed in the study

^aWorst severity per participant is reported TEAE, treatment-emergent adverse event De Nicola N et al. CMSC 2024 [DMT01]





Conclusion Cladribine tablets reduced the proinflammatory immune milieu in the CSF and in the periphery

Decreased protein levels of the B cell chemoattractant CXCL13 and the cytokine IFN γ were observed in the CSF after treatment with cladribine tablets

Exploratory analysis suggest a reduction of CXCL13 and CXCR5 transcript in peripheral B cells, and CXCR5 transcript in peripheral T cells at Month 12 after treatment with cladribine tablets



OCBs were reduced or eliminated in most patients (76.5%) treated with cladribine tablets

 Anti-inflammatory phenotype
Proinflammatory phenotype

These data suggest that immune reconstitution following treatment with cladribine tablets may **shift the immune system to a more homeostatic and less pathogenic state**



CSF, cerebrospinal fluid; CXCL, C-X-C chemokine ligand; CXCR, C-X-C chemokine receptor; IFN, interferon; OCB, oligoclonal bands

Thank you

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MAGNIFY MS