Tampere University

ILKKA RAUMA

Safety of Disease-Modifying Therapies for Highly Active Relapsing-Remitting Multiple Sclerosis

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ACADEMIC DISSERTATION
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PunaMusta Oy – Yliopistopaino
Joensuu 2022

To my family

ABSTRACT

The treatment of multiple sclerosis (MS) includes using disease-modifying therapies (DMTs) to prevent inflammatory disease activity. Patients with a highly active disease are at elevated risk for neurologic disability and are treated using DMTs with the highest expected efficacy. However, the use of these DMTs is limited by safety concerns. Measures to reduce medication-related harm in people with MS need to be further examined in the post marketing setting.

The aim of this thesis was to investigate the safety of four different DMTs for highly active relapsing-remitting MS in Finland using real-world patient data. The DMTs assessed in this thesis included the two anti-trafficking therapies, natalizumab (NTZ) and fingolimod (FNG), as well as the two immune reconstitution therapies, alemtuzumab (ALEM) and cladribine (CLAD) tablets. The specific aims of the four studies in this thesis included assessing disease reactivation after NTZ discontinuation (Study I), lipid profile alterations during FNG treatment (Study II), adverse events (AEs) after ALEM treatment (Study III) and clinical outcomes and treatment sequencing in patients treated with CLAD tablets (Study IV). Real-world data were acquired from patient information archives and the Finnish MS registry. Study I included data on 89 patients who had discontinued NTZ and had been followed-up for at least 12 months afterwards. Study II included data on 72 patients who had initiated FNG with a median follow-up of 12 months. Study III included data on 121 patients who had initiated ALEM with a median follow-up of 30.3 months. Study IV included data on 179 patients who had initiated CLAD tablets with a median follow-up of 19.0 months.

In Study I, we demonstrated that corticosteroid-treated relapses occurred in 20% and 30% of patients after six and 12 months after discontinuing NTZ respectively, whereas any relapse was observed in 27% and 36% of patients respectively. A higher number of relapses and an EDSS score of 5.5 or above prior to initiating NTZ predicted the risk of reactivation at six months. Initiating a subsequent DMT after a washout period longer than three months was associated with an increased risk for reactivation at six months when compared to not initiating any subsequent DMTs. In Study II, we observed minor elevation in the

concentrations of total cholesterol (0.40 mmol/L/year) and high-density lipoprotein (0.17 mmol/L/year) during a median follow-up of 12 months after the initiation of FNG. No statistically significant alterations in the concentrations of low-density lipoprotein or triglycerides were observed. In Study III, we showed that serious infusion-associated reactions (IARs) were observed in 11% of patients during the first course of ALEM, and in 0-3% of patients during subsequent courses. Serious adverse events (SAEs) other than IARs occurred in 23% of patients during a median follow-up of 30 months after the initiation of ALEM. Autoimmune AEs, infections, hepatobiliary AEs and malignancies were reported. In Study IV, we found that median time to first relapse was shorter in patients switching to CLAD tablets after receiving at least two previous DMTs when compared to patients with 0-1 previous DMTs prior to CLAD tablets. A subgroup analysis showed that this was most likely due to disease reactivation in patients switching from FNG. The most frequent AEs reported after receiving treatment with CLAD tablets were headache, *Herpes simplex* and nausea.

The main contributions of this thesis include describing disease reactivation after anti-trafficking therapies as well as safety issues associated with the use of immune reconstitution therapies. Exit strategies for patients discontinuing anti-trafficking therapies need to be developed, and the newly proposed risk factor for predicting the risk of reactivation after NTZ discontinuation based on EDSS should be taken into account when discontinuing NTZ in patients with early disability. We confirmed existing safety findings regarding the use of immune reconstitution therapies, but according to the results from Study III, considered ALEM a risky approach in the treatment of MS. Finally, the findings from Study II suggest that the overall risk of atherosclerosis may be unaltered by the small alterations in lipid concentrations observed during FNG treatment. However, more research is warranted to explore whether other similar drugs could have an effect on the lipoprotein concentrations or the risk of atherosclerosis in people with MS.

TIIVISTELMÄ

MS-tautia eli pesäkekovettumatautia hoidetaan taudinkulkua muuntavilla lääkehoidoilla. Näiden hoitojen tarkoitus on vähentää MS-taudin tulehduksellista tautiaktiivisuutta ja sitä kautta estää toimintakyvyn heikkenemistä. Osalla MS-tautia sairastavista henkilöistä tauti on kuitenkin poikkeuksellisen aktiivinen, jolloin usein turvaudutaan kaikista tehokkaimpina pidettyihin lääkehoitoihin. Tehokkaiden lääkkeiden käyttöön liittyy kuitenkin erityisiä turvallisuusriskejä. Jotta nämä riskit osattaisiin ottaa huomioon jo lääkehoitoa suunniteltaessa, tarvitaan tosielämän potilasaineistoihin perustuvaa tutkimustietoa eri lääkehoitojen turvallisuudesta.

Tämän väitöskirjan tavoitteena oli tutkia neljän eri MS-tautilääkkeen turvallisuutta. Tutkituista lääkkeistä natalitsumabi ja fingolimodi ovat niin sanottuja soluliikennöinnin estäjiä, kun taas alemtutsumabi ja kladribiini ovat niin sanottuja immuunirekonstituutiohoitoja. Väitöskirjaan kuuluu neljä osatyötä. Ensimmäisessä osatyössä tutkittiin natalitsumabin lopettamisen jälkeistä tautiaktiivisuuden lisääntymistä. Toisessa osatyössä tutkittiin veren rasva-arvojen muutoksia fingolimodin käytön aikana. Kolmannessa osatyössä tutkittiin alemtutsumabin haittavaikutuksia. Neljännessä osatyössä tutkittiin kladribiinin käyttöä, tehoa ja haittavaikutuksia. Tutkimusaineistot kerättiin sairaaloiden potilastietojärjestelmistä ja Suomen MS-rekisteristä. Ensimmäisen osatyön otos kattoi 89 natalitsumabin keskeyttänyttä potilasta, joilla seuranta-aika oli 12 kuukautta. Toisen osatyön otos kattoi 72 fingolimodin aloittanutta potilasta, joilla seuranta-ajan mediaani oli 12 kuukautta. Kolmannen osatyön otos kattoi 121 alemtutsumabin aloittanutta potilasta, joilla seuranta-ajan mediaani oli 30.3 kuukautta. Neljännen osatyön otos kattoi 179 kladribiinin aloittanutta potilasta, joilla seuranta-ajan mediaani oli 19.0 kuukautta.

Ensimmäisessä osatyössä osoitimme kliinisen tautiaktiivisuuden palaavan 20 % ja 30 % potilaista kuuden ja 12 kuukauden kuluessa natalitsumabin lopettamisesta, kun päätemuuttujana käytettiin kortisonihoidettuja pahenemisvaiheita. Ylipäätään pahenemisvaiheita todettiin vastaavasti 27 % ja 36 % potilaista kuuden ja 12 kuukauden kuluessa. Tautiaktiivisuuden lisääntymisen riski oli koholla niillä potilailla, joilla oli ollut useita pahenemisvaiheita tai alentunut toimintakyky ennen

natalitsumabin aloitusta (Expanded Disability Status Scale [EDSS] -pisteet vähintään 5.5). Uutta lääkehoitoa aloitettaessa yli kolmen kuukauden varoaika oli yhteydessä suurentuneeseen taudin aktivoitumisen riskiin verrattuna niihin potilaisiin, jotka eivät aloittaneet mitään uusia lääkehoitoja. Toisessa osatyössä havaitsimme, että kokonaiskolesterolin ja HDL-kolesterolin (high-density lipoprotein) pitoisuudet kohosivat hieman fingolimodin käytön aikana, kun taas LDL-kolesterolin (low-density lipoprotein) ja triglyseridien pitoisuudet eivät. Seuranta-ajan keston mediaani oli 12 kuukautta, ja tutkittuna ajanjaksona kokonaiskolesterolin pitoisuus nousi keskimäärin 0.40 mmol/L/vuosi ja HDL-kolesterolin pitoisuus nousi keskimäärin 0.17 mmol/L/vuosi. Kolmannessa osatyössä osoitimme, että vakavia infuusioreaktioita ilmeni 11 % alemtutsumabia saaneista potilaista ensimmäisen hoitokerran yhteydessä, mutta vain 0-3 % toisen hoitokerran yhteydessä. Muita vakavia haittoja ilmeni yhteensä 23 % potilaista 30 kuukauden mediaaniseurannan aikana. Tutkimuksessa raportoitiin muun muassa autoimmuunisairauksia, infektioita, maksa- ja sappiperäisiä haittoja sekä syöpiä. Neljännessä osatyössä havaitsimme, että niillä potilailla, jotka olivat käyttäneet aiemmin kahta tai useampaa lääkehoitoa, tuli nopeammin pahenemisvaihe kladribiinin aloittamisen jälkeen verrattuna niihin potilaisiin, jotka olivat käyttäneet korkeintaan yhtä aiempaa hoitoa. Alaryhmäanalyysin perusteella tämän katsottiin selittyvän fingolimodista kladribiiniin siirtyneillä potilailla, joista suurella osalla todettiin pahenemisvaihe pian fingolimodin lopettamisen jälkeen. Yleisimpiä kladribiinin haittavaikutuksia olivat päänsärky, *Herpes simplex* -infektio ja pahoinvointi.

Koska natalitsumabin ja fingolimodin lopetukseen liittyy taudin aktivoitumisen riski, tarvitaan yhteisesti sovittuja ohjeistuksia siitä, miten näitä lääkehoitoja lopetettaessa tulisi menetellä. Natalitsumabin käytön lopettamisen yhteydessä tulisi huomioida potilaan aiempi tautiaktiivisuus ja toimintakyky ennen natalitsumabin aloitusta. Vaikka toisen osatyön tulosten perusteella onkin epätodennäköistä, että fingolimodi vaikuttaisi merkittävästi valtimonkovettumataudin riskiin, tulisi asiaa tutkia vielä muilla vastaavan vaikutusmekanismin omaavilla MS-lääkkeillä erikseen. Alemtutsumabin ja kladribiinin käytön jälkeen ilmaantuneet haitat vastasivat enimmäkseen aiempaa tutkimusnäyttöä. Vakavien haittojen ilmaantuvuus alemtutsumabia saaneilla MS-tautipotilailla oli kuitenkin niin suuri, että hoitoa voidaan pitää riskialttiina.

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ABBREVIATIONS

AE	adverse event
ALC	absolute lymphocyte count
ALEM	alemtuzumab
ARR	annualized relapse rate
BBB	blood-brain barrier
CD	cluster of differentiation
CI	confidence interval
CIS	clinically isolated syndrome
CLAD	cladribine
CNS	central nervous system
CSF	cerebrospinal fluid
CYP	cytochrome P450
DMT	disease-modifying therapy
EBV	Epstein-Barr virus
EDSS	Expanded Disability Status Scale
EMA	European Medicines Agency
FNG	fingolimod
FS	Functional System
Gd	gadolinium
HARRMS	highly active relapsing-remitting multiple sclerosis
HCP	health-care practitioner
HDL	high-density lipoprotein
HLA	human leukocyte antigen
HPV	human papillomavirus
HR	hazard ratio
IAR	infusion-associated reaction
IM	intramuscular
IRT	immune reconstitution therapy
ITP	immune thrombocytopenic purpura

IV	intravenous
JCV	John Cunningham virus
LDL	low-density lipoprotein
MRI	magnetic resonance imaging
MS	multiple sclerosis
MSFC	Multiple Sclerosis Functional Composite
NEDA	no evidence of disease activity
Nrf2	nuclear factor erythroid 2-related factor 2
NTZ	natalizumab
PML	progressive multifocal leukoencephalopathy
PO	oral
PPMS	primary progressive multiple sclerosis
PRES	posterior reversible encephalopathy syndrome
pwMS	people with multiple sclerosis
pwRMS	people with relapsing multiple sclerosis
pwRRMS	people with relapsing-remitting multiple sclerosis
PY	patient-years
Q1	first quartile
Q3	third quartile
QoL	quality of life
QTc	corrected QT interval
RMS	relapsing multiple sclerosis
RRMS	relapsing-remitting multiple sclerosis
S1PR	sphingosine 1-phosphate receptor
SAE	serious adverse event
SC	subcutaneous
SPMS	secondary progressive multiple sclerosis
SSS	sick sinus syndrome
Th	T helper
TOP	Tysabri Observational Program
WOCBP	women of childbearing potential

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals I-IV.

I. Mustonen, T., Rauma, I., Hartikainen, P., Krüger, J., Niiranen, M., Selander, T., Simula, S., Remes, A.M., & Kuusisto, H. (2020). Risk factors for reactivation of clinical disease activity in multiple sclerosis after natalizumab cessation. *Multiple Sclerosis and Related Disorders*, *38*(May 2019), 101498. https://doi.org/10.1016/j.msard.2019.101498. *Reproduced with permission from Elsevier.*

II. Rauma, I., Huhtala, H., Soilu-Hänninen, M., & Kuusisto, H. Lipid Profile Alterations during Fingolimod Treatment in Multiple Sclerosis Patients. *Journal of Neuroimmune Pharmacology*, *15*(4), 567–569 (2020). https://doi.org/10.1007/s11481-020-09937-4. *Reproduced with permission from Springer Nature.*

III. Rauma I., Mustonen T., Seppä JM., Ukkonen M., Männikkö M., Verkkoniemi-Ahola A., Kartau M, Saarinen J.T., Luostarinen L., Simula S., Ryytty M., Ahmasalo R., Sipilä J.O.T., Pieninkeroinen I., Tapiola T., Remes A.M., Kuusisto H. (2022). Safety of alemtuzumab in a nationwide cohort of Finnish multiple sclerosis patients. *Journal of Neurology*, 269(2), 824-835. https://doi.org/10.1007/s00415-021-10664-w. *Reproduced under the terms of the Creative Commons CC BY 4.0 license.* (Creative Commons, n.d.)

IV. Rauma I., Viitala M., Atula S., Kuusisto H., Sipilä J.O.T., Ryytty M., Soilu-Hänninen M., Järvinen E. (2022) Finnish multiple sclerosis patients treated with cladribine tablets: a nationwide registry study. *Multiple Sclerosis and Related Disorders*, 61(January), 103755. https://doi.org/10.1016/j.msard.2022.103755. *Reproduced under the terms of the Creative Commons CC BY 4.0 license.* (Creative Commons, n.d.)

AUTHOR'S CONTRIBUTION

Ilkka Rauma is the first author of Studies II-IV and the corresponding author of Studies I-IV. The first authorship of Study I was shared between co-authors Tiina Mustonen and Ilkka Rauma, who contributed equally.

In Study I, Ilkka Rauma collected data from the patient information archives of Tampere University Hospital, participated in designing the statistical analyses together with Tiina Mustonen and Tuomas Selander while the statistical analyses were performed by Tiina Mustonen and Tuomas Selander, interpreted the study results, performed a literature search, wrote the first draft of the manuscript together with Tiina Mustonen, communicated with the research group and edited the manuscript according to the comments of co-authors, prepared the manuscript for submission, submitted the manuscript, coordinated the revision process and answered the reviewers' comments according to the comments of co-authors and applied for corrigendum after the name of one of the authors had been misspelt in the published version of the article.

In Study II, Ilkka Rauma collected data from the patient information archives of Kanta-Häme Central Hospital, designed the statistical analyses together with Heini Huhtala, performed some descriptive analyses using the study data while the primary analyses using a mixed effects model were performed by Heini Huhtala, interpreted the study results, performed a literature search, wrote the first draft of the manuscript, communicated with the research group and edited the manuscript according to the comments of co-authors, prepared the manuscript for submission, submitted the manuscript and revised the manuscript after the Editor-in-Chief requested it to be shortened to a Letter to the Editor.

In Study III, Ilkka Rauma developed the methodology for data acquisition, applied for institutional approvals in select hospital districts, coordinated actions of the study group, collected data from the patient information archives of Tampere University Hospital, Kanta-Häme Central Hospital and Turku University Hospital, assisted Marge Kartau and Auli Verkkoniemi-Ahola in collecting the data from the patient information archives of Helsinki University Hospital, designed the statistical analyses together with Heini Huhtala and Hanna Kuusisto, performed

descriptive analyses using the study data while the incidence rate calculations were performed by Heini Huhtala, interpreted the study results, performed a literature search, wrote the first draft of the manuscript, communicated with the research group and edited the manuscript according to the comments of co-authors, prepared the manuscript for submission, submitted the manuscript and revised the reviewed manuscript together with Anne Remes and Hanna Kuusisto.

In Study IV, Ilkka Rauma updated the Finnish MS registry using data from Tampere University Hospital prior to data extraction, participated in choosing the statistical methods, interpreted the study results, performed a literature search, wrote the first draft of the manuscript together with Matias Viitala and Elina Järvinen, edited the manuscript according to the comments of co-authors, prepared the manuscript for submission, submitted the manuscript and participated in answering the reviewers' comments and in revising the manuscript before final submission.

1 INTRODUCTION

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system (CNS) which causes disability especially in young adults (Dobson & Giovannoni, 2019; Frischer et al., 2009). The phenotypes of MS include clinically isolated syndrome (CIS), relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS) (Lublin et al., 2014). In addition to demyelination, which is the hallmark of the disease, the pathogenesis of MS includes neuroaxonal loss and CNS atrophy (Frohman et al., 2006; Lassmann, 2019; Trapp et al., 1998). Diagnostic evaluation is typically performed using clinical findings, magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) analysis (Thompson et al., 2018). Genetic and environmental risk factors have been identified but the cause of MS is still unclear (Nourbakhsh & Mowry, 2019).

As no cure exists for MS, people with multiple sclerosis (pwMS) are offered counselling, lifestyle modifications, rehabilitation and medical therapies. Disease-modifying therapies (DMTs) are drugs which aim to prevent clinical relapses and MS-related MRI lesions as well as reduce short term disability (McGinley et al., 2021). Early intensive therapy has been shown to be associated with better clinical outcomes in people with relapsing-remitting multiple sclerosis (pwRRMS) (Brown et al., 2019). However, treatment options for people with progressive forms of MS are currently limited to siponimod for SPMS and ocrelizumab for PPMS (European Medicines Agency, 2018, 2020a).

There are some special risks associated with the use of DMTs for MS, and safety monitoring is required for most therapies. Many of the currently available DMTs are relatively new and the treatment repertoire is constantly evolving. Therefore, evidence regarding the long-term safety of these therapies is needed. Treatment sequencing is another big question in the context of DMTs for MS. The discontinuation of certain DMTs has been associated with disease reactivation which sometimes exceeds the activity expected after treatment discontinuation (Barry et al., 2019; Prosperini et al., 2019). To mitigate the risk of such reactivation, better exit strategies are needed to guide treatment in high-risk individuals.

Choosing an optimal DMT for a person with MS requires careful consideration with regard to disease severity, disease activity, patient characteristics and comorbidities as well as drug safety and accessibility (Montalban et al., 2018). In order to provide pwMS the opportunity to participate in a shared decision-making process, healthcare practitioner (HCPs) need to have a good understanding about the pearls and pitfalls of each therapy. A subgroup of pwRRMS experience exceptionally high disease activity and require treatment using high-efficacy DMTs (Bowen, 2019). On the other hand, some of the highest efficacy therapies have been associated with serious adverse events (SAEs) and the safety of some of the newest therapies requires further investigation (European Medicines Agency, 2016, 2020c). Reducing medication-related harm is of utmost importance.

The purpose of this thesis was to investigate the safety of four DMTs used in the treatment of highly active relapsing-remitting multiple sclerosis (HARRMS) in Finland: natalizumab (NTZ), fingolimod (FNG), alemtuzumab (ALEM) and cladribine (CLAD) tablets (Multiple Sclerosis: Current Care Guidelines, 2020). NTZ and FNG are considered anti-trafficking therapies due to their mechanism of action, whereas ALEM and CLAD tablets are considered immune reconstitution therapies (IRTs) (Brinkmann et al., 2002; European Medicines Agency, 2009f, 2011, 2013b, 2017; Rice et al., 2005; Sellner & Rommer, 2020). The sphingosine 1-phosphate receptor (S1PR) modulator FNG causes lymphocyte sequestration into the lymphoid tissue but also has effects outside its target tissue. This has been thought to explain some of the adverse events (AEs) associated with its use (Camm et al., 2014). Whether the drug could also affect serum lipoproteins in pwMS has remained insufficiently explored.

The research data in this thesis is drawn from four individual studies focusing on the following aspects: I) risk factors for post-NTZ disease reactivation; II) lipid profile alterations during treatment with FNG; III) safety of ALEM and IV) clinical outcomes and treatment sequencing in patients treated with CLAD tablets. All studies were based on real-world data, which has certain advantages in terms of generalizability of results in contrast to clinical trials. The importance and originality of this thesis are that it explores both disease reactivation after discontinuation of anti-trafficking therapies and safety issues observed after the use of IRTs. This thesis will also generate fresh insight into the hypothesized association between lipoprotein levels and S1PR modulation.

2 REVIEW OF THE LITERATURE

2.1 Overview of multiple sclerosis

2.1.1 Clinical features and phenotypes

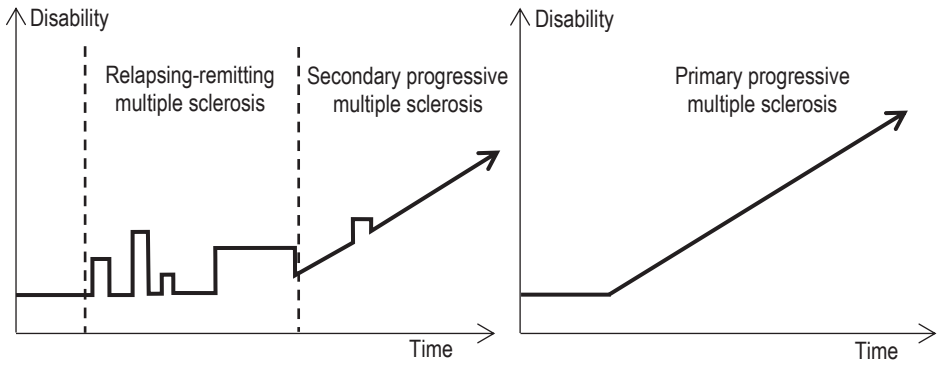
PwMS may present with various neurological symptoms or findings related to lesions localizing in the CNS. Typical clinical presentations of MS include optic neuritis, focal supratentorial syndromes, brainstem syndromes, cerebellar syndromes and spinal cord syndromes such as transverse myelitis (Miller et al., 2008; Thompson et al., 2018). In addition to clinical findings, subclinical deficits such as cognitive impairment may be attributable to MS (Compston & Coles, 2008). Regardless of disease course, MS may result in disability by inflicting cumulative lesions to the CNS over decades.

In pwMS, disability is accumulated due to either relapses or progression. MS relapses are the hallmark of relapsing forms of MS. A relapse is defined as a monophasic, acute or subacute onset clinical episode with patient-reported symptoms and objective findings typical of MS reflecting an inflammatory demyelinating event in the CNS (Thompson et al., 2018). The symptoms must last for at least 24 hours and be present in the absence of fever or infection (Thompson et al., 2018). It is important to be able to distinguish MS relapses from so called pseudo-relapses where the elevation of body temperature results in a temporary worsening of symptoms caused by pre-existing MS lesions (Bevan & Gelfand, 2015). Although most relapses are followed by at least partial recovery during the remission phase of the disease, incomplete recovery results in accumulation of disability referred to as relapse-associated worsening (Kappos et al., 2020). Progression independent of relapse activity, on the other hand, is characterized by a steadier accrual of disability between relapses or in the absence of relapses. It is commonly associated with progressing forms of MS (Kappos et al., 2020)

The clinical presentations of MS are heterogeneous. The first definitions for the clinical courses of the disease were described in 1996 (Lublin & Reingold, 1996). After the latest revision in 2013, the phenotypes of MS have been described as CIS, RRMS, SPMS and PPMS (Lublin et al., 2014). Disease activity and progression can be used as modifiers to the basic MS phenotypes. CIS is the first clinical presentation which has the characteristics of MS but has yet to fulfil the criteria for development of new lesions over time. The classic phenotype of MS is RRMS, in which symptoms manifest as clinical relapses followed by a recovery phase referred to as remission (Figure 1). The progressive phenotypes of MS include SPMS, in which progressive accumulation of disability occurs after an initial relapsing course, with or without relapses, and PPMS, in which accumulation of disability occurs without relapses from disease onset (Figure 1) (Lublin et al., 2014).

In a subgroup of pwMS, the level of disease activity is exceptionally high, and a high rate of disability worsening is seen. These patients require high-efficacy DMTs to prevent disability (Bowen, 2019). Certain factors have been shown to predict aggressive MS. These include demographic factors (male sex, onset after age 40, non-white race, smoking), relapse characteristics (number and interval of relapses, incomplete recovery, multifocal presentation), unfavourable symptoms (pyramidal, cerebellar, sphincter, cognitive), rapidly worsening disability, progressive disease course and CSF oligoclonal bands (Bowen, 2019).

Figure 1. The evolution of disability in different courses of multiple sclerosis.



2.1.2 Pathogenesis

The pathogenesis of MS is convoluted and includes both inflammation and neurodegeneration (Frischer et al., 2009). According to current understanding, the peripheral immune response of the acquired immune compartment begins with myelin antigen autoreactive cluster of differentiation (CD) 4 positive (CD4+) T lymphocytes in the periphery being activated by antigen-presenting dendritic cells (Hemmer & Selter, 2013). This occurs as a result to an unknown trigger, possibly due to 'molecular mimicry' or a process described as bystander activation (Fujinami & Oldstone, 1985; Geginat et al., 2017). The activated lymphocytes are able to cross the blood-brain barrier (BBB) into the CNS, where they become reactivated by antigen presenting cells such as dendritic cells, macrophages and B lymphocytes (Cencioni et al., 2021; Hemmer et al., 2002; Hemmer & Selter, 2013). The reactivated CD4+ T lymphocytes in the CNS produce pro-inflammatory cytokines which result in an increase in the permeability of the BBB, the recruitment of new peripheral immune cells and the activation of CNS resident microglia.

In MS, cytokines produced by the innate immune system skew the CD4+ T lymphocyte response towards pro-inflammatory lymphocyte subsets such as T helper (Th) 1 and Th17 cells as opposed to regulatory Th2 cells (Frohman et al., 2006; Hemmer et al., 2015). Although CD4+ T lymphocytes are involved in the initiation of the peripheral immune response in MS, they may actually have a lesser role in the effector stage of CNS inflammation (Lassmann, 2019). Lymphocytes and monocyte-derived macrophages recruited through the impaired BBB together with CNS resident microglia initiate the events which lead to the development of confluent demyelinated areas indicating a loss of oligodendrocytes and myelin sheaths. Together with astrocytic scarring, these changes produce the pathologic markers of MS: demyelinated plaques (Frohman et al., 2006; Nourbakhsh & Mowry, 2019).

It has been considered unlikely that the tissue injury in MS lesions is triggered by a direct interaction of lymphocytes with the target tissue, but rather induced by soluble factors (Lassmann, 2018). Demyelination in acute MS lesions may be due to an antibody-mediated reaction resulting in the phagocytosis of the myelin sheath (Frohman et al., 2006; Genain et al., 1999). Neuroaxonal damage is elicited through CD8+ T lymphocytes releasing perforin and granzyme as well as other mechanisms including glutaminergic excitotoxicity, cytokine release, hypoxia and oxidative stress (Frohman et al., 2006; HU et al., 1997; Lassmann, 2013; Neumann et al., 2002; Nourbakhsh & Mowry, 2019; Werner et al., 2001). Lately it has become

evident that B lymphocytes are also a major contributor to MS immunopathology, and therapies against the cell-surface antigen CD20 have been shown efficacious as DMTs for MS (Cencioni et al., 2021). In addition to the production of pathogenic autoantibodies by plasma cells differentiated from B lymphocytes, B lymphocytes may also present antigens for T lymphocytes, take part in immune cell regulation through the release of a range of cytokines and release toxic secretory factors (Cencioni et al., 2021). As demyelination and neuroaxonal damage continue, new CNS antigens become released and presented to T lymphocytes. This 'epitope spreading' may lead to the additional autoantigens becoming involved in the pathogenesis of MS (Vanderlugt & Miller, 2002).

The cellular mechanisms described above result in demyelination. Demyelination of axons leads to reduced conduction velocity, spontaneous discharges, increased mechanical sensitivity and ephaptic transmission, all of which may attribute to the symptoms of MS (Compston & Coles, 2008). Compensatory changes aimed at preserving conduction velocity come at the cost of increased energy demand and susceptibility to temperature changes (Compston & Coles, 2008). Oligodendrocyte precursor cells migrate to MS lesions and differentiate following tissue damage, which results in partial or complete remyelination after an attack (Compston & Coles, 2008; Frohman et al., 2006). Axonal loss results in irreversible neurological deficits, and the activation of astrocytes results in gliotic scarring (Frohman et al., 2006; Trapp et al., 1998). Although neuroaxonal loss has traditionally been associated with progressive disease, it has been shown to occur in early RRMS as well (Trapp et al., 1998).

The focal demyelinated lesions of MS typically accumulate in the periventricular white matter, occurring often at sites with high venous density and in the so-called 'watershed areas' of the brain (Haider et al., 2016). Lesions of the white matter include classical active lesions, chronic active or slowly expanding lesions, inactive lesions and remyelinated lesions, also referred to as 'shadow plaques' (Frischer et al., 2009; Lassmann, 2019). Classical active lesions are characterized by lymphocytic involvement together with the infiltration of macrophages containing myelin debris (Frischer et al., 2009; Kuhlmann et al., 2017). They are abundant in pwRRMS but rare in people with progressive MS (Kutzelnigg et al., 2005). Slowly expanding lesions are sometimes referred to as 'smouldering lesions' (Frischer et al., 2015; Kuhlmann et al., 2017). They are characterized by an inactive demyelinated lesion centre surrounded by a rim of activated microglia and infiltration of macrophages. Axonal injury is seen at the active edge of these slowly expanding lesions (Frischer et al., 2015). Inactive lesions are sharply demarcated hypocellular areas of

demyelination without inflammatory activity, and 'shadow plaques' represent remyelinated lesions (Frischer et al., 2015; Kuhlmann et al., 2017).

In addition to white matter lesions, grey matter demyelination is also seen in areas such as the cerebral cortex, the deep grey matter nuclei and the spinal cord (Cifelli et al., 2002; Gilmore et al., 2009; Kutzelnigg et al., 2005; Peterson et al., 2001). Subpial cortical lesions have a strong relation to meningeal inflammation, as they are adjacent to large follicle-like structures in the meninges and the perivascular spaces, most likely driving demyelination in the underlying cortex (Cencioni et al., 2021; Kutzelnigg et al., 2005; Lucchinetti et al., 2011; Magliozzi et al., 2007). Although cortical demyelination is a characteristic of progressive MS, it occurs already during early-stage RMS (Kutzelnigg et al., 2005; Lucchinetti et al., 2011).

The pathogenic mechanisms which drive progression in MS are not clear, and there is substantial overlap in pathological features between relapsing and progressive forms of MS (Lassmann, 2019). Brain atrophy, which is evident from the earliest stages of MS, seems to be an important determinant of disability in pwMS (Bergsland et al., 2012; Rudick et al., 2009). Studies have suggested that some form of compartmentalized inflammation driven by B lymphocytes and microglia occurs even in the setting of an intact BBB (Hemmer et al., 2015; Lassmann, 2019; Magliozzi et al., 2007). Furthermore, diffuse inflammation and axonal loss can be observed not only in active MS lesions, but also in the normal-appearing white matter in progressive MS (Kutzelnigg et al., 2005). Other involved mechanisms may include mitochondrial injury and oxidative stress, which are aggravated by mechanisms related to ageing and the accumulation of both disease and lesion burden (Haider et al., 2016; Mahad et al., 2015; Witte et al., 2014). Also, age-related iron accumulation in the human brain appears to have a role in increasing the susceptibility of the CNS to oxidative damage (Lassmann, 2013).

Of note, it has been under debate whether MS is truly a primary autoimmune disease or an immunological reaction to an underlying neurodegenerative disorder (Stys et al., 2012). It has been suggested that the disease may originate in the CNS, and the focal inflammatory events described earlier are actually an epiphenomenon to the primary neuroaxonal loss (Giovannoni et al., 2022). Although the primary trigger of MS is yet to be determined, inflammation seems to drive neurodegeneration in all stages of the disease (Frischer et al., 2009).

2.1.3 Risk factors

Studies investigating risk factors for MS have provided evidence that the pathogenesis of MS involves an interaction between genetic and environmental risk factors (Van Der Mei et al., 2016). The estimated heritability for MS was 19.2% in a recent genome-wide association study and 50% in a meta-analysis of eight twin studies (Fagnani et al., 2015; International Multiple Sclerosis Genetics Consortium, 2019). Of all genetic risk factors, the human leukocyte antigen (HLA) associated genetic variants are the strongest to affect the risk for MS. The variant with the strongest association with MS risk is the HLA class II variant HLA-DRB1*15:01, whereas the HLA class I variant HLA-A*02 is associated with a protective effect (Brynedal et al., 2007; Schmidt et al., 2007). Genome-wide association studies have detected over 200 independent genetic variants, which together with other suggestive variants, explain approximately 48% of the estimated genetic predisposition to MS (International Multiple Sclerosis Genetics Consortium, 2019).

Of the environmental factors which might have a role in the pathogenesis of MS, Epstein-Barr virus (EBV), a herpesvirus which resides in the B lymphocytes of an infected host, is the most promising candidate (Bjornevik et al., 2022; Thorley-Lawson, 2015). There is extensive evidence that EBV infection increases the risk for MS, but it has been challenging to establish a causal link. According to a meta-analysis of 18 studies, infectious mononucleosis results in a more than 2-fold increase in the risk for MS (relative risk 2.17) (Handel et al., 2010). Furthermore, in a recent study including a cohort of more than 10 million young adults, it was shown that EBV seroconversion greatly increases the risk for MS when compared to persistent EBV seronegativity (hazard ratio [HR] 32.4) (Bjornevik et al., 2022). This is the strongest known association between a suspected risk factor and the susceptibility to MS so far, and suggests that EBV has a major role in MS.

Other environmental factors which have been shown to increase the risk for MS include obesity in adolescence (Mokry et al., 2016; Munger et al., 2009), smoking (Degelman & Herman, 2017), vitamin D deficiency (Rhead et al., 2016), low sunlight exposure (Bäärnhielm et al., 2012) and shift work (Hedström et al., 2015). Sunlight exposure and vitamin D status have been shown to have independent roles in the risk for CNS demyelination (Lucas et al., 2011). Also, a Finnish maternity cohort study concluded that insufficient vitamin D intake during pregnancy may increase the risk for MS in the offspring (Munger et al., 2016). Certain environmental risk factors, such as smoking, EBV infection and obesity may interact with HLA risk variants and further increase the risk for MS (Olsson et

al., 2016). Smoking, in addition to being a risk factor for MS, may also be causally associated with disease progression in pwMS (Degelman & Herman, 2017).

2.1.4 Epidemiology and comorbidity

MS is the most common cause for non-traumatic neurological disability in young adults and has a female to male ratio of 2:1 (Dobson & Giovannoni, 2019; Walton et al., 2020). It has been estimated to affect approximately 2.8 million people worldwide, and the global prevalence of MS has increased in comparison to earlier estimates (Walton et al., 2020). In January 2022, there were approximately 11 000 pwMS included in the Finnish MS registry, in which most Finnish hospital districts are included (StellarQ, n.d.).

According to a widely accepted theory, a latitudinal increase exists in the incidence and prevalence of MS (S. Simpson et al., 2019). However, this theory has been questioned in North America and Europe (Koch-Henriksen & Sørensen, 2010). In Finland, there is currently no latitude gradient of MS, but regional differences have been observed with high prevalence of MS in the western and southwestern parts of the country (Pirttisalo et al., 2020).

PwMS use more healthcare services when compared to the general population and have increased mortality (Campbell et al., 2014; Manouchehrinia et al., 2016). The prevalence of cardiovascular, psychiatric, neurological and autoimmune comorbidities are increased in pwMS, while mixed results have been demonstrated with regard to the prevalence of metabolic conditions and malignancies (Hauer et al., 2021). The existence of comorbidities not only increases the risk of diagnostic delay, but also influences adversely some MS-related outcomes (Kappus et al., 2015; Kowalec et al., 2017; Tettey et al., 2016; Thormann et al., 2017; Tobin, 2019). As a result, a holistic and multidisciplinary approach has been suggested in the treatment of MS (Hauer et al., 2021).

2.1.5 Diagnostics

The diagnosis of MS is based on the combination of clinical and paraclinical evidence of CNS demyelination with dissemination in both space and time. The diagnostic criteria of MS have evolved from the Schumacher criteria in 1965 (Schumacher et al., 1965) to the most recent revisions of the McDonald criteria in 2017 (Tables 1 and 2) (Thompson et al., 2018). Dissemination in space refers to the

development of lesions in different CNS locations, whereas dissemination in time refers to the appearance of lesions over time (Thompson et al., 2018).

In addition to acquiring a history and performing a neurological examination, diagnostic tools such as MRI, CSF analysis, visual evoked potentials and optical coherence tomography can be utilized when MS is suspected. Brain and spinal cord MRI while using gadolinium (Gd) as a marker for active inflammation is recommended for establishing a diagnosis of MS (Wattjes et al., 2021). MRI is also used to monitor disease activity, treatment response and drug safety (Wattjes et al., 2021). Typical MS lesions are round or ovoid areas of focal hyperintensity on a T2-weighted or a proton density weighted sequence (Filippi et al., 2019). The threshold for performing a lumbar puncture when suspecting MS should be low, since CSF analysis not only contributes to the differential diagnosis, but according to the most current revisions of the McDonald criteria, the presence of CSF-specific oligoclonal bands can substitute for the requirement for demonstration of dissemination in time (Thompson et al., 2018). Visual evoked potentials or optical coherence tomography may be used as objective paraclinical evidence of optic neuritis, but only in patients presenting with symptoms or signs consistent with optic neuritis (Thompson et al., 2018).

The 2017 McDonald criteria include definitions for both RRMS and PPMS but lack a definition for SPMS (Thompson et al., 2018). Due to the nature of SPMS it is most often diagnosed retrospectively by a history of gradual worsening with or without relapses after an initial relapsing course of disease (Lublin et al., 2014). In recent years, criteria for SPMS based on clinical attributes have been suggested by at least two groups (Table 3) (Kopp et al., 2021; Lorscheider et al., 2016).

Additional evaluation is advised to avoid misdiagnosis of MS especially in patients with atypical and challenging presentations or in the presence of red flags (Miller et al., 2008; Solomon et al., 2019; Thompson et al., 2018). The differential diagnosis of MS includes other idiopathic inflammatory demyelinating diseases of the CNS as well as a myriad of infectious, inflammatory, malignant, genetic, metabolic, vascular and other diseases (Dobson & Giovannoni, 2019; Olek, 2021; Solomon et al., 2019). Table 4 summarizes the differential diagnosis of MS based on three existing review articles (Dobson & Giovannoni, 2019; Olek, 2021; Repovic, 2019).

Table 1. The 2017 McDonald criteria for relapsing-remitting multiple sclerosis.

The 2017 McDonald criteria for relapsing-remitting multiple sclerosis		
	Number of lesions with objective clinical evidence	Additional data needed for diagnosis
≥2 clinical attacks	≥2	None
≥2 clinical attacks	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location)	None
≥2 clinical attacks	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI
1 clinical attack	≥2	Dissemination in time demonstrated by an additional clinical attack or by MRI *OR* Demonstration of CSF-specific oligoclonal bands
1 clinical attack	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI *AND* Dissemination in time demonstrated by an additional clinical attack or by MRI *OR* Demonstration of CSF-specific oligoclonal bands

CNS, central nervous system; CSF, cerebrospinal fluid, MRI, magnetic resonance imaging. If the criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is multiple sclerosis.
Reprinted from The Lancet. Vol. 17, Thompson et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. 162-173. Copyright (2018) with permission from Elsevier.

Table 2. The 2017 McDonald criteria for primary progressive multiple sclerosis.

The 2017 McDonald criteria for primary progressive multiple sclerosis
1 year of disability progression (retrospectively or prospectively determined) independent of clinical relapse
AND
At least two of the following criteria
One or more T2-hyperintense lesions characteristic of multiple sclerosis in one or more of the following brain regions: periventricular; cortical or juxtacortical or infratentorial
Two or more T2-hyperintense lesions in the spinal cord
Presence of CSF-specific oligoclonal bands

CSF, cerebrospinal fluid. *Reprinted from The Lancet. Vol. 17, Thompson et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. 162-173. Copyright (2018) with permission from Elsevier.*

Table 3. Suggested criteria for secondary progressive multiple sclerosis.

MSBase criteria / Lorscheider criteria for secondary progressive multiple sclerosis (Lorscheider et al., 2016)
Disability progression in the absence of a relapse
1 EDSS step in patients with EDSS ≤5.5
OR
0.5 EDSS steps in patients with EDSS ≥6.0
A minimum EDSS score of 4.0
A pyramidal FS score of 2
Confirmed progression over ≥3 months including confirmation within the leading FS
Modified EXPAND criteria for secondary progressive multiple sclerosis (Kopp et al., 2021)
An EDSS from 3.0 to 6.5 (both inclusive) at index date ±6 months
Disability progression within the last two years in the absence of relapses six months prior to progression
≥1 EDSS step in patients with EDSS <6.0
OR
≥0.5 EDSS steps in patients with EDSS ≥6.0
A minimum EDSS score of 3.0 at time of progression
Confirmed progression over ≥6 months

EDSS, Expanded Disability Status Scale; FS, Functional System.

Table 4. Differential diagnosis of multiple sclerosis.

Other demyelinating diseases of the CNS
Acute disseminated encephalomyelitis (ADEM)
Chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids (CLIPPERS)
Myelin oligodendrocyte glycoprotein IgG-associated encephalomyelitis (MOG-EM)
Neuromyelitis optica spectrum disorder (NMOSD)
Other inflammatory or autoimmune diseases
Bickerstaff's encephalitis
Sarcoidosis
Sjögren's syndrome
Susac syndrome
Vasculitis
Infectious diseases
Brucellosis
Human immunodeficiency virus infection
Human T lymphotropic virus infection
Lyme disease
Syphilis
Genetic diseases
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)
Hereditary ataxias
Hereditary spastic paraplegia
Leber hereditary optic neuropathy
Leukodystrophies
Neoplasia
CNS lymphoma
Glioma
Metastatic disease
Paraneoplastic syndromes
Metabolic diseases
Copper deficiency
Tobacco-alcohol amblyopia
Vitamin B12 / folate deficiency
Vitamin E deficiency
Zink toxicity
Vascular disorders
Ischaemic events
Vascular malformations/fistula
Other
Migraine
Motor neuron disease
Radiation myelopathy
Somatoform disorders

CNS, central nervous system. A summary of data from three existing review articles. (Dobson & Giovannoni, 2019; Olek, 2021; Repovic, 2019).

2.1.6 Methods for quantifying disability

The Expanded Disability Status Scale (EDSS) is a widely used tool for quantifying disability in pwMS using a scale from 0 to 10 (Kurtzke, 1983; Piri Cinar & Guven Yorgun, 2018). An EDSS score 0 refers to a normal neurological examination, and an EDSS score 10 refers to death due to MS (Figure 2). The EDSS is calculated after assessing ambulation and seven distinct Functional Systems (FSs): visual; brainstem; pyramidal; cerebellar; sensory; bowel / bladder and cerebral. An EDSS score from 0 to 5.5 indicates ambulation without aid (Kurtzke, 1983; Piri Cinar & Guven Yorgun, 2018).

In addition to the EDSS, several other methods for quantifying disability in pwMS have also been developed, including tests for ambulation, upper extremity function and cognition (Piri Cinar & Guven Yorgun, 2018). The Multiple Sclerosis Functional Composite (MSFC) is a combination of tests including the assessment of ambulation (using the Timed 25-Foot Walk test [T25FW]), upper extremity function (using the 9 Hole Peg test) and cognition (using the Paced Auditory Serial Addition test [PASAT]) (Cutter, 1999). Both the EDSS and the MSFC have been used to quantify disability in pwMS in clinical trials (Cohen et al., 2010, 2012, 2019; Coles et al., 2012; Comi et al., 2019; Confavreux et al., 2014; Fox et al., 2012; Giovannoni et al., 2010; Hauser et al., 2017, 2020; Johnson et al., 1995; Kappos et al., 2010, 2018; Montalban et al., 2017; P. O'Connor et al., 2011; Polman et al., 2006).

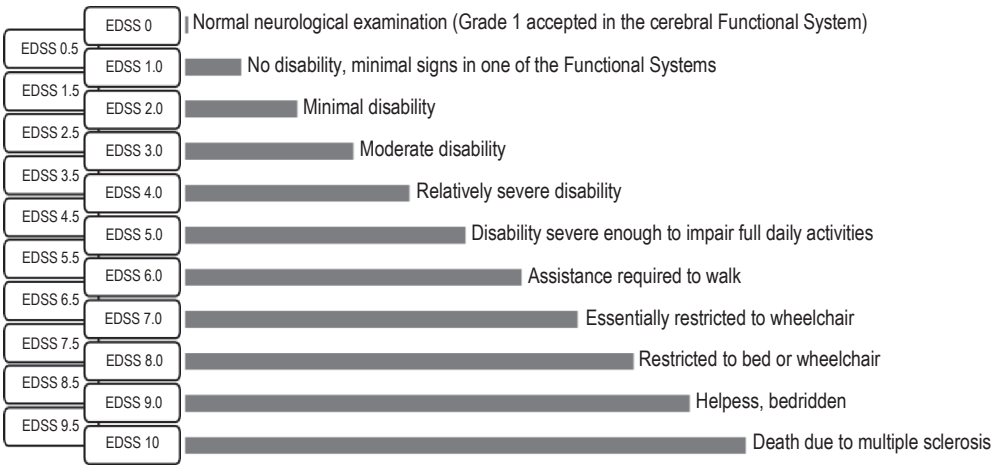


Figure 2. The Expanded Disability Status Scale (EDSS). For clarity, only the full steps have descriptions in this illustration. (Kurtzke, 1983).

2.2 Treatment of multiple sclerosis

2.2.1 Overview of treatment

Treatment of MS may include counselling, lifestyle modifications, rehabilitation and medical therapies. Medical therapies which aim to prevent disease activity and disability progression are called DMTs. They are reviewed more in detail under the subheading 2.2.2. In addition to DMTs, several medical therapies as well as nonpharmacological methods can be used in MS to provide symptomatic management (Tobin, 2019). Adequate information should be offered to pwMS during all stages of their disease. According to a Cochrane Review published in 2018, providing information to pwMS increases disease-related knowledge, but the results regarding decision making and quality of life (QoL) have been less clear (Köpke et al., 2018). Management of associated comorbidities is also important to improve QoL and MS-related outcomes (Tobin, 2019).

PwMS are encouraged to engage in both aerobic and strength-training exercise, but the intensity, duration and frequency of these activities should be considered in relation to each patient's symptoms, heat intolerance, strength and endurance (Narayan et al., 2021). Evidence about the effect of dietary interventions on MS-related outcomes is insufficient (Parks et al., 2020). Whether vitamin D should be offered to pwMS has been under debate. In a recent systematic review and meta-analysis, it was shown that vitamin D supplementation had no significant effect on relapse rate or disability in pwMS (Hanaei et al., 2021). Another systematic review focusing on mental health concluded that vitamin D supplementation may have a positive effect on the QoL in pwMS (Głąbska et al., 2021). In Finland, the 2020 Current Care Guidelines recommend vitamin D supplementation (50-100 μg daily) for pwMS who have low serum concentrations of vitamin D (Multiple Sclerosis: Current Care Guidelines, 2020).

The effectiveness of rehabilitation interventions for pwMS was evaluated in an overview of Cochrane Reviews in 2019 (Amatya et al., 2019). There was moderate-quality evidence that structured, multidisciplinary rehabilitation programmes and physical therapy improved functional outcomes and QoL in pwMS, and interventions that provide information improved patient knowledge. Also, there was low-quality evidence that neuropsychological interventions, symptom-management programs, whole-body vibration and telerehabilitation were beneficial (Amatya et al., 2019). According to another systematic review and meta-analysis,

mindfulness-based interventions improved mental well-being in pwMS (R. Simpson et al., 2019).

Acute MS relapses are typically treated with corticosteroids (Citterio et al., 2000). Both oral (PO) and intravenous (IV) formulations are used, and with the exception of insomnia associated with the PO route of administration, no clear-cut differences in the overall efficacy and safety have been observed between these two formulations (Lattanzi et al., 2017). For patients who cannot tolerate corticosteroids, adrenocorticotropic hormone is an option, although not in routine use (Citterio et al., 2000). In patients who suffer from acute relapses unresponsive to corticosteroids, plasma exchange or immunoadsorption may be considered (Bevan & Gelfand, 2015; Lipphardt et al., 2020). Off-label monotherapy with IV immunoglobulins has also been suggested for the treatment of acute MS relapses especially in patients in whom corticosteroids or plasma exchange are contraindicated (Elovaara et al., 2011).

2.2.2 Disease-modifying therapies

The DMTs approved by the European Medicines Agency (EMA) are presented in Table 5. In Finland, reimbursement for medicine expenses is currently available for CLAD, dimethyl fumarate, diroximel fumarate, FNG, glatiramer acetate, interferons, ofatumumab, ponesimod and teriflunomide (The Social Insurance Institution of Finland, n.d.). For medicines administered in hospitals (ALEM, mitoxantrone, NTZ and ocrelizumab) the expenses are included in the price of the hospital day. Currently, there is no reimbursement available for ozanimod or siponimod in Finland (The Social Insurance Institution of Finland, n.d.). Also, although some of the interferons have an EMA indication for high-risk CIS, reimbursement for this indication is currently not available in Finland (The Social Insurance Institution of Finland, n.d.). In addition to the DMTs approved by EMA, rituximab is sometimes used as an off-label drug for pwMS (Airas et al., 2020; Alping et al., 2016; Chisari et al., 2022).

DMTs aim to reduce the inflammatory activity of MS by affecting the cells of the peripheral immune system (Hemmer & Selter, 2013). This can be achieved for example through sequestration of lymphocytes, shifting the T helper lymphocyte balance from Th1 to Th2, interference with deoxyribonucleic acid synthesis, depletion of immune cells or changes in cytokine secretion patterns (Figure 3) (European Medicines Agency, 2009b, 2009c, 2016, 2017, 2018, 2020a, 2020b,

2021b, 2021c, 2021a, 2009a, 2009e, 2009f, 2011, 2013b, 2013a, 2014b, 2014a; McGinley et al., 2021). Most DMTs require ongoing therapy to maintain efficacy, but some require only a limited number of short courses which are followed by long-term changes in the immune system (Baker et al., 2017; Stuve et al., 2019). The term 'immune reconstitution therapy' (IRT) has been used to describe these therapies (Sellner & Rommer, 2020). A commonly used goal for DMT in people with relapsing multiple sclerosis (pwRMS) is the so-called no evidence of disease activity (NEDA) concept. The core features of NEDA include being relapse-free with no worsening of disability measured by the EDSS scale (NEDA-1 and -2), no new or enlarging T2-hyperintense lesions and no new Gd-enhancing lesions (NEDA-3) and no brain volume loss over a period of observation (NEDA-4) (Dobson & Giovannoni, 2019).

Clinical trials have demonstrated that DMTs reduce the level of inflammatory disease activity compared to placebo (Calabresi, Kieseier, et al., 2014; Confavreux et al., 2014; Ebers, 1998; Fox et al., 2012; Giovannoni et al., 2010; Johnson et al., 1995; Kappos et al., 2010; P. O'Connor et al., 2011; Polman et al., 2006; The IFNB Multiple Sclerosis Study Group, 1993) or active comparator in pwRMS (Cohen et al., 2010, 2012, 2019; Coles et al., 2012; Comi et al., 2019; Hauser et al., 2017, 2020). Also, a reduced risk of disability progression compared to placebo has been demonstrated in people with PPMS treated with ocrelizumab and people with SPMS treated with siponimod (Kappos et al., 2018; Montalban et al., 2017). Comparison between DMTs based on results from clinical trials is difficult due to differences in study protocols, patient populations and inclusion and exclusion criteria. Due to the limited number of direct head-to-head clinical trials, comparison between different DMTs has been performed using observational data and network meta-analyses (Cohen et al., 2010, 2012, 2019; Coles et al., 2012; Comi et al., 2019; Hauser et al., 2017, 2020; Tur et al., 2019). In a 2021 position statement of the Multiple Sclerosis Therapy Consensus Group, ALEM, CLAD, NTZ, ocrelizumab, ofatumumab and the S1PR modulators FNG, ozanimod and ponesimod were considered high-efficacy DMTs for RRMS (Wiendl et al., 2021). In the EU, an EMA indication for HARRMS is included in the Summary of Product Characteristics of ALEM, CLAD, FNG, mitoxantrone and NTZ (European Medicines Agency, 2009f, 2011, 2013b, 2016, 2017).

Generally, two alternative treatment strategies have been utilized: escalation and induction (Wiendl et al., 2021). Escalation refers to initiating treatment using a moderate-efficacy DMT and switching to a high-efficacy DMT if inflammatory disease activity persists, whereas induction refers to initiating treatment with a

high-efficacy DMT early in the disease course. The latter approach is also referred to as early intensive therapy. In a prospective cohort study of 1555 pwRRMS after propensity-score matching, it was shown that initial therapy with ALEM, FNG or NTZ was associated with a lower risk of conversion to SPMS when compared to initial therapy with glatiramer acetate or interferon beta (Brown et al., 2019). Randomized controlled trials aiming to compare escalation and induction strategies in pwRRMS are under way (ClinicalTrials.gov, 2018b, 2018a). Also, new agents are constantly being developed and evaluated. For example, Bruton's tyrosine kinase inhibitors, which target B cell driven inflammation and modulate cells of the innate immune system, are currently being investigated in clinical trials (García-Merino, 2021).

Choosing an optimal DMT for each patient is often far from straightforward. HCPs must consider disease severity and activity, patient characteristics and comorbidities, drug safety and accessibility (Montalban et al., 2018). In Finland, neurologists follow the Finnish Current Care Guidelines, which include recommendations on choosing DMTs for pwMS according to disease course and the assessed level of inflammatory disease activity (Multiple Sclerosis: Current Care Guidelines, 2020). After diagnosis, pwRRMS are categorized into having either 'active' or 'highly active' disease according to relapses and MRI lesions, and an induction approach is recommended for people with HARRMS (Table 6) (Multiple Sclerosis: Current Care Guidelines, 2020). Notably, according to these recommendations, ocrelizumab can be used in both active and highly active RRMS as well as active PPMS.

As always, safety and tolerability issues need to be considered when choosing DMTs for pwMS. Besides glatiramer acetate and ofatumumab, all current DMTs officially require some form of laboratory testing during treatment, and other forms of monitoring are also recommended for some DMTs (Table 5). Most DMTs are not recommended during pregnancy or breastfeeding, and some DMTs are even shown to be teratogenic (Table 5) (European Medicines Agency, 2011, 2013a, 2016, 2017, 2020b, 2020a, 2021b). However, all interferons and glatiramer acetate can be used during pregnancy and breastfeeding if clinically needed (Electronic medicines compendium, 2019; European Medicines Agency, 2009a, 2009e, 2009b, 2009c, 2014a). The most common AEs and notable risks and precautions for use for each DMT are summarized in Table 5. The safety profiles of the four DMTs on which this thesis focuses (NTZ, FNG, ALEM and CLAD) are reviewed more in detail under the subheadings 2.2.3-2.2.6.

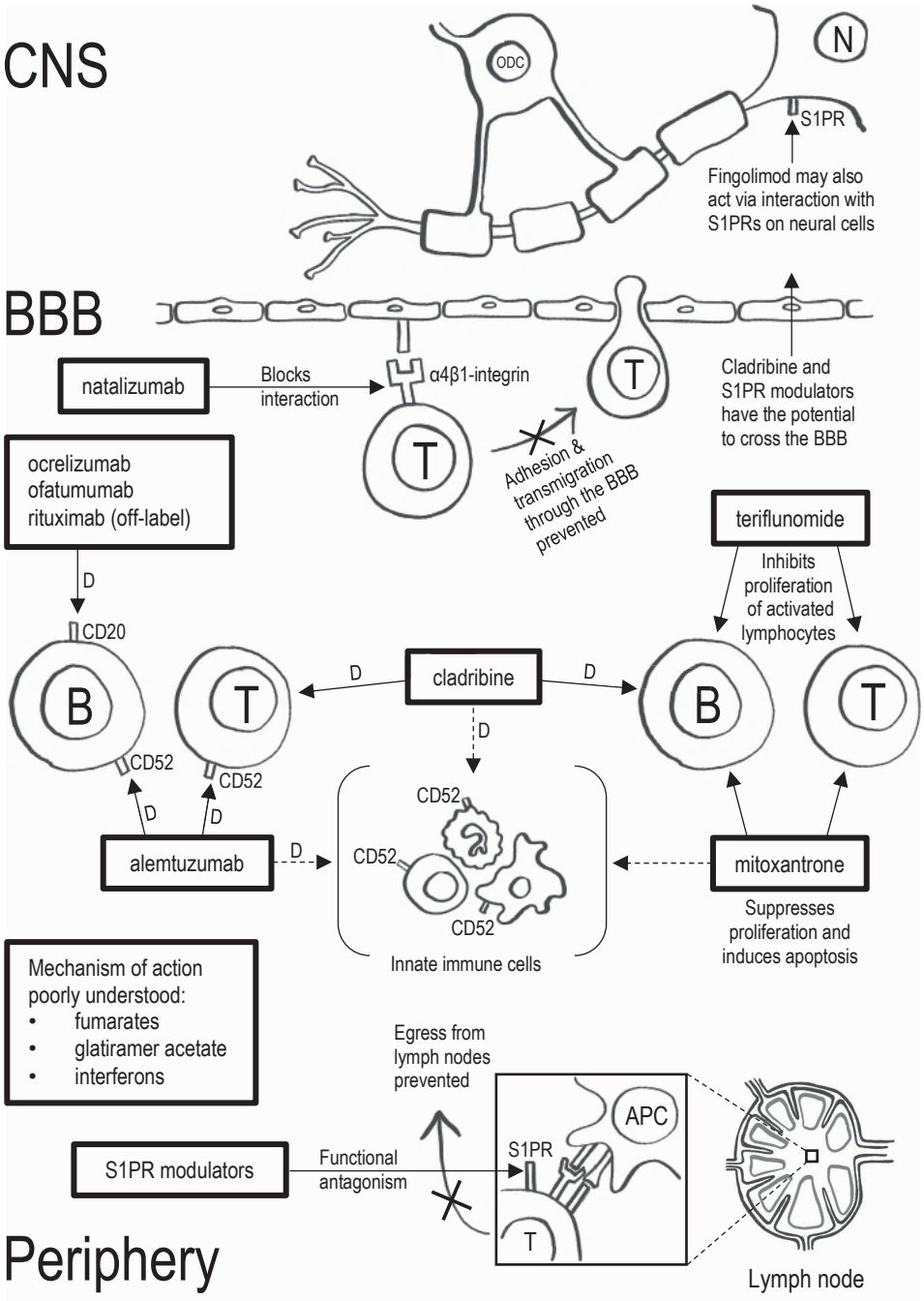


Figure 3. An illustration of the cells and/or receptors acting as the main target of disease-modifying therapies. Some therapies may have several mechanisms of action. APC, antigen-presenting cell; BBB, blood-brain barrier; CD, cluster of differentiation; CNS, central nervous system; D, depletion; N, neuron; ODC, oligodendrocyte; S1PR, sphingosine 1-phosphate receptor.

Table 5. Summary of disease-modifying therapies approved by the European Medicines Agency.

Therapy, route (reference)	EMA Indication	Mechanism of action	Most common adverse events	Contraindications	A summary of special warnings and precautions for use
Alemtuzumab, IV (European Medicines Agency, 2013b)	HARRMS despite a full and adequate course of treatment with at ≥1 disease-modifying therapy and rapidly evolving severe RRMS	CD52 antibody Immune cell depletion	IARs (headache, tachycardia, flushing, nausea, urticaria, rash, pruritus, pyrexia, fatigue, chills Infections (upper respiratory tract, urinary tract, herpetic) Thyroid disorders Leukopenia	Hypersensitivity Human immunodeficiency virus infection Active severe infection Uncontrolled hypertension Coagulopathy (or patients on anti-thrombotic therapy) History of any of the following: arterial dissection of the cervicocephalic arteries; stroke; angina pectoris; myocardial infarction Other concomitant autoimmune disease (besides multiple sclerosis)	Special risks: secondary autoimmune or immune system diseases, IARs, other serious reactions temporally associated with infusion, infections (including opportunistic infections and PML), changes in laboratory parameters (cytopenias), acute acalculous cholecystitis Precautions and monitoring: screening, vaccinations, premedication, contraception, anti-herpes prophylaxis, dietary changes at the time of infusions to prevent listeriosis, infusion-related monitoring, HPV testing, periodic laboratory testing until at least 48 months after the last infusion
Cladribine, PO, (European Medicines Agency, 2017)	HARRMS	Nucleoside analogue of deoxy-adenosine Immune cell depletion	Lymphopenia Herpes zoster	Hypersensitivity, Human immunodeficiency virus infection Active chronic infection Immunocompromised patients Active malignancy Moderate or severe renal impairment Pregnancy or breastfeeding	Special risks: opportunistic infections (*herpes zoster* in particular, PML has been reported for parenteral cladribine in patients treated for hairy cell leukaemia), malignancies, liver injury, changes in laboratory parameters (lymphopenia, liver enzymes), teratogenicity Precautions and monitoring: screening, vaccination, contraception (in males also), anti-herpes prophylaxis if severe lymphopenia occurs, irradiation of cellular blood components if blood transfusions are needed, laboratory testing before each course and at 2 and 6 months after each course

Table 5. Summary of disease-modifying therapies approved by the European Medicines Agency, *continued*.

Therapy, route (reference)	EMA Indication	Mechanism of action	Most common adverse events	Contraindications	A summary of special warnings and precautions for use
Dimethyl fumarate, PO (European Medicines Agency, 2014b)	RRMS	Activation of the Nrf2 transcriptional pathway Immuno-modulation	Flushing Gastrointestinal events (diarrhoea, nausea, abdominal pain) Ketones in urine	Hypersensitivity Suspected PML	Special risks: liver injury, infections (risk of herpetic infections and PML), flushing, anaphylactic reactions, Fanconi syndrome, changes in laboratory parameters (renal function, liver enzymes, lymphopenia) Precautions and monitoring: screening, contraception, discontinuation if prolonged severe lymphopenia occurs, suspending treatment should be considered if serious infection occurs, periodic laboratory testing
Diroximel fumarate, PO (European Medicines Agency, 2021c)	RRMS	Similar to dimethyl fumarate	Similar to dimethyl fumarate	Hypersensitivity Suspected PML	Special risks: expected to be similar to those reported for dimethyl fumarate even though not all of them have been observed specifically for diroximel fumarate Precautions and monitoring: similar to dimethyl fumarate
Fingolimod, PO (European Medicines Agency, 2011)	HARRMS and rapidly evolving severe RRMS	S1PR modulator Peripheral lymphocyte redistribution	Infections (sinusitis, influenza) Headache Cough Diarrhoea Back pain Elevation of liver enzymes	Hypersensitivity Immunodeficiency syndrome Patients with increased risk for opportunistic infections Active severe or chronic infection Active malignancy Severe liver impairment Certain cardiac or cerebrovascular conditions in the previous six months Severe cardiac arrhythmias treated with certain therapies QTc interval ≥500 msec During pregnancy and in WOCBP without effective contraception	Special risks: cardiorespiratory effects, infections (including opportunistic infections and PML), malignancies (mainly lymphomas and cutaneous malignancies), macular oedema, liver injury, PRES, relapses with tumefactive lesions, changes in laboratory parameters (liver enzymes, lymphopenia), teratogenicity, rebound risk Precautions and monitoring: screening, vaccinations, contraception, protection against sunlight, first dose monitoring, periodic laboratory testing and skin evaluations, regular blood pressure monitoring, ophthalmological evaluation

Table 5. Summary of disease-modifying therapies approved by the European Medicines Agency, *continued*.

Therapy, route (reference)	EMA Indication	Mechanism of action	Most common adverse events	Contraindications	A summary of special warnings and precautions for use
Glatiramer acetate, SC (Electronic medicines compendium, 2019)	RMS	Random polymer of four amino acids Presumably immuno-modulation	Infections (influenza) Anxiety Depression Headache Vasodilatation Nausea Rash Arthralgia Back pain Asthenia Chest pain Injection-site reactions Pain	Hypersensitivity	Special risks: injection-associated reactions, injection-site lipoatrophy, hypersensitivity, liver injury Precautions and monitoring: -
Interferons					
Interferon β-1a and β-1b, SC or IM (European Medicines Agency, 2009a, 2009b, 2009c, 2009e)	RMS and high-risk CIS	Cytokines Immuno-modulation	Flu-like symptoms Injection-site reactions Headache Pyrexia Chills Sweating Arthralgia Elevation of liver enzymes Cytopenias	Hypersensitivity Current severe depression and/or suicidal ideation Decompensated liver disease (only for interferon β-1b)	Special risks: depression and suicidal ideation, injection site reactions (including injection site necrosis), hypersensitivity reactions, pancreatitis, liver injury, thrombotic microangiopathy, nephrotic syndrome, thyroid abnormalities, changes in laboratory parameters (liver enzymes, cytopenias), neutralizing antibodies Precautions and monitoring: antipyretic analgesic may be administered, periodic laboratory testing

Table 5. Summary of disease-modifying therapies approved by the European Medicines Agency, *continued*.

Therapy, route (reference)	EMA Indication	Mechanism of action	Most common adverse events	Contraindications	A summary of special warnings and precautions for use
Pegylated interferon β-1a, SC or IM (European Medicines Agency, 2014a)	RRMS	Cytokine Immuno-modulation	Flu-like symptoms, Injection-site reactions Myalgia Arthralgia Pyrexia Chills Asthenia	Hypersensitivity Current severe depression and/or suicidal ideation	Special risks: depression and suicidal ideation, injection site reactions (including injection site necrosis), hypersensitivity reactions, liver injury, changes in laboratory parameters (liver enzymes, cytopenias), interferon beta class effects (nephrotic syndrome, thrombotic microangiopathy), neutralizing antibodies Precautions and monitoring: periodic laboratory testing
Mitoxantrone, IV (European Medicines Agency, 2016)	HARRMS with rapidly evolving disability and no other treatment options	Topoiso-merase inhibitor Immuno-suppression	Infections (urinary tract, upper respiratory tract) Nausea Alopecia Amenorrhea	Hypersensitivity Breastfeeding women Pregnant women	Special risks: cardiotoxicity, myelosuppression, infections, secondary acute myeloid leukaemia and myelodysplastic syndrome, changes in laboratory parameters (cytopenias, liver enzymes), potential carcinogenicity, teratogenicity Precautions and monitoring: screening, vaccinations, contraception (in males also), periodic laboratory testing and echocardiogram for up to 5 years after the end of treatment

Table 5. Summary of disease-modifying therapies approved by the European Medicines Agency, *continued*.

Therapy, route (reference)	EMA Indication	Mechanism of action	Most common adverse events	Contraindications	A summary of special warnings and precautions for use
Natalizumab, IV or SC (European Medicines Agency, 2009f)	HARRMS and rapidly evolving severe RRMS	α4β1 integrin antibody Prevention of immune cell migration into the CNS Inhibition of interaction between α4-expressing leukocytes and ligands in the CNS	Infections (nasopharyngitis, urinary tract) IARs Nausea Fatigue Dizziness Headache Arthralgia Safety profile of SC formulation was consistent with the IV formulation except for injection site pain	Hypersensitivity PML Patients with increased risk for opportunistic infections Combination with other disease-modifying therapies Active malignancy (except for cutaneous basal cell carcinoma)	Special risks: infections (PML and herpetic infections in particular), infusion-/injection-associated reactions and hypersensitivity, anti-drug antibodies, liver injury, thrombocytopenia (including immune thrombocytopenic purpura), special issues regarding treatment discontinuation Precautions and monitoring: screening, discontinuation should be considered if a woman becomes pregnant while using natalizumab, monitoring platelet counts is recommended in neonates born to women exposed to natalizumab during pregnancy, infusion-/injection-related monitoring, periodic laboratory testing (including JCV serology), special MRI monitoring for patients at higher risk of PML
Ocrelizumab, IV (European Medicines Agency, 2018)	RMS and PPMS	CD20 antibody Immune cell depletion	Infections (respiratory) IARs Decrease in blood immunoglobulins	Hypersensitivity Severely immunocompromised patients Active infection Active malignancy	Special risks: IARs and hypersensitivity, infections (including respiratory tract infections and herpetic infections, a risk of PML cannot be ruled out), late neutropenia, malignancies Precautions and monitoring: screening, vaccinations, premedication, contraception, infusion-related monitoring, periodic laboratory testing before each course and 3 months after each course

Table 5. Summary of disease-modifying therapies approved by the European Medicines Agency, *continued*.

Therapy, route (reference)	EMA Indication	Mechanism of action	Most common adverse events	Contraindications	A summary of special warnings and precautions for use
Ofatumumab, SC (European Medicines Agency, 2021a)	RMS	CD20 antibody Immune cell depletion	Infections (upper respiratory tract, urinary tract) Local and systemic injection reactions	Hypersensitivity Severely immunocompromised patients Severe active infection Active malignancy	Special risks: injection-associated reactions, infections (may increase susceptibility to infections, PML has been reported at higher doses in oncology indications) Precautions and monitoring: screening, vaccination, contraception, first dose under guidance of a HCP
Ozanimod, PO (European Medicines Agency, 2020b)	RRMS	S1PR modulator Peripheral lymphocyte redistribution	Infections (nasopharyngitis) Lymphopenia	Hypersensitivity Immunodeficient state Certain cardiac or cerebrovascular conditions in the previous six months Certain atrioventricular blocks or SSS unless patient has a pacemaker Active severe or chronic infection Active malignancy Severe liver impairment During pregnancy and in WOCBP without effective contraception	Special risks: cardiovascular effects, infections (including opportunistic infections and PML), cutaneous malignancies, macular oedema, PRES, changes in laboratory parameters (liver enzymes, lymphopenia), teratogenicity Precautions and monitoring: screening, vaccinations, contraception, ophthalmological evaluation in patients with an increased risk for macular oedema, protection against sunlight, first-dose monitoring in patients with certain cardiac conditions, periodic laboratory testing, regular blood pressure monitoring
Ponesimod, PO (European Medicines Agency, 2021b)	RMS	S1PR modulator Peripheral lymphocyte redistribution	Infections (upper respiratory tract) Elevation of liver enzymes	Hypersensitivity Immunodeficient state Certain cardiac or cerebrovascular conditions in the previous six months Certain atrioventricular blocks or SSS unless patient has a pacemaker Active severe or chronic infection Active malignancy Moderate or severe liver impairment During pregnancy and in WOCBP without effective contraception	Special risks: cardiorespiratory effects, infections (including opportunistic infections), macular oedema, liver injury, cutaneous malignancies, changes in laboratory parameters (liver enzymes, lymphopenia), teratogenicity Precautions and monitoring: screening, vaccinations, contraception, ophthalmological evaluation, protection against sunlight, first-dose monitoring in patients with certain cardiac conditions, periodic laboratory testing, regular blood pressure monitoring

Table 5. Summary of disease-modifying therapies approved by the European Medicines Agency, *continued*.

Therapy, route (reference)	EMA Indication	Mechanism of action	Most common adverse events	Contraindications	A summary of special warnings and precautions for use
Siponimod, PO (European Medicines Agency, 2020a)	SPMS	S1PR modulator Peripheral lymphocyte redistribution	Headache Hypertension Elevation of liver enzymes	Hypersensitivity Immunodeficiency syndrome History of PML or cryptococcal meningitis Active malignancy Severe liver impairment Certain cardiac or cerebrovascular conditions in the previous six months Certain atrioventricular or sinoatrial blocks or SSS unless patient has a pacemaker Homozygosity for CYP2C9*3 During pregnancy and in WOCBP without effective contraception	Special risks: cardiorespiratory effects, infections (including opportunistic infection), macular oedema, cutaneous malignancies, changes in laboratory parameters (liver enzymes, lymphopenia), teratogenicity Precautions and monitoring: screening (including CYP2C9 genotyping), vaccinations, contraception, first-dose monitoring in patients with certain cardiac conditions, periodic laboratory testing and skin evaluations, ophthalmological evaluation
Teriflunomide, PO (European Medicines Agency, 2013a)	RRMS	Inhibition of the mitochondrial enzyme dihydro-orotate dehydro-genase Immuno-modulation	Headache Diarrhoea Nausea Alopecia Elevation of liver enzymes	Hypersensitivity Severe liver impairment Severe immunodeficiency Significantly impaired bone marrow function or significant cytopenias Active severe infection Severe renal impairment and dialysis Severe hypoproteinaemia During pregnancy, breastfeeding, and in WOCBP without effective contraception if plasma levels above 0.02 mg/l	Special risks: elevation of blood pressure, liver injury, respiratory reactions, skin reactions, peripheral neuropathy, changes in laboratory parameters (liver enzymes, cytopenias), teratogenicity Precautions and monitoring: screening, contraception, suspending treatment should be considered if serious infection occurs, periodic laboratory testing and blood pressure monitoring Other: eliminated slowly from the plasma (an accelerated elimination procedure can be utilized)

CD, cluster of differentiation; CIS, clinically isolated syndrome; CYP, cytochrome P450; HARRMS, highly active relapsing-remitting multiple sclerosis; HCP, healthcare practitioner ; HPV, human papillomavirus; IAR, infusion-associated reaction; IM, intramuscular; IV, intravenous; JCV, John Cunningham virus; Nrf2, nuclear factor erythroid 2-related factor 2; PML, progressive multifocal leukoencephalopathy; PO, oral; PPMS, primary progressive multiple sclerosis; PRES, posterior reversible encephalopathy syndrome; QTc, corrected QT interval; RMS, relapsing multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; S1PR, sphingosine 1-phosphate receptor; SC, subcutaneous; SPMS, secondary progressive multiple sclerosis; SSS, sick sinus syndrome; WOCBP, women of childbearing potential.

Table 6. The 2020 Finnish Current Care Guidelines regarding the use of disease-modifying therapies for multiple sclerosis.

Guidelines on determining the level of inflammatory disease activity in people with RRMS

		Active	Highly active
Before initiation of disease-modifying therapy			
Relapses during the last 12 months		1	≥ 1 considering relapse severity
		AND	*AND*
T2-hyperintense MRI lesions		1-8	≥ 9
		OR	*OR*
T1 Gd-enhancing MRI lesions		1	≥ 1
	Stable	Active	Highly active
During disease-modifying therapy			
Relapses during the last 12 months	0	1	≥ 1 considering relapse severity
		OR	*AND*
T2-hyperintense MRI lesions	0	1-4	≥ 5
		OR	*OR*
T1 Gd-enhancing MRI lesions	0	1	≥ 1

Recommended therapies according to disease course and determined level of inflammatory disease activity

Active RRMS	Highly active RRMS
Interferon beta	Alemtuzumab
Dimethyl fumarate	Fingolimod
Glatiramer acetate	Cladribine
Ocrelizumab	Mitoxantrone
Teriflunomide	Natalizumab
	Ocrelizumab
Active PPMS	
Ocrelizumab	

Currently available therapies which were not included in the 2020 recommendations as they were not available in Finland during the time when these recommendations were made

Diroximel fumarate
Ofatumumab
Ozanimod
Ponesimod
Siponimod

Gd, gadolinium; MRI, magnetic resonance imaging; PPMS, primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis. (Multiple Sclerosis: Current Care Guidelines, 2020)

2.2.3 Safety of natalizumab

NTZ is a humanized monoclonal antibody of α4 integrin (Figure 3) (European Medicines Agency, 2009f). It prevents immune cell transmigration across the BBB into the CNS parenchyma (European Medicines Agency, 2009f; Rice et al., 2005). In addition to the IV formulation approved by EMA in 2006, a subcutaneous (SC) formulation is now available (European Medicines Agency, 2009f). Officially, NTZ is administered once every four weeks, although extended interval dosing (once every six weeks) has also been utilized to reduce the risk of progressive multifocal leukoencephalopathy (PML) in patients with anti-John Cunningham virus (JCV) antibodies (Ryerson et al., 2019). The official contraindications, most common AEs and special risks and precautions for use for NTZ are summarized in Table 5.

In the two-year phase 3 placebo-controlled trial of NTZ for pwRMS (AFFIRM), AEs which were significantly more frequent in the NTZ group included fatigue (27%) and allergic reactions (9%) (Polman et al., 2006). NTZ has also been studied as combination therapy with interferon beta-1a, and the AEs associated with the use of the combination therapy included anxiety (12%), pharyngitis (7%), sinus congestion (6%) and peripheral oedema (5%) during a two-year phase 3 trial (SENTINEL) (Rudick et al., 2006). In this trial, two patients were diagnosed with PML, resulting in a temporarily suspension of administration of NTZ in 2005. After the therapy was reintroduced and approved with a risk management plan, several observational studies as well as the Tysabri Observational Program (TOP) have assessed the safety of NTZ in the post marketing setting (Butzkueven et al., 2020; European Medicines Agency, 2009f; van Pesch et al., 2016). According to the 10-year interim analysis of TOP, the most common SAEs during a median follow-up of 5.2 years were infections and infestations (4.1%), with PML, pneumonia, urinary tract infection and herpes zoster being the most commonly reported serious infections (Butzkueven et al., 2020). Also, serious hypersensitivity reactions were reported in 0.7% of patients. In observational studies, SAEs have been reported in 0-6% of patients treated with NTZ (van Pesch et al., 2016). Another issue regarding NTZ use is the risk for development of anti-NTZ antibodies, which may have potential effects on the safety and efficacy of the drug (Polman et al., 2006).

PML is a rare and potentially fatal viral disease of the CNS caused by the reactivation of JCV during suppression of cell-mediated immunity (Kartau et al., 2019). In addition to NTZ, numerous drugs have been associated with cases of PML (Kartau et al., 2019). In a pooled analysis with data from four clinical trials,

the incidence of PML was 4.19 / 1000 patients after treatment with NTZ (Ho et al., 2017). Major risk factors for PML during NTZ therapy include the presence of anti-JCV antibodies, prior immunosuppression and long treatment duration (Vivekanandan et al., 2021). A risk stratification system has been established to minimize the risk of PML in patients receiving NTZ. This includes anti-JCV antibody testing every six months and intermittent MRI screening with the recommended screening interval dictated by the assessed risk of PML (European Medicines Agency, 2009f). Fear or risk of PML has been the most common reason for NTZ discontinuation in several studies (Prosperini et al., 2019).

Multiple studies have demonstrated that discontinuation of NTZ may result in a return of disease activity, which has been demonstrated to occur in 7-87% of patients, depending on the definition of reactivation (Prosperini et al., 2019). Even rebound activity, where inflammatory disease activity increases beyond pre-treatment levels, has been suggested, although there is considerable heterogeneity in the definition for this phenomenon (González-Suarez et al., 2017; Prosperini et al., 2019). It has been suggested that a high number of relapses and Gd-enhancing lesions prior to initiating NTZ, short exposure to NTZ, young age and the existence of comorbidities are associated with an increased risk for post-NTZ disease reactivation (Butzkueven et al., 2021; Iaffaldano et al., 2015; Prosperini et al., 2019). There are no established guidelines on switching to other DMTs from NTZ, but there is evidence that a subsequent DMT should be initiated within three months after NTZ discontinuation to reduce the risk of post-NTZ disease reactivation (Iaffaldano et al., 2015). In the Finnish Current Care Guidelines, a two-month washout is recommended (Multiple Sclerosis: Current Care Guidelines, 2020). According to existing literature, FNG has traditionally been suggested as a preferred subsequent DMT, although it is apparently less effective in this scenario than ALEM, ocrelizumab or off-label rituximab (Alping et al., 2016; Bigaut et al., 2022; Iaffaldano et al., 2015; Pfeuffer et al., 2019; Sellner & Rommer, 2019). More data about switching to the more recently approved DMTs is needed.

According to the Summary of Product Characteristics, the use of NTZ during pregnancy should be considered only if clearly needed, and breastfeeding should be discontinued during treatment with NTZ (European Medicines Agency, 2009f). The placental transport of immunoglobulins reaches its maximum during the third trimester, and newborns exposed to NTZ during that time may present with haematological abnormalities after delivery (Haghikia et al., 2014; Kane & Acquah, 2009). Recent recommendations have suggested that continuation of NTZ may be considered up to week 32 in people with HARRMS, while extended interval dosing

has been suggested to reduce exposure to the foetus (Canibaño et al., 2020; Wiendl et al., 2021). A recent small study has also suggested that NTZ might be safe during breastfeeding (Ciplea et al., 2020).

2.2.4 Safety of fingolimod

FNG acts as a functional antagonist of S1PRs and results in lymphocytes being sequestered inside the lymphoid tissue (Figure 3) (Brinkmann et al., 2002; Chun & Hartung, 2010). In addition to lymphocytes, S1PRs are found in other tissues as well, most importantly in the cardiovascular system, which explains some of the safety issues reviewed here. FNG is administered orally once daily, and first-dose monitoring is required due to risk of bradycardia or conduction block. The official contraindications, most common AEs and special risks and precautions for use for FNG are summarized in Table 5.

The efficacy and safety of FNG for RMS has initially been evaluated in two 24-month placebo-controlled phase 3 trials (FREEDOMS and FREEDOMS II) as well as in one 12-month trial using interferon beta-1a as an active comparator (TRANSFORMS) (Calabresi, Radue, et al., 2014; Cohen et al., 2010; Kappos et al., 2010). In the clinical trials, AEs that were related to FNG included infections, elevated liver enzymes, lymphopenia, hypertension, first-dose bradycardia or atrioventricular block, skin cancer and macular oedema (Calabresi, Radue, et al., 2014; Cohen et al., 2010; Kappos et al., 2010). Observational studies in the post marketing setting have demonstrated safety findings similar to the clinical trials, with SAEs occurring in 5-12.5% of patients during up to five years of follow-up (Biernacki et al., 2022; Ziemssen et al., 2022). According to these post marketing studies, infections are the most frequent AE during FNG therapy (20-33%).

Some risks regarding the use of FNG require special attention and precautions. By affecting S1PRs in atrial myocytes, FNG may result in changes in heart rate and can cause atrioventricular conduction block, which is why first-dose monitoring is required (Camm et al., 2014). Macular oedema, although rare in the clinical trials, needs to be ruled out with an ophthalmologic examination (Calabresi, Radue, et al., 2014; European Medicines Agency, 2011). PML, which is more typically associated with NTZ treatment, is also rarely associated with the use of FNG with an estimated risk of 0.069 / 1000 patients (Berger et al., 2018; Kartau et al., 2019). FNG is teratogenic and should not be used during pregnancy or breastfeeding

(European Medicines Agency, 2011). Contraception should be continued for two months after treatment discontinuation (European Medicines Agency, 2011).

Similar to NTZ, discontinuation of FNG has also been associated with reactivation of inflammatory disease activity, which may have variable clinical and radiologic presentations and is often referred to as 'rebound' in the literature (Barry et al., 2019). Recent cohort studies have demonstrated that about one third of patients experience relapses during a follow-up of 12 months after FNG discontinuation (Landi et al., 2022; Malpas et al., 2022). Potential prognostic factors for disease reactivation include young age, high annualized relapse rate (ARR) prior to discontinuation of FNG, switching to low-efficacy DMTs and delaying the initiation of a subsequent therapy beyond two months (Malpas et al., 2022). Data on choosing the optimal subsequent therapy are still limited but using ALEM and other cell-depleting agents as a subsequent therapy have been suggested. Conflicting results have been published on switching to ALEM, whereas off-label rituximab has shown a better clinical outcome when compared to switching to CLAD tablets (Ferraro et al., 2022; Frau et al., 2019; Nygaard et al., 2022; Pfeuffer et al., 2021). There has been concern about whether the efficacy of immune cell depleting agents could be reduced when initiated such therapies while the absolute lymphocyte count (ALC) is still recovering from the effect of FNG. However, this may not be the case, since the risk of relapse increases with the washout duration length when switching to immune cell depleting agents (Ferraro et al., 2022).

In the context of this thesis, it is also relevant to discuss the proposed association between FNG treatment and lipoprotein levels in pwMS. Preclinical studies have shown that FNG inhibits atherosclerosis in apolipoprotein E-deficient and low-density lipoprotein (LDL) receptor-deficient mice while on a high-cholesterol diet (Keul et al., 2007; Nofer et al., 2007). It is still unknown whether FNG reduces atherogenesis in humans, but an increase in high-density lipoprotein (HDL) level has been reported in a retrospective study including 29 patients treated with FNG (Blumenfeld Kan et al., 2019). Another earlier study aiming to evaluate lipid profiles in 26 FNG-treated patients was underpowered to give conclusive answers on whether FNG affects lipid levels (Hovi & Airas, 2016). It has been suggested that targeting the S1PR 3 ($S1P_3$) in particular might provide opportunities to develop new drugs against atherosclerosis, but more basic research on this subject is needed (Tölle et al., 2007).

2.2.5 Safety of alemtuzumab

ALEM is a humanized monoclonal antibody of the cell-surface antigen CD52 (Figure 3) (European Medicines Agency, 2013b). It is a pulsed IRT administered intravenously in two courses 12 months apart, although additional courses may be considered if clinically needed (European Medicines Agency, 2013b). Infusion of ALEM leads to depletion and repopulation of peripheral T and B lymphocytes, and the subsequent reconstitution of immune function has been thought to mediate the therapeutic effect of ALEM (Coles et al., 2008; Sellner & Rommer, 2020). The official contraindications, most common AEs and special risks and precautions for use for ALEM are summarized in Table 5. Prior to its approval for pwRRMS, ALEM was authorised for the treatment of patients with B-cell chronic lymphocytic leukaemia (European Medicines Agency, 2009d).

The efficacy and safety of ALEM in pwRRMS were initially demonstrated in three randomized controlled trials (Cohen et al., 2012; Coles et al., 2008, 2012). In the two phase 3 core trials (CARE-MS I and II), AEs associated with ALEM included infusion-associated reactions (IARs), infections and autoimmune disorders. The most common symptoms of an IAR were headache, rash, pyrexia and nausea (Cohen et al., 2012; Coles et al., 2012). A pre-treatment protocol is utilized during courses of ALEM to prevent IARs (European Medicines Agency, 2013b). The secondary autoimmune disorders reported in the clinical trials included thyroid disease, renal disease, ITP and autoimmune haemolytic anaemia, with thyroid disease being most common autoimmune AE (16-18% during the two-year trials) (Cohen et al., 2012; Coles et al., 2012). Extension studies of the core trials demonstrated similar AEs over six years of follow-up, with the incidence of infections peaking at year 1 (59.9%) and the incidence of thyroid AEs peaking at year 3 (16.2%) (Coles et al., 2021). The 5-year incidence of thyroid AEs reported after ALEM is 40-41% (Coles et al., 2017; Havrdova et al., 2017). The incidence of IARs is largest during the first course of ALEM (84.7%) and decreases with subsequent courses (40.0-68.8%) (Coles et al., 2021). There is limited data about the use of ALEM during pregnancy or breastfeeding, and effective contraception as well as discontinuation of breastfeeding are recommended for four months after each treatment course (European Medicines Agency, 2013b).

In the post marketing setting, rare but severe AEs have been reported in patients treated with ALEM, eventually resulting in a referral procedure initiated by the European Commission (European Medicines Agency, 2019). In the assessment report of the Pharmacovigilance Risk Assessment Committee, it was concluded

that ALEM causes secondary autoimmune diseases and serious immunological reactions such as haemophagocytic lymphohistiocytosis (European Medicines Agency, 2020c). Events which may be related to cytokine release such as acute coronary syndrome, cerebrovascular events, pulmonary haemorrhage and transient thrombocytopenia were identified as risks associated temporally with ALEM (European Medicines Agency, 2020c). Also, reactivation of EBV was considered related to ALEM (European Medicines Agency, 2020c). The final decision of the European Commission introduced new restrictions on the use of ALEM together with updated recommendations regarding monitoring (European Medicines Agency, 2020d). The current therapeutic indication effectively prevents the use of ALEM as a first-line therapy unless a patient has rapidly evolving severe RRMS (European Medicines Agency, 2013b). The updated contraindications of ALEM are presented in Table 5.

2.2.6 Safety of cladribine tablets

CLAD is a nucleoside analogue of deoxyadenosine which causes selective depletion of peripherally circulating T and B lymphocytes followed by immune cell repopulation (Figure 3) (European Medicines Agency, 2017). A greater effect is seen on B cells than T cells (Stuve et al., 2019). A SC formulation of CLAD has also been approved for the treatment of people with hairy cell leukaemia (European Medicines Agency, 2006). For clarity, the term "CLAD tablets" is used in this thesis to distinguish the PO formulation approved for the treatment of people with HARRMS from the SC formulation. Similar to ALEM, treatment with CLAD tablets is often referred to as IRT, as long-term therapeutic efficacy has been demonstrated after only two annual doses each divided into two separate courses (Sellner & Rommer, 2020). The official contraindications, most common AEs and special risks and precautions for use for CLAD tablets are summarized in Table 5.

In the initial placebo-controlled 96-week phase 3 trial (CLARITY), AEs which were more frequent in the CLAD groups included lymphopenia (21.6% in the 3.5 mg/kg group) and *herpes zoster* (1.8% in the 3.5 mg/kg group) (Giovannoni et al., 2010). The safety of treatment with CLAD tablets was further assessed in an integrated analysis of clinical trials, in which no new safety issues were reported, but periods of severe lymphopenia were shown to be associated with an increased frequency of infections (Cook et al., 2019). It has been under investigation whether

the use of CLAD tablets is associated with an increased risk for malignancy, especially since in the initial phase 3 clinical trial there was a numerical imbalance in the number of malignancies between CLAD tablets and placebo (Giovannoni et al., 2010). However, long-term safety data have shown that the rate of malignancies do not differ from the expected rate in a matched reference population (Leist et al., 2020).

In 2022, recommendations for liver function monitoring were introduced for the use of CLAD tablets due to cases of liver injury reported in the post marketing setting (European Medicines Agency, 2022). Also, skin reactions may develop after the use of CLAD tablets, as demonstrated by a real-world case series of 77 patients (Rolfes et al., 2021). CLAD tablets are contraindicated during pregnancy and breastfeeding, and women of childbearing potential (WOCBP) and males who could father a child need to be counselled on the potential risks to the foetus and the need for contraception for six months after the last dose (European Medicines Agency, 2017). Breastfeeding may be continued one week after the last dose.

3 AIMS OF THE STUDY

The purpose of this thesis was to investigate the safety of DMTs for HARRMS in Finland. The specific aims of each of the four studies were as followed:

Study I: To investigate risk-factors for clinical disease reactivation after discontinuation of NTZ.

Study II: To investigate lipid profile alterations during treatment with FNG in order to assess whether treatment with FNG may increase the risk for atherosclerosis.

Study III: To investigate the safety of ALEM especially with regard to SAEs.

Study IV: To investigate demographic details, clinical outcomes and treatment sequencing in patients treated with CLAD tablets.

4 PATIENTS AND METHODS

4.1 Setting

The research data in this thesis is drawn from four retrospective case series conducted during 2017-2021 (Table 7). The studies represent real-world data of pwMS treated and followed by neurologists in the public health care system of Finland. The geographical coverage of the studies ranged from covering two hospital districts in Study II to 18 hospital districts in Study III. In mainland Finland, there are currently 20 hospital districts in total, five of which include a university hospital. Ålands hälso- och sjukvård, which is the authority responsible for organizing healthcare in Åland, an autonomous region located in an archipelago southwest of Finland, was not included in any of the four studies.

Analyses were based on the secondary use of data recorded prospectively during clinical practice. Patients had received treatment during the 21st century, during which high-efficacy DMTs were already available in Finland but new therapeutic options were emerging rapidly. Of the four DMTs investigated in this thesis, three were already commercially available in 2017 when the project began (NTZ, FNG and ALEM). CLAD tablets became commercially available in Finland in 2018. Studies II-IV investigated data of patients while they were receiving the studied DMT, while Study I investigated data of patients after they had discontinued the studied DMT. Therefore, the contribution of this thesis is not restricted to issues of ongoing therapy, but also the period following treatment discontinuation.

Table 7. Characteristics of Studies I-IV.

	Study I	**Study II**	**Study III**	**Study IV**
Reference	(Mustonen et al., 2020)	(Rauma et al., 2020)	(Rauma, Mustonen, et al., 2022)	(Rauma, Viitala, et al., 2022)
DMT in focus	Natalizumab	Fingolimod	Alemtuzumab	Cladribine tablets
Study topic	Risk of disease reactivation after discontinuation	Lipid profile alterations	Safety	Efficacy, safety and treatment sequencing
Data source	Patient information systems	Patient information systems + The Finnish MS registry	Patient information systems	The Finnish MS registry
Data coverage	Four hospital districts including three university hospitals	Two hospital districts including one university hospital	18 hospital districts[a] including all five university hospitals	16 hospital districts including all five university hospitals
Timeframe when the DMT was either initiated or discontinued	2009–2016 (treatment discontinued)	2011–2015 (treatment initiated)	2013–2019 (treatment initiated)	2018–2020 (treatment initiated)
Year of final data extraction	2017	2018	2019	2021

DMT, disease-modifying therapy; MS, multiple sclerosis. [a]15 participating hospital districts and three non-participating hospital districts wherefrom patients had been referred to one of the participating hospital districts.

4.2 Inclusion and exclusion criteria

The inclusion and exclusion criteria in each study are presented in Table 8. In Study I, patients with a follow-up time shorter than 12 months were excluded in order to assess the incidence of reactivation at both six and 12 months (Mustonen et al., 2020). In Study II, patients were included if they had initiated FNG during the years when lipid profile monitoring had been applied to new patients (from 2011 to 2015). The four exclusion criteria presented in Table 8 were applied to minimize bias introduced by missing data, the use of lipid-lowering medication or previous treatment with FNG (Rauma et al., 2020). In Studies III and IV, a nationwide perspective was pursued, which is why no exclusion criteria were applied (Rauma, Mustonen, et al., 2022; Rauma, Viitala, et al., 2022).

Table 8. Inclusion and exclusion criteria in Studies I-IV.

Study I: Natalizumab (Mustonen et al., 2020)	
Inclusion criteria	A diagnosis of MS
	Previous treatment with natalizumab for MS (at least six consecutive infusions)
	Discontinuation of natalizumab (three months without any infusions at minimum)
Exclusion criteria	Less than 12 months of follow-up after the last infusion of natalizumab
Study II: Fingolimod (Rauma et al., 2020)	
Inclusion criteria	A diagnosis of MS
	Treatment with fingolimod for MS initiated between 2011 and 2015
Exclusion criteria	Less than two lipid profile measurements performed during treatment period
	No lipid profile measurements performed during the first year of treatment
	Use of lipid-lowering medication at the time of fingolimod initiation
	Previous use of fingolimod during the last 12 months before the studied period
Study III: Alemtuzumab (Rauma, Mustonen, et al., 2022)	
Inclusion criteria	A diagnosis of MS
	Treatment with alemtuzumab for MS
Exclusion criteria	None
Study IV: Cladribine tablets (Rauma, Viitala, et al., 2022)	
Inclusion criteria	A diagnosis of MS
	Treatment with cladribine tablets for MS initiated between 2018 and 2020
Exclusion criteria	None

MS, multiple sclerosis.

4.3 Collection of study data

Data were collected retrospectively from patient information systems of participating hospitals, the Finnish MS registry or both, depending on study (Table 7). The Finnish MS registry is a browser-based quality registry with the possibility for integration directly into the electronic patient information system of a participating hospital (Laakso et al., 2019; StellarQ, n.d.). The registry is used to aid decision making during clinical practice but can also be used for research purposes.

In Study I, data were collected from the electronic patient information systems and paper archives of four Finnish hospitals, including three university hospitals and one central hospital (Mustonen et al., 2020). Patients who had received NTZ for MS were identified. Each patient's records were reviewed systematically by the authors, and clinical attributes of those matching the inclusion criteria were manually entered into predesigned paper charts. The variables used in Study I are listed in Table 9.

Table 9. Variables used in Study I.

Variables in Study I (Mustonen et al., 2020)
Age
Sex category
Time of MS diagnosis
Onset symptom of MS
Course of disease
EDSS at diagnosis, initiation and discontinuation of natalizumab
MRI findings prior to initiating natalizumab
Previous and subsequent disease-modifying therapies
Medication names
Time of initiation and discontinuation
Washout period lengths
Use of natalizumab
Number of infusions
Time and primary reason for initiation and discontinuation
Adverse events during treatment
Relapses

EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; MS, multiple sclerosis.

In Study II, data were collected using the electronic patient information systems of two Finnish hospitals as well as the Finnish MS registry, demonstrating how hospital records can be used collectively with registry data to perform observational studies (Rauma et al., 2020; StellarQ, n.d.). Patients were identified using a diagnosis of MS and treatment with FNG as search criteria. In the Kanta-Häme Hospital District, data were extracted solely from the electronic patient information system of Kanta-Häme Central Hospital, whereas in The Hospital District of Southwest Finland, data were first extracted from the Finnish MS registry, after which the electronic patient information system of Turku University Hospital was used to complement data on whether study patients had used concomitant lipid-lowering medications. The variables used in Study II are listed in Table 10.

Table 10. Variables used in Study II.

Variables in Study II (Rauma et al., 2020)
Age
Sex category
Time of fingolimod initiation (and discontinuation, if applicable)
Time of lipid-lowering medication initiation, if applicable
Lipid profile measurements (values and dates)
Total cholesterol
High-density lipoprotein
Low-density lipoprotein
Triglycerides

In Study III, data were collected from the electronic patient information systems of 15 Finnish hospital districts where ALEM had been administered to pwMS. These included all five university hospitals in Finland as well as ten central hospitals (Rauma, Mustonen, et al., 2022). The final study data covered altogether 18 hospital districts, as in three non-participating hospital districts patients requiring ALEM had been referred to one of the participating hospital districts. In one of the two non-participating hospital districts, no patients had been treated with ALEM. In the other non-participating hospital districts, patients had been treated with ALEM, but data could not be collected due to difficulties in recruiting a local physician. Each patient's records were reviewed systematically by the authors and clinical attributes were entered into pre-designed spreadsheets. Unlike in Study I, where data were collected first into paper charts and then digitalized prior to data

analysis, a spreadsheet designed specifically for Study III was utilized to aid systematic data collection and ensure consistency of data. The variables used in Study III are listed in Table 11.

Table 11. Variables used in Study III.

Variables in Study III (Rauma, Mustonen, et al., 2022)
Age
Sex category
Time of MS diagnosis
Course of disease
Pre-existing comorbidities
Smoking status
Use of Vitamin D
EDSS at initiation of alemtuzumab
Previous and subsequent disease-modifying therapies
Medication names
Time of initiation and discontinuation
Use of alemtuzumab
Number of infusions
Time of infusions
Adverse events (event descriptions and timing)
Infusion-associated reactions
Other adverse events (autoimmune events, infections, malignancies, etc.)
Outcomes of adverse events
Impact on alemtuzumab therapy
Patient outcome
Interventions
Examinations

EDSS, Expanded Disability Status Scale; MS, multiple sclerosis.

In Study IV, data were collected from the Finnish MS registry (StellarQ, n.d.), which at the time of data extraction on 31st May 2021 included data from 16 hospital districts in Finland. Patients were identified using treatment with CLAD tablets as a search criterion. In the five hospital districts represented by the authors of Study IV, the Finnish MS registry was updated prior to data acquisition to reduce the impact of missing data. The variables used in Study IV are listed in Table 12.

Table 12. Variables used in Study IV.

Variables in Study IV (Rauma, Viitala, et al., 2022)
Age
Sex category
Time of MS diagnosis
Course of disease
Previous and subsequent disease-modifying therapies
Medication names
Time of discontinuation
Reason for discontinuation
Total number of previous therapies
Use of cladribine tablets
Time of initiation
Time of discontinuation, if applicable
Reason for discontinuation, if applicable
Clinical relapses
Annualized relapse rate
EDSS
Adverse events
Absolute lymphocyte counts

EDSS, Expanded Disability Status Scale; MS, multiple sclerosis.

4.4 Patient characteristics and demographic details

Table 13 summarizes the patient characteristics and demographic details of the four study samples. All cohorts were female predominant, as can be expected in data consisting of pwRMS (Walton et al., 2020). Reflecting the real-world setting, where DMTs are often used sequentially, most patients in Studies I, III and IV had used at least one previous DMT prior to the DMT in focus (data concerning previous DMT use was not assessed in Study II) (Mustonen et al., 2020; Rauma, Mustonen, et al., 2022; Rauma, Viitala, et al., 2022). Moreover, as the studies focused on different DMTs and had partially overlapping timeframes, it is possible that some patients are included in more than one of the four studies.

The study sample of Study I included data on 89 pwMS who had discontinued NTZ and had been followed for at least 12 months afterwards (Mustonen et al., 2020). They had received a mean number of 26.9 infusions of NTZ before discontinuation. The most common reason for discontinuing NTZ in the study sample was the risk of PML (57.7%).

The study sample of Study II included data on 72 pwMS who had initiated FNG and had been followed for a median 12 months since treatment initiation

(Rauma et al., 2020). Baseline characteristics of the study sample were limited to age and gender (Table 13). Altogether 328 lipid profile measurements were available in the study data. The baseline values of lipid concentrations are presented collectively with the study results under the subheading 5.2.

The study sample of Study III included data on 121 pwMS who had received treatment with ALEM and had been followed for a median 30.3 months since treatment initiation (Rauma, Mustonen, et al., 2022). Study III was the only study in this thesis which reported the incidence of smoking as a demographic detail. At least some amount of smoking was reported in 27 patients (22.3%) during ALEM therapy. However, underreporting may have occurred, since smoking status is often not systematically recorded in patient information archives.

The study sample of Study IV included data on altogether 179 pwMS who had initiated treatment with CLAD tablets and had been followed for a median 19.0 months since treatment initiation (Rauma, Viitala, et al., 2022). Interestingly, the female to male ratio in this study sample was rather high when compared to the study samples of Studies I-III (Table 13). In fact, the subgroup of treatment-naive patients in Study IV consisted almost exclusively of females (94.3%). Moreover, the proportion of treatment-naive patients in Study IV (29.6%) was somewhat larger than in studies I and III (14.6% and 17.4% respectively).

Table 13. A summary of patient characteristics, demographic details and follow-up times in Studies I-IV.

		Study I	Study II	Study III	Study IV
Reference		(Mustonen et al., 2020)	(Rauma et al., 2020)	(Rauma, Mustonen, et al., 2022)	(Rauma, Viitala, et al., 2022)
DMT in focus		Natalizumab	Fingolimod	Alemtuzumab	Cladribine tablets
Number of patients		89	72	121	179
Sex category					
Female	%	70.8	77.8	74.4	85.5
Male	%	29.2	22.2	25.6	14.5
Age					
at diagnosis	years, mean	30.4	-	26.6 (median)	29.6
at initiation of studied DMT	years, mean	36.0	40	32.0 (median)	35.9
Disease duration at initiation of studied DMT	years, mean	5.5	-	5.3 (median)	4.2 (median)
Course of disease					
RRMS	%	95.5	-	98.3	98.9
SPMS	%	4.5	-	1.7	1.1
EDSS at initiation of studied DMT	median	3.6 (mean)	-	3.0	2.0
Treatment-naive patients	%	14.6	-	17.4	29.6
Patients who had used previous DMTs	%	85.4	-	82.6	70.4
Follow-up time	years, median [Q1, Q2]	1.0[a]	1.0 [0.5, 2.4[b]]	2.5 [1.7, 3.5]	1.6 [1.0, 2.2]

DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS; secondary progressive multiple sclerosis; Q1 and Q3, quartiles. [a]In Study I, follow-up time was always 12 months. [b]Due to missing data, the third quartile for low-density lipoprotein was 2.3 years.

4.5 Study outcomes

In Study I, although all relapses were documented and reported, the primary outcome was 'clinical disease reactivation', defined as a corticosteroid-treated relapse within six or 12 months after NTZ discontinuation (Mustonen et al., 2020). As a secondary outcome, the study aimed to identify cases of rebound, defined in the study as an increase in the yearly number of all relapses during the year following NTZ discontinuation when compared to the year before NTZ initiation. In this analysis, the onset symptom of MS was also regarded as a relapse. Severity of relapses was not assessed due to limited EDSS data. Additionally, Study I reported AEs and reasons for treatment discontinuation.

In Study II, the longitudinal evolution of lipid parameters was evaluated to detect changes which could have the potential to adversely affect the risk of atherosclerosis (Rauma et al., 2020). The study outcomes included concentrations of total cholesterol, HDL, LDL and triglyceride. In the two hospital districts where data was acquired from, enzymatic colorimetric tests had been used to determine concentrations of total cholesterol, HDL and triglycerides. However, concentrations of LDL were determined differently at the two hospitals: the Friedewald method (Friedewald et al., 1972) was used in The Hospital District of Southwest Finland, and an enzymatic colorimetric test at Kanta-Häme Hospital District.

In Study III, the assessed outcomes included AEs and SAEs. All events were categorized into either IARs or other AEs (Rauma, Mustonen, et al., 2022). Other SAEs were further subcategorized into clinically meaningful groups: infections; secondary autoimmunity; hepatobiliary AEs; neoplasms; and unclassified AEs. To avoid data fragmentation, infections and cases of acute acalculous cholecystitis were analysed as other AEs regardless of their temporal connection to ALEM infusions. Similar to clinical studies, SAEs were defined as AEs which were life-threatening, resulting in death, requiring or prolonging hospitalization, disabling, resulting in a congenital anomaly or requiring intervention to prevent one of these outcomes (*ClinicalTrials.Gov*, n.d.).

In Study IV, both efficacy and safety outcomes were assessed (Rauma, Viitala, et al., 2022). Efficacy outcomes included clinical relapses and EDSS scores. Safety outcomes included AEs and ALCs. Reasons for treatment discontinuation were also reported, and treatment sequencing was investigated by categorizing patients according to the last preceding DMT.

4.6 Statistical methods

Study I used the occurrence of clinical relapses to measure disease activity after NTZ discontinuation (Mustonen et al., 2020). Univariate Cox regression model was used to identify individual risk factors for reactivation, after which the variables significantly affecting the risk for reactivation were re-assessed using multivariate Cox regression. In a post hoc analysis, the effect of subsequent DMTs on the risk for reactivation was assessed using univariate Cox regression with patient as a random effect. In this analysis, patients were stratified according to subsequent treatment strategy and the length of washout period. Cases of rebound, AEs and reasons for treatment discontinuation were only described on a patient level. Summary statistics were utilized to describe the study sample. Results from the regression analyses were displayed as HRs with 95% confidence intervals (CIs).

Study II analysed the longitudinal development of lipid concentrations in patients treated with FNG (Rauma et al., 2020). Each patient's lipid measurements were included from the initiation of FNG to either the last available measurement, discontinuation of FNG or initiation of a lipid-lowering medication, whichever was first. Two patients' last available LDL concentrations and one patient's last available HDL concentration were excluded due to changes having occurred in the methods for determining these lipid concentrations in 2017-2018. Baseline values were defined as 0 to 3 months before or up to 2 weeks after the first dose of FNG. If no eligible baseline measurements were available but a previous measurement from up to 3 months before the initiation of treatment was available, linear interpolation was used to determine what the values would have been at the exact time of treatment initiation. Data were visualized on a patient level using multiple line plots, and changes in lipid concentrations were analysed using a linear mixed-effects model with patient as a random effect. A mixed-effects model was chosen instead of repeated-measures ANOVA due to variance in the intervals between measurements and differences in the number of eligible measurements between patients. In addition to analysing the entire data, a separate analysis using only the first 12 months of follow-up was performed.

Study III was mostly descriptive in nature and included little statistical testing. Incidences were reported for IARs stratified according to treatment course. Incidence rates for other AEs were calculated until first event, and person-years at risk were used as denominator. The timing of an AE was defined as the day of symptom manifestation or when a HCP recognized the condition. Missing dates were imputed. In order to assess whether patients with a pre-existing autoimmune

disease were at risk for developing secondary autoimmunity, a Mantel-Haenszel-type method was used (Rauma, Mustonen, et al., 2022).

In Study IV, data regarding clinical outcomes were analysed using summary statistics, and treatment-sequencing was visualized using a Sankey diagram (Rauma, Viitala, et al., 2022). Clinically relevant subgroups were identified according to patients' previous DMT use. Subgroups of patients switching from the most common previous DMTs were compared in post hoc analyses. A more detailed description of the statistical methods used in Study IV is included in the published article.

4.7 Approvals and data management

As the studies in this thesis were observational and no patients were contacted during any stage, a research ethics committee approval or patient consent was not required. However, for Study I, an approval from the Research Ethics Committee of the Northern Savo Hospital District had been acquired. An institutional approval was obtained from each participating hospital wherefrom data was extracted. For Study IV, where data was extracted solely from the Finnish MS registry, permission from the Finnish National Institute for Health and Welfare was acquired. All studies were conducted according to Finnish legislation. The General Data Protection Regulation entered into force in 2016 and became applicable in Finland in 2018, affecting Studies III-IV. The Act on Secondary Use of Health and Social Data (552/2019) entered into force in 2019, affecting Study IV. Patient data was carefully protected throughout the studies, and anonymized or pseudo-anonymized data was used during the analyses. Management of study data was done by author Tiina Mustonen in Study I, author Ilkka Rauma in Studies II-III and StellarQ Ltd. in Study IV.

5 RESULTS

5.1 Clinical disease reactivation after natalizumab discontinuation (Study I)

After discontinuation of NTZ, clinical disease reactivation was documented in 20.2% and 30.3% of patients at six and 12 months respectively (Mustonen et al., 2020). For comparison, any kind of relapse was documented in 27.0% and 35.6% of patients at six and 12 months respectively (Mustonen et al., 2020). In the multivariate Cox regression model, two clinical biomarkers were shown to be associated with the risk for reactivation. First, the number of relapses during the year preceding NTZ initiation was associated with an increased risk for reactivation at both six months (HR 1.65, 95% CI 1.26-2.15, $p<0.001$) and 12 months (HR 1.54, 95% CI 1.21-1.96, $p<0.001$). Second, an EDSS score of 5.5 or higher at the time of NTZ initiation was associated with an increased risk for reactivation at six months (HR 3.70, 95% CI 1.23-11.15, $p=0.020$), while an EDSS score of 5.5 or higher at the time of NTZ discontinuation was associated with an increased risk for reactivation at 12 months (HR 2.63, 95% CI 1.12-6.20, $p=0.027$) (Table 14).

According to a univariate Cox regression model, the following factors were not associated with the risk for reactivation: sex category; age at NTZ initiation; time from diagnosis; number of infusions; and multifocal onset symptom when compared to other onset symptoms. Whether the primary reason for discontinuing NTZ affected the risk of reactivation was not statistically tested. Notably, 57.1% of patients discontinuing NTZ due to family planning and 43.8% of patients discontinuing NTZ due to inefficacy experienced at least one relapse during the first six months of follow-up (Table 1 in the original publication) (Mustonen et al., 2020).

A subsequent DMT had been initiated in 77.5% of patients during the 12 months following NTZ discontinuation. Additionally, 10.1% of the patients had received preventive high-dose corticosteroids within three months after discontinuation of NTZ. Most patients initiating a subsequent DMT after NTZ discontinuation did so after a washout period exceeding three months in length

(81.2% of all patients initiating DMTs) (Table 15). Of the 13 patients who had initiated a subsequent DMT within three months, only one (7.7%) experienced reactivation during the first six months of follow-up. However, in a post hoc analysis where patients were stratified according to subsequent therapies and washout period length, the risk of reactivation in patients initiating a subsequent DMT within three months after NTZ discontinuation was no different from patients who did not initiate any DMTs (HR 1.38, 95% CI 0.30-6.23, p=0.679 at six months and HR 1.74, 95% CI 0.55-5.50, p=0.349 at 12 months for first-line therapies; HR 0.00, 95% CI not calculable, p=0.984 at six months and HR 0.00, 95% CI not calculable, p=0.986 at 12 months for second-line therapies). Initiating a DMT after a washout period longer than three months was associated with an increased risk of reactivation at six months when compared to patients who did not initiate any DMTs during follow-up (Table 14).

Rebound was observed in 9.0% of patients in this cohort. These patients had used 2-3 previous DMTs before NTZ, had received a median number of 12 infusions (range 6-41) before discontinuation, and had a median EDSS score of 4.0 at both initiation (range 2.0-6.5) and discontinuation (range 1.5-7.0) of NTZ. The yearly number of all relapses in these patients increased from a median of two relapses during the year before NTZ initiation (range 0-5) to a median of three relapses during the year after NTZ discontinuation (range 2-7). The level of disability caused by these relapses was not assessed due to limited EDSS data.

AEs reported during the use of NTZ included fatigue (7.9%), skin symptoms (4.5%), nausea (3.4%), arrhythmia (3.4%), headache (2.2%) and fever (1.1%). NTZ was discontinued due to AEs in three patients (3.4%) (fatigue, fever and exacerbation of atopic dermatitis). No cases of PML were reported.

Table 14. The results of the statistical analyses of Study I.

	6 months HR (95 % CI)	12 months HR (95 % CI)
Univariate analysis		
No. of relapses during the year before initiation of NTZ	1.71 (1.29-2.27)***	1.66 (1.29-2.14)***
EDSS 5.5 or higher at the initiation of NTZ	2.84 (1.05-7.69)*	-
EDSS 5.5 or higher at the discontinuation of NTZ	3.64 (1.38-9.57)**	2.94 (1.28-6.72)*
Gender	-	-
Age at the initiation of NTZ	-	-
Time from diagnosis	-	-
Number of NTZ infusions	-	-
Multifocal onset symptoms	-	-
Multivariate analysis		
No. of relapses during the year before initiation of NTZ	1.65 (1.26-2.15)***	1.54 (1.21-1.96)***
EDSS 5.5 or higher at the initiation of NTZ	3.7 (1.23-11.15)*	-
EDSS 5.5 or higher at the discontinuation of NTZ	-	2.63 (1.12-6.20)*
The effect of subsequent DMTs (univariate Cox regression with patient as a random effect)		
First-line DMT 0-3 months of washout	-	-
First-line DMT >3 months of washout	7.69 (1.40-42.19)*	-
Second-line DMT 0-3 months of washout	-	-
Second-line DMT >3 months of washout	3.94 (1.11-14.08)*	-

DMT, disease-modifying therapy (in the original publication, the term 'disease-modifying drug' was used instead); NTZ, natalizumab; HR, hazard ratio; EDSS, Expanded Disability Status Scale. Hazard ratios and 95 % confidential intervals (95 % CI) are displayed for the statistically significant findings. For clarity, results with p>0.05 have been removed. *p<0.05, **p<0.01, ***p<0.001. *From the original publication, reproduced and modified with permission from Elsevier.* (Mustonen et al., 2020)

Table 15. The distribution of patients in Study I according to use of subsequent disease-modifying therapies and length of washout.

	No. of patients in group	Corticosteroid-treated relapse at 0-6 months, n (%)	Corticosteroid-treated relapse at 0-12 months, n (%)
No subsequent DMTs	20	4 (20.0 %)	8 (40.0 %)
DMT initiated after 0-3 months of washout	13	1 (7.7 %)	4 (30.8 %)
DMT initiated after >3 months of washout	56	13 (23.2 %)	15 (26.8 %)
All patients	89	18 (20.2 %)	27 (30.3 %)

DMT, disease-modifying therapy (in the original publication, the term 'disease-modifying drug' was used instead). The proportions of patients with corticosteroid-treated relapses at six and 12 months are presented within each group. *From the original publication, reproduced and modified with permission from Elsevier.* (Mustonen et al., 2020)

5.2 Lipid profile alterations during fingolimod treatment (Study II)

Mean lipid concentrations at FNG initiation in Study II are presented in Table 16 together with the results from the mixed-effects model. The mixed-effects model demonstrated a clinically small but statistically significant elevation of total cholesterol and HDL both during the first 12 months of follow-up and during the entire follow-up (Table 16) (Rauma et al., 2020). The coefficients reflecting the magnitude of this change were largest during the first 12 months of follow-up (Table 16). No statistically significant alterations of LDL or triglyceride concentrations were observed, although a positive trend was observed in LDL concentrations when examining the entire follow-up (coefficient=0.0064 mmol/L/month, p=0.053).

During the studied period, three patients (4.2%) initiated a lipid-lowering medication. These patients' lipid parameters were not examined beyond the initiation of lipid-lowering medication. Inter-individual variance was observed in the evolution of lipid parameters, as illustrated in Figure 4. Of note, a high concentration of serum triglycerides was observed in one patient at 30 months (6.4 mmol/L) (Figure 4).

Table 16. Baseline values of lipid parameters together with results from the mixed-effects model in Study II.

	Baseline values		Results from the mixed-effects model			
			First 12 months		Entire follow-up	
	Mean (mmol/L)	SD (mmol/L)	coefficient (mmol/L/month)	p	coefficient (mmol/L/month)	p
Total cholesterol	5.31	1.23	0.0332	0.004	0.0101	0.001
HDL	1.74	0.53	0.0138	<0.001	0.0034	0.002
LDL	3.00	1.08	0.0133	0.258	0.0064	0.053
Triglycerides	1.32	0.67	0.0133	0.259	0.0033	0.160

HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation. *Previously unpublished table containing data from Study II.*

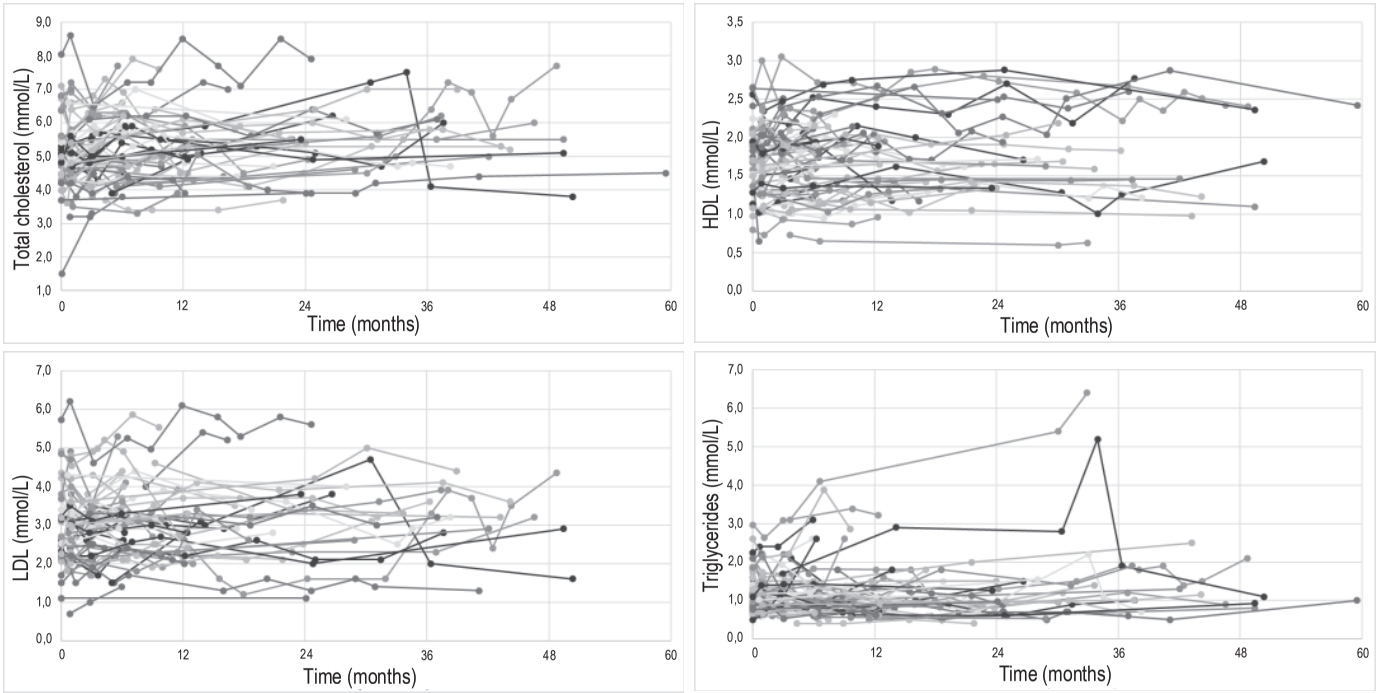


Figure 4. Evolution of lipid parameters after the initiation of fingolimod in Study II. HDL, high-density lipoprotein; LDL, low-density lipoprotein. *Previously unpublished figure containing data from Study II.*

5.3 Safety of alemtuzumab (Study III)

AEs were observed in 95.5% of patients who had received treatment with ALEM in Study III (Rauma, Mustonen, et al., 2022). Respectively, SAEs were observed in 32.3% of patients. Incidence rates of AEs of interest are presented in Table 17. The most frequent AE categories were IARs (90.1%), autoimmune AEs (30.6%) and infections (24.8%). Treatment with ALEM was discontinued in six patients (5.0 %) due to AEs and two patients (1.7%) due to lymphopenia (Table 18). Half of all ALEM discontinuations were due to hepatobiliary AEs. Of note, all AEs which resulted in treatment discontinuation were also SAEs. The deaths of two patients were reported (1.7%): one due to pneumonia with MS as the underlying cause of death; and another due to haemophagocytic lymphohistiocytosis.

Table 17. Incidence rates of adverse events of interest in Study III.

	Patients with event		Incidence rate
	n	%	number of events per 100 patient-years
Any AE	116	95.9	
Any IAR	109	90.1	
Any AE excluding IARs	65	53.7	33.1
Any AE leading to treatment discontinuation	6[a]	5.0	
Any serious AE	39	32.2	
Any serious IAR	15	12.4	
Any SAE excluding IARs	28	23.1	10.2
Death	2	1.7	0.6
Any infection event	30	24.8	11.8
Serious infection event	10	8.3	3.4
Any autoimmune AE	37	30.6	13.8
Autoimmune thyroid event	32	26.4	11.8
Serious autoimmune thyroid event	5	4.1	1.6
ITP	2	1.7	0.6
Acute acalculous cholecystitis	3	2.5	1.0
Malignant disease	4	3.3	1.3

AE, adverse event; IAR, infusion-associated reaction; ITP, immune thrombocytopenic purpura SAE, serious adverse event. [a]In addition, two patients (1.7 %) discontinued due to lymphopenia, which we did not regard as an adverse event. *From the original publication, reproduced under the terms of the Creative Commons Attribution 4.0 International License.* (Creative Commons, n.d.; Rauma, Mustonen, et al., 2022)

Table 18. Reasons for discontinuation of alemtuzumab in Study III.

	n
Adverse events resulting in discontinuation	
Acute acalculous cholecystitis	2
Hepatic or hepatobiliary reaction	2
Pulmonary reaction with oedema	1
Pyelonephritis	1
Other reasons for discontinuation	
Acute lymphopenia	1
Prolonged lymphopenia	1

5.3.1 Infusion-associated reactions after alemtuzumab

IARs were observed in 71.4% of treatment courses administered. They were most frequently observed during the first course of treatment (84.3%) and less frequently during subsequent courses (57.1-57.3%) (Table 19). The most common symptoms or findings during IARs were urticaria or rash, headache and hyperthermia or fever. Of all IARs observed in this cohort, 39% did not require any extra interventions, examinations, prolonged hospitalization or re-hospitalization.

Serious IARs were observed in 10.7% of patients during the first treatment course and 0-2.9% of patients on subsequent courses (Table 19). These included 16 serious IARs affecting altogether 15 patients (6.9% of all treatment courses administered). All serious IARs resulted in either prolongation of hospitalization or re-hospitalization after discharge. No IAR-related deaths were observed. Additional corrective or symptomatic medical therapy was administered in 62.5% of all serious IARs. A consultation to another medical specialty was performed after 12.5% of all serious IARs, and an outpatient visit was reported to have occurred after 18.8% of all serious IARs. Patients experiencing serious IARs received various symptomatic therapies, additional doses of corticosteroids and sometimes empiric antibiotics due to suspected infection. At least three serious IARs were managed by slowing or temporarily discontinuing the infusion, although this information was not systematically collected in the study.

None of the patients in this cohort had discontinued therapy due to an event classified as an IAR. However, some therapy was missed due to IARs in five

patients during the first course of therapy (4.2%) and in one patient during the second course of therapy (1.0%). Two cases of acute acalculous cholecystitis and one case of pyelonephritis resulted in discontinuation of ALEM during an ongoing treatment course. However, owing to study definitions, these AEs were not classified as IARs even though they occurred in close relation to administration of ALEM. Also, one of the two patients discontinuing therapy due to lymphopenia did so during a treatment course (Table 18).

Table 19. Incidences of infusion-associated reactions in Study III according to treatment course.

	1st course		2nd course		3rd course	
	n	%	n	%	n	%
Any infusion-associated reaction	102	84.3	59	57.3	4	57.1
Urticaria or rash	63	52.1	22	21.3	1	25.0
Headache	22	18.2	20	19.4	1	25.0
Hyperthermia or fever	18	14.9	13	12.6	2	50.0
Alterations in heart rate or palpitations	21	17.4	9	8.7	0	
Neurological symptoms	19	15.7	8	6.6	0	
Serious infusion-associated reaction	13	10.7	3	2.9	0	
Patients receiving alemtuzumab in each course	121		103		7	

Only the most frequently observed symptoms or findings are presented separately. *From the original publication, reproduced under the terms of the Creative Commons Attribution 4.0 International License.* (Creative Commons, n.d.; Rauma, Mustonen, et al., 2022)

5.3.2 Adverse events other than infusion-associated reactions after alemtuzumab

AEs excluding IARs were observed in 53.7% of patients with an incidence rate of 33.1/100 patient-years (PYs) (Table 17). Respectively, SAEs excluding IARs were observed in 23.1% of patients with an incidence rate of 10.2/100 PYs. All SAEs excluding IARs are listed in Table 20.

All autoimmune AEs are presented in Table 21. Hyperthyroidism was the most common autoimmune AE (20 patients, 6.9/100 PYs) as well as the most common autoimmune SAE (5 patients, 1.6/100 PYs). As shown in a visual illustration in Figure 5, the cumulative incidence of first autoimmune AE started to increase around the time of the second course of ALEM. Pre-existing autoimmune comorbidity was not associated with a higher risk of secondary autoimmunity (stratified rate ratio 1.03, 95% CI 0.40-2.64).

Herpes zoster reactivation was the most common infection (10 patients, 3.4/100 PYs) followed by *Herpes simplex* infection or reactivation (8 patients, 2.7/100 PYs) and pneumonia (5 patients, 1.6/100 PYs). The most common serious infection was pneumonia (4 patients, 1.3/100 PYs). As discussed earlier, one patient discontinued treatment due to pyelonephritis, and this interrupted an ongoing treatment course (Table 18).

Hepatobiliary AEs were reported in six patients, all of which were SAEs (acute acalculous cholecystitis, acute calculous cholecystitis and hepatic or hepatobiliary reactions). Hepatobiliary AEs were also the most common cause for treatment discontinuation (Table 18). Malignant neoplasms were reported in four patients and cervical dysplasia in one patient. Among unclassified AEs, three AEs were considered SAEs: ischemic stroke; pulmonary drug reaction; and sarcoidosis. The pulmonary drug reaction resulted in treatment discontinuation (Table 18).

Table 20. All serious adverse events excluding infusion-associated reactions in Study III.

	Number of patients with a serious event
Serious infections	
Pneumonia	4
Herpes Zoster reactivation	3
Pyelonephritis	2
Dental infection	1
Unspecified infection (strong suspicion of bacterial aetiology)	1
Serious autoimmune adverse events	
Autoimmune thyroid event	5
Haemophagocytic lymphohistiocytosis	1
Immune thrombocytopenic purpura	1
Thrombotic thrombocytopenic purpura	1
Type 1 diabetes	1
Serious hepatobiliary adverse events	
Acute acalculous cholecystitis	3
Acute calculous cholecystitis	1
Unspecified hepatic reaction	1
Unspecified hepatobiliary reaction	1
Serious neoplasms	
Breast cancer	2
Cervical cancer or carcinoma in situ	2
Cervical dysplasia	1
Unclassified serious adverse events	
Ischemic stroke	1
Pulmonary drug reaction with oedema	1
Sarcoidosis with pulmonary and renal manifestations	1

Previously unpublished table containing data from Study III.

Table 21. All autoimmune adverse events reported in Study III.

	Number of patients with	
	event	serious event
Autoimmune thyroid event	32	5
Immune thrombocytopenic purpura	2	1
Asthma	1	
Haemophagocytic lymphohistiocytosis	1	1
Psoriasis	1	
Thrombotic thrombocytopenic purpura	1	1
Type 1 diabetes	1	1
Vitiligo	1	

Previously unpublished table containing data from Study III.

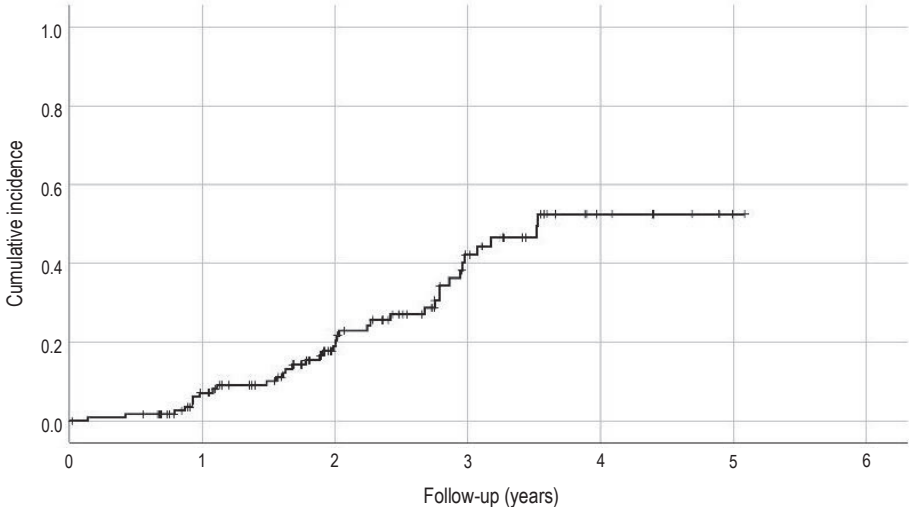


Figure 5. A survival curve displaying the cumulative incidence of first autoimmune adverse event in Study III. *From the original publication, reproduced under the terms of the Creative Commons Attribution 4.0 International License, text and numbers rewritten for clarity.* (Creative Commons, n.d.; Rauma, Mustonen, et al., 2022)

5.4 Clinical outcomes in patients treated with cladribine tablets (Study IV)

Our analysis demonstrated that a variety of different DMTs (Figure 6) had been used prior to initiating CLAD tablets, and 51.4% of the patients in Study IV had used ≥2 previous DMTs before CLAD tablets (Rauma, Viitala, et al., 2022). In contrast to patients with 0-1 previous DMTs, these patients were older, had longer disease duration, higher baseline EDSS score, lower baseline ARR and were more often relapse-free during the year before initiating CLAD tablets (Table 22). Also, two of these patients had SPMS as their disease course (2.2%). The most common reasons for discontinuing previous DMT were inefficacy (51.6%), AEs (25.4%) and the presence of anti-JC virus antibodies (7.1%). Treatment with CLAD tablets was discontinued in nine patients (5.0%), after which four patients (2.2%) initiated subsequent DMTs (Figure 6).

Mean ARR was 1.0 (SD 0.89) at baseline and 0.1 (SD 0.3) during follow-up. Median time to first relapse was 6.8 months (interquartile range 1.3-12.2) in patients with relapses during follow-up (n=25, 14.0%). Time to first relapse was shorter in patients with ≥2 previous DMTs in comparison to patients with 0-1 previous DMTs (Table 22, Figure 7). Mean ARR during follow-up was similar in both groups (Table 22).

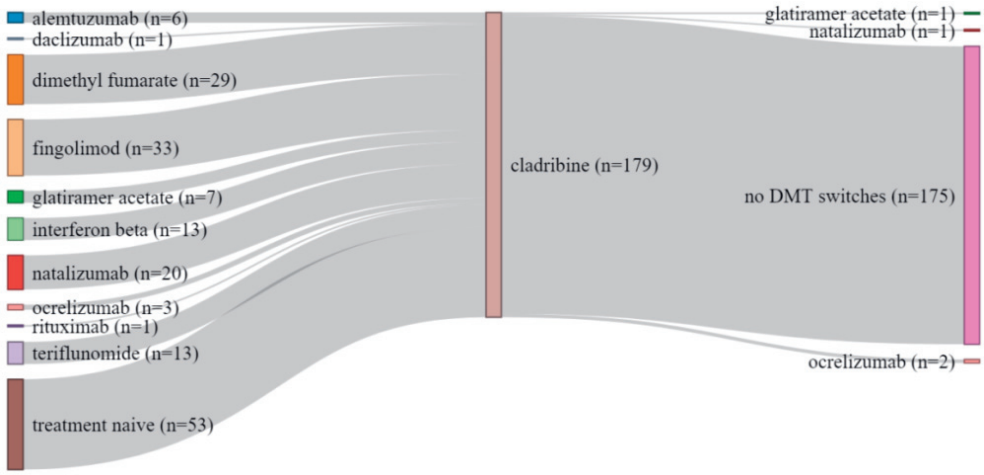


Figure 6. Treatment sequencing before and after cladribine tablets as demonstrated in Study IV. DMT, disease-modifying therapy. *From the original publication, reproduced under the terms of the Creative Commons Attribution 4.0 International License.* (Creative Commons, n.d.; Rauma, Viitala, et al., 2022)

Table 22. Patients stratified into two groups according to the number of previous disease-modifying therapies in Study IV.

	0-1 previous DMTs n = 87		2 or more previous DMTs n = 92		*p*
Before cladribine tablets initiation					
Sex category female, n (%)	76	(87.4)	77	(83.7)	0.550
Age at cladribine initiation, years, mean (SD)	33.5	(8.73)	38.2	(10.35)	0.007
Disease duration, years, median [Q1, Q3]	0.3	[0.2, 2.9]	9.5	[5.7, 14.0]	<0.001
Reason for discontinuing last preceding DMT, n (%)					
Inefficacy	26	(76.5)[a]	39	(42.4)	
Adverse events	4	(11.8)[a]	28	(30.4)	
JC virus	2	(5.9)[a]	7	(7.6)	
Patient's wish	1	(2.9)[a]	5	(5.4)	
Pregnancy	2	(5.9)[a]	3	(3.3)	
Alteration of disease course	1	(2.9)[a]	1	(1.1)	
Other or unknown	2	(5.9)[a]	12	(13)	
EDSS at baseline, median [Q1, Q3]	1.5	[1.0, 2.5]	2.0	[1.5, 3.5]	0.028
Relapses at baseline[b], n (%)					<0.001
No relapses	17	(19.5)	42	(45.7)	
1 relapse	36	(41.4)	35	(38.0)	
2 or more relapses	34	(39.1)	15	(16.3)	
ARR at baseline[b], mean (SD)	1.3	(0.89)	0.7	(0.81)	<0.001
After cladribine tablets initiation					
Relapses during follow-up, n (%)					0.031
No relapses	80	(92.0)	74	(80.4)	
1 relapse	7	(8.0)	11	(12.0)	
2 relapses	-		7	(7.6)	
ARR at follow-up, mean (SD)					
at entire follow-up	0.1	(0.18)	0.2	(0.38)	0.063
at 0-12 mo	0.1	(0.2)	0.2	(0.5)	0.063
at 12-24 mo	0.0	(0.2)	0.1	(0.3)	0.550
Time to first relapse[c], months, median [Q1, Q3]	11.4	[8.7, 13.1]	2.5	[0.6, 9.3]	0.013
Number of patients discontinuing cladribine, n (%)	2	(2.3)	7	(7.6)	0.208

ARR, annualized relapse rate; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; Q1 and Q3, quartiles; SD, standard deviation. [a]In the group of patients with 0-1 previous DMTs, the proportion displayed here is relative to patients with one previous DMT (n=34) and not the whole group. [b]During the last 12 months before initiation of cladribine tablets. [c]In patients with relapses during follow-up. *From the original publication, reproduced under the terms of the Creative Commons Attribution 4.0 International License.* (Creative Commons, n.d.; Rauma, Viitala, et al., 2022)

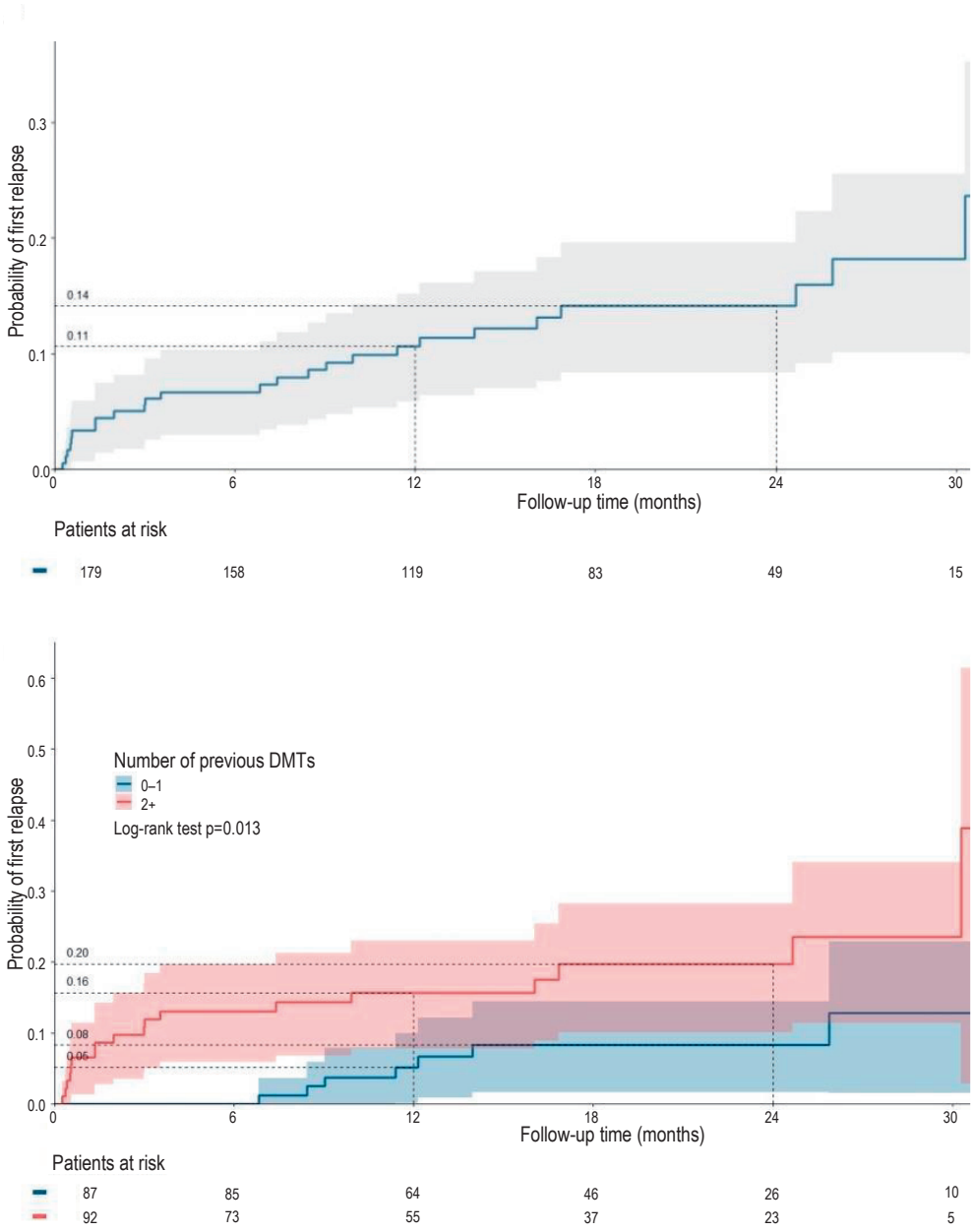


Figure 7. A cumulative events curve displaying probability of first relapse (and 95% confidence intervals) during follow-up in Study IV: a) all patients, b) according to the number of previous disease-modifying therapies (DMTs). *From the original publication, reproduced under the terms of the Creative Commons Attribution 4.0 International License, text and numbers rewritten for clarity.* (Creative Commons, n.d.; Rauma, Viitala, et al., 2022)

Stratification according to last previous DMT showed that patients who switched from FNG had a particularly short median time to first relapse when compared to patients switching from platform therapies (glatiramer acetate, interferon beta and teriflunomide), dimethyl fumarate or NTZ (Table 23). It was hypothesized that the difference in time to first relapse between the two main subgroups in this study ('≥2 previous DMTs' and '0-1 previous DMTs') was driven by early relapses in patients switching from FNG, since a majority of patients switching from FNG had used ≥2 previous DMTs. To test this theory, a post hoc analysis was performed, in which all patients switching from FNG to CLAD tablets were excluded. In this analysis, no statistically significant difference was observable in time to first relapse between patients with ≥2 previous DMTs and 0-1 previous DMTs (Figure 8, p=0.252).

Furthermore, data regarding relapses prior to initiation of CLAD tablets demonstrated that nine out of 33 patients switching from FNG (27.3%) experienced relapses during their washout period. In contrast, only one out of 20 patients switching from NTZ (5.0%) experienced relapses during their washout period, despite the median washout period lengths being similar among these two subgroups (Table 23). In a further analysis from which the results were not included in the final published manuscript of Study IV, the first six months after the discontinuation of a preceding DMT were analysed regardless of the timing of CLAD initiation (as opposed to only analysing the washout periods). In this analysis, relapses were observed in 42.2% of patients switching from FNG and 10.0% of patients switching from NTZ during the first six months following discontinuation. This further highlights the differences between these two groups.

AEs were reported in 16.8% of patients after receiving treatment with CLAD tablets (Table 24). The most common AE was headache (n=14, 7.8%). One fatality due to cardiac arrest was reported (0.6%). Lymphopenia, which is related to the mode of action of cladribine tablets, was reported in 124 patients out of the 166 patients from whom ALCs were available (74.7%). No grade IV lymphopenia (ALC <0.2 x 109/L) was reported. Interestingly, 42 patients (25.3%) had normal ALCs during follow-up. In these patients, mean ARR during follow-up was in line with the results from the total cohort (mean ARR 0.1, SD 0.3).

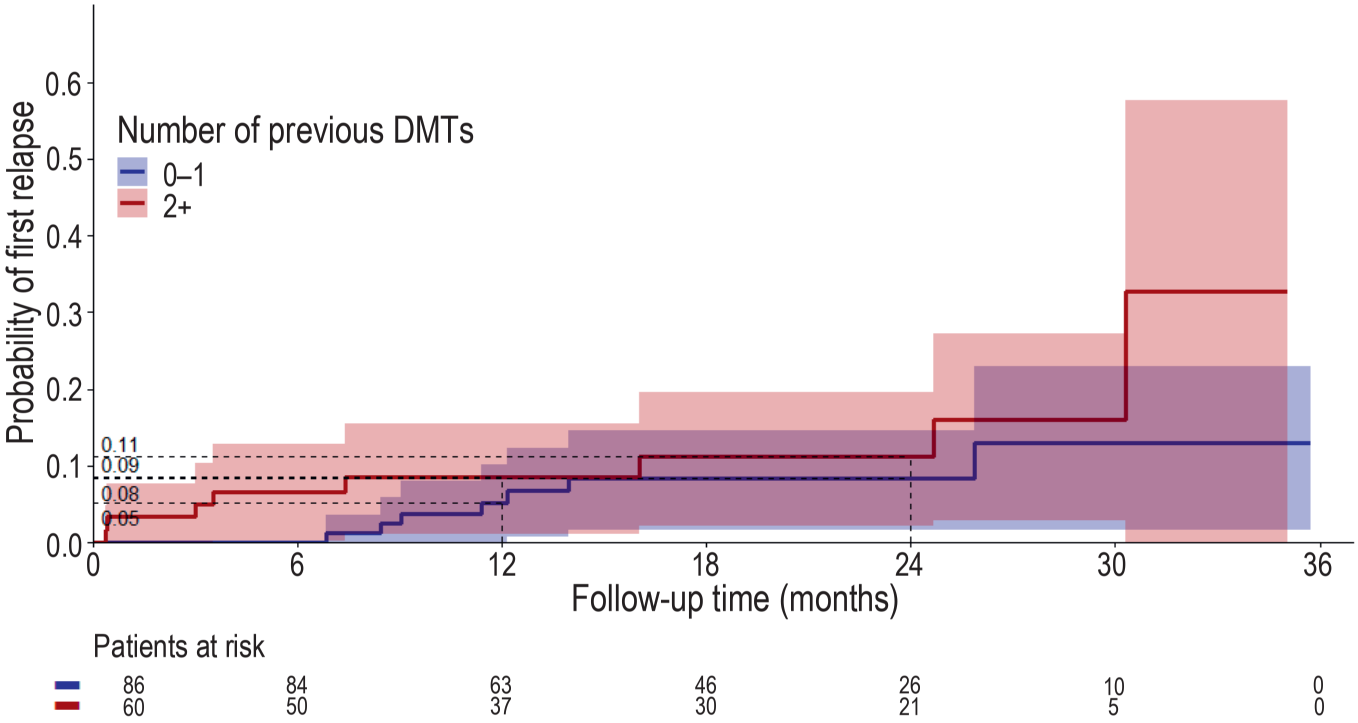


Figure 8. A cumulative events curve displaying probability of first relapse (and 95% confidence intervals) in Study IV after removing patients switching from fingolimod. DMT, disease-modifying therapy. *Previously unpublished figure containing data from Study IV.*

Table 23. Patient switching from the most common previous disease-modifying therapies as well as treatment-naive patients in Study IV.

Last preceding DMT before cladribine tablets	Platform therapies[a] (n=33)		Dimethyl fumarate (n=29)		Fingolimod (n=33)		Natalizumab (n=20)		Treatment-naive (n=53)	
Before cladribine tablets initiation										
Sex category female, n (%)	27	(81.8)	22	(75.9)	27	(81.8)	17	(85.0)	50	(94.3)
Disease duration, years, median [Q1, Q3]	7.2	[3.1, 10.1]	3.5	[2.3, 10.4]	11.2	[5.9, 13.8]	7.9	[4.6, 14.7]	0.2	[0.1, 0.3]
Previous history of at least two DMTs, n (%)	22	(66.7)	15	(51.7)	32	(96.7)	13	(65.0)	-	
Washout from previous DMT, months, median [Q1, Q3]	1.6	[0.7, 3.0]	0.9	[0.2, 2.1]	3.1	[2.2, 4.8]	3.1	[1.3, 6.3]	-	
Patients with relapses during washout[b], n (%)	1	(3.0)	1	(3.4)	9	(27.3)	1	(5.0)	-	
Relapses at baseline[c], n (%)										
No relapses	11	(33.3)	12	(41.4)	13	(39.4)	15	(75.0)	2	(3.8)
1 relapse	17	(51.5)	11	(37.9)	13	(39.4)	5	(25.0)	20	(37.7)
2 or more relapses	5	(15.2)	6	(20.7)	7	(21.2)	-		31	(58.5)
ARR at baseline[c], mean (SD)	0.8	(0.76)	0.8	(0.77)	0.9	(0.89)	0.2	(0.44)	1.7	(0.77)
After cladribine tablets initiation										
Relapses during follow-up, n (%)										
No relapses	30	(90.9)	25	(86.2)	23	(69.7)	17	(85.0)	49	(92.5)
1 relapse	3	(9.1)	2	(6.9)	7	(21.2)	1	(5.0)	4	(7.5)
2 relapses	-		2	(6.9)	3	(9.1)	2	(10.0)	-	
ARR at entire follow-up, mean (SD)	0.1	(0.22)	0.2	(0.41)	0.2	(0.37)	0.2	(0.42)	0.1	(0.20)
Time to first relapse[d], months, median [Q1, Q3]	6.8	[3.6, 15.8]	5.7	[2.4, 10.3]	1.3	[0.6, 2.7]	7.4	[5.5, 16.6]	11.8	[10.8, 12.6]
Patients discontinuing cladribine tablets, n (%)	-		1	(3.4)	6	(18.2)	1	(5.0)	1	(1.9)

ARR, annualized relapse rate; DMT, disease-modifying therapy; Q1 and Q3, quartiles. Patients switching from alemtuzumab (n=6), daclizumab (n=1), ocrelizumab (n=3) and rituximab (n=1) are not shown. Null values are not shown. [a]Glatiramer acetate (n=7), interferons (n=13) and teriflunomide (n=13). [b]During the first six months of any washout period. [c]During the last 12 months before cladribine tablets. [d]In patients with relapses. *From the original publication, reproduced under the terms of the Creative Commons Attribution 4.0 International License and edited in order to fit on a single page, specific rows removed. For the full table, please refer to the original article.* (Creative Commons, n.d.; Rauma, Viitala, et al., 2022)

Table 24. Adverse events reported during follow-up in Study IV.

	Patients with event, n (%)	
Any adverse event	30	(16.8)
Infections and infestations		
Herpetic skin infection	8	(4.5)
Herpes simplex	7	(3.9)
Herpes zoster	1	
Other skin or mucocutaneous infection (abscess, furunculus, ringworm, tinea pedis)	7	(3.9)
Upper respiratory infection	5	(2.8)
Unspecified infection	1	
Urinary tract infection	1	
Vaginal infection	1	
Nervous system disorders		
Headache	14	(7.8)
Dysesthesia	1	
Gastrointestinal disorders		
Nausea	7	(3.9)
Abdominal pain	2	(1.1)
Diarrhoea	2	(1.1)
Dyspepsia	1	
Skin disorders		
Alopecia	2	(1.1)
Acne	1	
Dermatitis	1	
General disorders		
Malaise	2	(1.1)
Musculoskeletal disorders		
Back pain	2	(1.1)
Cardiovascular disorders		
Cardiac arrest	1	
Hepatobiliary disorders		
Hepatitis, aetiology not reported	1	
Psychiatric disorders		
Insomnia	1	

For clarity, proportions are not shown in categories with only 1 patient (0.6%). *From the original publication, reproduced and modified under the terms of the Creative Commons Attribution 4.0 International License, footnotes have been included in the table.* (Creative Commons, n.d.; Rauma, Viitala, et al., 2022)

6 DISCUSSION

This thesis investigated the clinical use of four different DMTs for HARRMS in Finland. The research data is drawn from four real-world case series which, unlike clinical trials, are not limited by rigorous inclusion and exclusion criteria.

First, we aimed to investigate disease reactivation in patients discontinuing NTZ. In Studies I and IV, we observed that clinical relapses occurred in 10-27% of patients during the first six months after discontinuing NTZ. As shown in Study IV, clinical disease reactivation was also observed in patients discontinuing FNG. Our results support the current understanding that this phenomenon may be a special concern when discontinuing DMTs, which act via lymphocyte sequestration (so-called anti-trafficking therapies) and the risk should ideally be discussed with the patient already when initiating such therapies. The risk factors identified in Study I may be utilized when planning exit strategies for patients discontinuing NTZ.

Study II aimed to assess whether unfavourable lipid profile alterations could be observed during treatment with FNG. It is particularly important to reduce the risk of atherosclerosis in pwMS due to their increased risk for cardiovascular comorbidity (Hauer et al., 2021). Interestingly, we observed a modest increase in total cholesterol and HDL during follow-up. Although the results of Study II were deemed clinically insignificant in terms of overall cardiovascular risk, the results suggest that further studies should explore whether or not the more recently approved S1PR modulators may affect lipoprotein concentrations in pwMS.

Studies III and IV investigated the safety of ALEM and CLAD tablets, both of which are considered IRTs. As they are both relatively new therapies, there is a need for real-world studies to supplement data from earlier clinical trials and their extensions. The concept of IRT has been promising in the sense that patients treated with these therapies may not require ongoing treatment after a limited number of courses. However, the safety of this approach still needs to be assessed in a real-world setting. The results presented in our two case series provide patients and neurologists with new information about the risks associated with the use of ALEM and CLAD tablets in Finland.

6.1 Disease reactivation after discontinuation of anti-trafficking therapies (Studies I and IV)

Several studies have reported reactivation of clinical or radiological disease activity soon after discontinuation of NTZ or FNG, sometimes with catastrophic consequences (Barry et al., 2019; Butzkueven et al., 2021; González-Suarez et al., 2017; Iaffaldano et al., 2015; Kerbrat et al., 2011; Landi et al., 2022; Lo Re et al., 2015; Malpas et al., 2022; Prosperini et al., 2019; Sorensen et al., 2014). The literature so far lacks a precise definition for disease reactivation after these therapies, and the terminology for describing the phenomenon has not been uniform. In our study investigating the risk of clinical disease reactivation after NTZ cessation (Study I), we used the term 'post-NTZ disease reactivation', as did an earlier systematic review and meta-analysis (Prosperini et al., 2019). Another term, 'rebound', is often used to describe patients in whom disease activity increases even beyond pre-treatment levels (Sorensen et al., 2014). The use of the term 'rebound' merely to refer to severe relapses or impressive MRI activity has occasioned criticism (Prosperini et al., 2019). In the literature on disease reactivation after FNG discontinuation, this phenomenon is characterized more loosely, and the term 'rebound' is more typically used to describe severe relapses (Barry et al., 2019). For the sake of clarity, the term 'post-FNG disease reactivation' is used in this chapter similar to 'post-NTZ disease reactivation'.

Post-NTZ disease reactivation was the main outcome in Study I (Mustonen et al., 2020). Additionally, a subgroup of patients who had discontinued NTZ was investigated in Study IV (Rauma, Viitala, et al., 2022). Supporting the earlier literature, the most commonly reported reason for discontinuing NTZ was the presence of anti-JCV antibodies or the risk of PML (Mustonen et al., 2020; Prosperini et al., 2019; Rauma, Viitala, et al., 2022). Although our studies had differing methodology, they both provide epidemiologic data and interesting insights into post-NTZ disease reactivation. Initially, we did not seek to investigate post-FNG disease reactivation in isolation, but in the subgroup analysis of Study IV, early relapses were seen particularly often in patients switching from FNG to CLAD tables (Rauma, Viitala, et al., 2022). Our interpretation was that these patients had likely experienced post-FNG disease reactivation or even rebound. Therefore, this thesis was able to investigate disease reactivation after two anti-trafficking therapies.

Considering post-NTZ disease reactivation, our studies reported the occurrence of relapses in 10-27% of patients during the first six months and 36% of patients

during the first 12 months after discontinuation of NTZ (Mustonen et al., 2020; Rauma, Viitala, et al., 2022). Also, corticosteroid-treated relapses were observed in 20% and 27% of patients discontinuing NTZ at six and 12 months respectively (Mustonen et al., 2020). These proportions are in line with the existing literature, where clinical relapses have been observed in 9-80% of patients during follow-up times ranging from one to 24 months after discontinuation of NTZ (Prosperini et al., 2019). Although there is considerable heterogeneity with regard to discrepancy between study designs, study samples, follow-up period lengths and definitions for post-NTZ disease reactivation, the most recent literature unequivocally suggests that post-NTZ disease reactivation is a phenomenon which must be taken into account when taking patients off NTZ.

Post-NTZ disease rebound, on the other hand, has clearly been a subject of debate. Not all studies investigating NTZ discontinuation have demonstrated rebound (P. W. O'Connor et al., 2011), and it is not clear whether this term should be used to indicate high relapse rates, tumefactive MRI lesions, the occurrence of disabling relapses, or a combination of any of these (Kerbrat et al., 2011; Lo Re et al., 2015; Sorensen et al., 2014). In Study I, a definition based solely on the number of relapses was used to define rebound, as EDSS and MRI data were insufficient (Mustonen et al., 2020). This approach may well have overestimated rebound incidence in patients who had few or no relapses during the year before NTZ, and underestimated rebound incidence in patients with a high number of relapses prior to NTZ initiation. Nevertheless, the results from Study I showed that an increase in the yearly number of relapses when compared to the pre-NTZ state occurred in patients with considerable variability with regard to the number of relapses before NTZ (0-5), length of treatment (6-41 infusions), or level of disability when discontinuing NTZ (EDSS 1.5-7.0). Thus, although the total number of observations was small, rebound was not limited to patients with certain clinical characteristics.

As for post-FNG disease reactivation, Study IV demonstrated that 42% of patients switching from FNG to CLAD tablets experienced a relapse within six months after discontinuation of FNG. This is a higher proportion than has previously been reported, and an especially interesting finding since in the same study, only 10% of patients switching from NTZ suffered relapses within the same timeframe (Nygaard et al., 2022; Rauma, Viitala, et al., 2022). Some of this discrepancy may be attributable to different patient characteristics, as patients switching from FNG in our study sample had a long disease duration and more often switched treatment due to AEs or inefficacy. It could be argued that the

median washout period length (approximately three months) may have been too long when switching from FNG to CLAD tablets. In fact, the risk of relapses has been shown to increase with the length of washout period (Ferraro et al., 2022). It may be difficult to achieve short washout periods when switching from FNG to CLAD tablets due to having to wait for ALCs to return to acceptable levels. Frequent ALC monitoring may make it easier to avoid such delays if performed around the time when the ALC typically reaches the lower limit of normal (6-8 weeks) (Francis et al., 2014). This approach could be quite challenging in patients with prolonged lymphopenia, and thus, other options will need to be considered.

In order to develop individualized strategies to mitigate the risk of disease reactivation after discontinuation of anti-trafficking drugs, patients at highest risk must first be identified. In Study I, we demonstrated that a higher number of relapses during the year before NTZ initiation and an EDSS score of 5.5 or higher at the time of NTZ initiation were associated with an increased risk for clinical disease reactivation at six months (HR 1.7 and 3.7 respectively) (Mustonen et al., 2020). Although an association between with high EDSS prior to NTZ initiation and the risk for disease reactivation had not previously been reported, a similar finding was later published from the Tysabri Observational Program (TOP) (Butzkueven et al., 2020). According to their results, a higher risk of relapses after switching to PO therapies was predicted by an EDSS score over 3.5 at NTZ initiation. This potential new risk factor should be taken into account when planning to discontinue NTZ. Finally, patients in whom the primary reason for discontinuing NTZ was family planning may require extra caution, although this was not assessed statistically in Study I.

Study I failed to identify treatment strategies which could reduce the risk of clinical disease reactivation, but a washout period over three months in length was shown to be associated with an increased risk for reactivation when compared to no DMT. Most likely the study sample included patients who remained untreated due to having been determined to be low-risk patients on the basis of unmeasured variables. Patients who remained untreated due to having converted to SPMS during NTZ therapy and patients who had discontinued due to family planning were likely to affect the results. Also, the statistical model in the post hoc analysis was not controlled for confounding variables, and the residual effect of NTZ during the first months after discontinuation may have affected the results. Consequently, there may have been a protective effect associated with DMT use which the statistical model failed to capture. Nevertheless, the clinical implications could be that if a patient is considered to require further therapy after

discontinuing NTZ on the basis of clinical judgement, the next therapy should be initiated within three months. The results from Study IV may indicate that CLAD tablets could be a suitable exit strategy for patients discontinuing NTZ, but a larger study is needed to assess this. Given that NTZ does not induce lymphopenia, it is also easier to optimize washout when switching from NTZ to CLAD tablets in contrast to switching from FNG.

As evidence regarding disease reactivation after the discontinuation of these anti-trafficking drugs grows, so also does the need for terminological consistency and common criteria between studies. In the future, it would be wise to distinguish between disease reactivation and severe rebound to avoid overlapping terminology. In terms of disease rebound, incorporating variables such as EDSS increase during relapses may be beneficial to better take into account the severity of relapses, as some studies have already done. Thus, we could identify patients who are at risk for suffering catastrophic relapses after treatment discontinuation, and offer close monitoring and high-efficacy therapies to these patients. Such cohort studies will be made possible in the future due to EDSS scores being currently documented in MS registries such as the Finnish MS registry. Also, more research is needed to better understand the pathogenesis of rebound.

6.2 Safety of immune reconstitution therapies (Studies III and IV)

There are currently two authorized IRTs for MS which can produce long term changes in the immune cell populations of pwMS without needing continuous therapy: ALEM and CLAD tablets (Sellner & Rommer, 2020). The safety outcomes of these two DMTs were assessed in Studies III and IV, although due to different study design the results should not be used for direct comparison (Rauma, Mustonen, et al., 2022; Rauma, Viitala, et al., 2022). Instead, they may be used, for example, as a reference when explaining to patients which safety issues have been observed in the past while using these therapies. We demonstrated that in Finland, ALEM and CLAD tablets have been most commonly used to treat patients with a history of at least two previous DMTs in contrast to clinical trials, where most patients had typically used one previous DMT at most (Coles et al., 2012; Giovannoni et al., 2010). Although unsurprising considering the indications of these drugs and the current reimbursement criteria, it emphasizes the need for re-assessing the safety of ALEM and CLAD tables in a real-world setting. Interestingly, the proportion of treatment-naive patients in Study IV was higher

than expected, which may indicate that Finnish neurologists are increasingly utilizing an induction approach when choosing DMTs for pwRRMS Here, the safety aspects of these therapies are discussed with a focus on some of the special risks of each therapy as well as some of the similarities in terms of susceptibility for viral infections.

The main safety issues identified with ALEM in Study III included IARs, autoimmune AEs and infections (Rauma, Mustonen, et al., 2022). Although not particularly frequent, hepatobiliary AEs and malignancies were also reported. Two deaths were reported: one due to an infection and the other due to an immunological reaction. The high incidence of serious IARs, especially during the first course of ALEM, was concerning. Better strategies are therefore needed to control the symptoms associated with an IAR and to guarantee the safety of patients during the first course. These could include using symptomatic treatment more routinely or increasing the amount of corticosteroids administered during the course, as done in some hospitals (Herman et al., 2021; Šega-Jazbec et al., 2017). Slowing or temporarily discontinuing infusions may be useful in these instances, since some serious IARs were successfully managed this way in Study III. Additional examinations may be required to rule out other aetiologies in the occurrence of severe symptoms. Patients may be reluctant to continue treatment after experiencing a serious IAR during their first course of ALEM. However, they should be counselled that the incidence of IARs and serious IARs is lower during subsequent courses, and in our study, only one patient experienced a serious IAR in both courses (Rauma, Mustonen, et al., 2022).

A second special safety concern in Study III was the occurrence of autoimmune AEs or other immunological reactions after ALEM (Rauma, Mustonen, et al., 2022). These included AEs with considerable variability in terms of disease presentation and time to onset. Thus, clinical alertness and a maintaining a low consultation threshold are advised. Although thyroid AEs were the most common autoimmune AE in our study, their full extent was probably not captured as the median follow-up time fell short of three years, which is when the incidence usually peaks (Coles et al., 2021). Interestingly, having a pre-existing autoimmune disease was not associated with an increased risk for first autoimmune AE (Rauma, Mustonen, et al., 2022). Similar results have recently been published in a study where the authors concluded that pre-existing autoimmune disease should not preclude administering ALEM (Coles et al., 2022). The pathogenesis of secondary autoimmunity is still unclear, and managing several concomitant autoimmune diseases in one patient could be challenging. Identifying risk factors for

autoimmune multimorbidity in pwMS would be important, since autoimmune comorbidity has been associated with undesirable outcomes in terms of brain volume (Zivadinov et al., 2016). We did not recommend deviating from the current contraindication regarding pre-existing autoimmune disease based on the results from Study III (European Medicines Agency, 2013b), but if our findings and the findings of Coles and colleagues were to be replicated in future studies, this contraindication may need to be re-assessed.

Both ALEM and CLAD tablets cause lymphopenia and may result in an increased risk for infections (European Medicines Agency, 2013b, 2017). As in earlier studies, herpetic infections were observed after treatment with ALEM or CLAD tablets (Cohen et al., 2012; Coles et al., 2012; Giovannoni et al., 2010; Rauma, Mustonen, et al., 2022; Rauma, Viitala, et al., 2022). Informing patients about the possibility and typical presentations of herpetic infections is advised. No cases of PML or other rare opportunistic infections were observed. The severity of AEs was reported only in Study III, in which the incidence rate of serious infections was slightly higher than previously reported (Coles et al., 2017; Rauma, Viitala, et al., 2022). Both our studies likely underestimated the incidence of mild infections due to the retrospective setting.

Malignancies were observed at slightly higher rates in patients treated with ALEM when compared to the results of an earlier clinical trial, and in no patients treated with CLAD tablets (Coles et al., 2017; Rauma, Mustonen, et al., 2022; Rauma, Viitala, et al., 2022). However, without a comparison to a matched reference population, these results are mainly descriptive in nature. Of note, all the malignancies reported in Study III were also SAEs, but due to the limited follow-up, the long-term outcomes of these potentially life-threatening AEs remained unconfirmed at the time of data acquisition. Therefore, the overall mortality observed in Study III must be considered relative to the length of follow-up. It is recommended to perform annual human papillomavirus (HPV) screening in patients treated with ALEM, but no recommendations have been made on mammography (European Medicines Agency, 2013b). Considering that two patients in Study III had breast cancer, patients could be encouraged to participate in the organized mammography screening, which in Finland is funded by the municipality. Interventions to prevent and reduce smoking in pwMS are encouraged, as smoking not only increases the risk for certain infections and malignancies, but can also have adverse effects on some MS-related outcomes (Baskaran et al., 2019; Degelman & Herman, 2017; Park et al., 2021).

The overall safety findings observed after receiving treatment with CLAD tablets in Study IV were comparable to those reported in the existing literature (Leist et al., 2020). Two individual AEs observed in Study IV were of particular interest. One patient died of cardiac arrest. There are no previous reports of serious cardiac disorders associated with the use of CLAD tablets, but pwMS are at higher risk for cardiovascular disease (Hauer et al., 2021; Leist et al., 2020). Another noteworthy AE was the case of hepatitis, since recommendations for monitoring of liver enzymes and bilirubin were recently added to the Summary of Product Characteristics following reports of liver injury in patients treated with CLAD tablets (European Medicines Agency, 2022).

It would have been interesting to shed more light on the subject of skin-related AEs after CLAD tablets. After all, non-herpetic skin or mucocutaneous infections were as common as upper respiratory infections in Study IV, and non-infectious skin disorders such as alopecia, acne and dermatitis were also reported (Rauma, Viitala, et al., 2022). Unfortunately, our data did not allow for more specific exploration of these AEs. Nevertheless, these findings support the existing literature by confirming that skin-related AEs may arise after taking CLAD tablets (Aruta et al., 2020; Rolfes et al., 2021).

Treatment discontinuation was relatively infrequent after ALEM and CLAD tablets. However, ALEM was most often discontinued due to AEs, while CLAD tablets had been discontinued due to a variety of reasons (Rauma, Mustonen, et al., 2022; Rauma, Viitala, et al., 2022). Lastly, it should once more be emphasized that the individual incidences of AEs are not comparable between Studies III and IV due to differing methodologies. Both studies relied heavily on the comprehensiveness of the source data, and especially in Study IV, in which data was acquired from the Finnish MS registry, underreporting may have occurred.

6.3 Lipid profile alterations during fingolimod treatment (Study II)

We demonstrated that minor elevation of total cholesterol and HDL could be observed during treatment with FNG (Rauma et al., 2020). Our results are in line with those of an earlier study conducted in Israel, where an increase in total cholesterol and HDL concentrations was also seen in a small study sample including 29 patients treated with FNG (Blumenfeld Kan et al., 2019). The patients in their cohort had a similar female-to-male ratio but slightly lower mean baseline values of all four lipid level parameters when compared to our study sample.

During a 12-month follow-up, they demonstrated an 8.4% increase in total cholesterol and a 15% increase in HDL. Direct comparison to our study is not straightforward as bias is a possibility in both studies due to unmeasured variables such as lifestyle factors. Also, the methods for determining lipid concentrations were not reported in the Israeli study (Blumenfeld Kan et al., 2019). In another study conducted in Finland, no significant alterations in lipid concentrations were observed during a six-month follow-up (Hovi & Airas, 2016). However, this study had a smaller sample size and a shorter follow-up time than our study and we used a different statistical method from either of these studies (Blumenfeld Kan et al., 2019; Hovi & Airas, 2016). A mixed-effects model was more suitable than repeated measures ANOVA due to a differing number of measurements and irregular intervals between measurements in our study sample.

The coefficients observed in Study II regarding the longitudinal evolution of total cholesterol and HDL were rather small, translating to a yearly mean elevation of 0.40 mmol/L for total cholesterol and 0.17 mmol/L for HLD during the first 12 months of follow-up, and 0.12 mmol/L and 0.04 mmol/L respectively during the entire follow-up (Rauma et al., 2020). Relative to the baseline values of total cholesterol and HDL in our study sample, the coefficients from the mixed-effects model during the first 12 months translate into an approximately 7.5-9.5% elevation in these variables. However, these percentages should be interpreted with caution as they are derived from the results of the statistical model and not from exact measurements performed at baseline and at 12 months. Visual illustration showed substantial inter-individual variance in the lipid concentrations during the examined period (Figure 4). Potential confounding variables could include changes in lifestyle factors, smoking, body weight, comorbidities and other medications. The inclusion of confounding variables was not possible using the data available although we did exclude all patients on lipid-lowering medication to reduce bias. It is possible that patients were counselled to pursue healthier habits at the time of initiation of FNG.

The initial study hypothesis in Study II was that exposure to FNG might adversely affect lipid concentrations in pwMS, and consequently increase the risk for atherosclerosis. However, it is unlikely for the lipid profile alterations observed in Study II to have had a major effect on the overall risk for atherosclerosis in a person with MS. For comparison, according to data from a Cochrane Review, the lipid-lowering medication atorvastatin, when used at doses 10-40 mg daily, decreases total cholesterol by 27-34%, LDL by 37-47% and triglycerides by 18-29% and increases HDL by 4-5% (Adams et al., 2015). Also, statin therapy reduces

the five-year incidence of major atherosclerotic cardiovascular events by about one fifth per 1 mmol/L reduction in LDL (Baigent et al., 2005). In light of these data, the alterations of lipid concentrations observed in our study seem modest. However, the fact that mean LDL and triglyceride levels remained unchanged is also noteworthy, as MS is associated with an increased risk for cardiovascular comorbidities (Hauer et al., 2021). A hypothetical antiatherogenic effect could be supported by the fact that patients using S1PR modulators in randomized controlled trials have been shown to have higher incidence of bradyarrhythmia and hypertension, but no increased incidence of coronary disease (Zhao et al., 2021).

If the elevation in HDL observed in two studies to date is a reliable finding, more basic research is warranted to describe the underlying mechanism (Blumenfeld Kan et al., 2019; Rauma et al., 2020). Also, whether potential changes in lipid parameters – perhaps using more advanced lipid analyses – could be used as biomarkers for treatment response remains to be investigated. It would also be interesting to study whether the use of the more recently approved S1PR modulators could affect the lipid profiles or the risk of atherosclerosis in real-world patients.

6.4 Strengths and limitations

The studies in this thesis share the many strengths and limitations associated with retrospective real-world studies. In contrast to clinical trials, where generalizability and sample sizes are limited by inclusion and exclusion criteria, real-world studies are able to investigate large study samples and patients with various backgrounds and comorbidities. In the studies included in this thesis, the generalizability of results is further increased by the existence of the Finnish Current Care Guidelines which increase concordance between hospital districts with regard to DMT use. We also had the advantage of using the Finnish MS registry, which gives access to nearly nationwide real-world data of pwMS in a structured format.

The most important limitations were the potential for missing data and the lack of control groups. It is possible that not all events had been documented in our source data, and underreporting, especially of mild AEs, may have been an issue in Studies III and IV. The data in the Finnish MS registry is contributed on a voluntary basis by treating physicians during clinical practice and not systematically, as in clinical trials. This may increase the likelihood of missing data in a registry-based study. The Finnish MS registry is currently used only by neurologists and

does not extend to primary health care. Therefore, AEs diagnosed and treated by physicians from other medical specialities may not have been recorded in the registry. Due to the lack of a control group or comparison to a matched reference population in each study, many of our findings need to be interpreted as mainly descriptive.

Regarding efficacy outcomes, Studies I and IV would have benefitted greatly from EDSS and MRI data in order to further assess the risk of disease reactivation. However, these were either lacking or were not systematically documented in the original data and therefore could not be utilized to a full extent. Ideally, evaluating efficacy should not be restricted to assessing relapse outcomes as it is known that radiological disease activity may be present even without clinical relapses in a person with MS. Also, our studies did not take into account that it may take a considerable amount of time for the therapeutic effect of a subsequent DMT to reach its full extent. 'Re-baselining' after six months, for example, is a technique which is often utilized in this setting but was not used in our studies since we aimed specifically to assess the incidence of relapses during the transition phase from one DMT to another.

Some study-specific limitations are also to be considered. In Study I, more individual variables could have been assessed in the primary analysis investigating the risk of reactivation. In retrospect, it would have also been interesting to study whether age or disease duration at the time of NTZ discontinuation affected the risk for disease reactivation since older patients typically have fewer relapses and are more likely to have SPMS. Also, the effect of preventive high-dose corticosteroids was not assessed. Given the current state of the literature with regard to disease reactivation or rebound, our definitions for these phenomena were not uniform with those of some other studies.

There are also some limitations to be considered in the subgroup analyses in Study I, in which we aimed to compare different exit strategies after NTZ. There was no controlling for potential confounding variables, and the number of patients initiating a subsequent DMT within three months after NTZ discontinuation was very small. It could be argued that more evenly sized subgroups (for example "0-4 months" and ">4 months") might have been more useful. Findings from other studies may also have unintentionally guided the authors towards interpreting the results of the subgroup analyses as favouring shorter washout times despite the limitations caused by small subgroups since it is widely acknowledged that longer washout times after NTZ discontinuation are associated with an increased risk for disease reactivation. However, the statistical method may actually have

underestimated the efficacy of initiating early subsequent DMT since in the statistical model all patients were first in the "no subsequent treatment" group and then moved to either of the two treatment groups when a DMT was initiated. Thus, the "no subsequent treatment" group included the first months of follow-up of most patients who were to be treated with other DMTs in the near future. Potential residual effect of NTZ may have reduced the overall occurrence of relapses during the first months of follow-up in these patients thereby skewing the analyses towards favouring "no subsequent treatment".

In Study II, the use of a mixed-effects model was beneficial in terms of statistical power but resulted in a dilemma: were the findings also clinically significant? Also, due to the retrospective nature of the study, we could not choose the methods for determining lipid concentrations and had no access to potential confounding variables. The tendency of lipid parameters to change during normal ageing may also have affected the results. Without a control group, we could not say whether the changes observed in total cholesterol and HDL were due to FNG or to some other factor.

In Study III, missing data may have been an issue, but measures were made to minimize it during data acquisition. These included systematic data collection and not including certain benign infections in the analyses. The authors believed that SAEs were most likely detected with reasonable accuracy. Study III would have also benefitted from comparison to patients using other DMTs or a matched reference population, which would have made it possible to assess whether the incidences of rare AEs, such as malignancies, were within expected rates in pwMS.

Study IV was heavily dependent on the accuracy of the Finnish MS registry at the time of data acquisition. The completeness of data in the registry may have varied between hospital districts due to differences in local practices. For example, hospital districts may have had different follow-up protocols, which may have affected the likelihood for reporting relapses or AEs the registry. The effect of missing data in Study IV was reduced by updating the registry in some, but not all, hospital districts immediately prior to data acquisition. Unlike in Study III, where exposure-adjusted incidence rates were reported, only the proportions of patients with AEs were reported in Study IV. We concede that a case series setting is considered inferior to controlled trials when assessing efficacy outcomes and relapse rates may have been affected by regression to mean in Study IV. The study also lacked sufficient EDSS and MRI data, which is why all efficacy analyses had to be based solely on relapse outcomes. Similar to the other studies in this thesis, there was no control group or a matched reference population.

6.5 Ethical considerations

Even though patients are not typically contacted in real-world studies, there are ethical issues to be considered. In general, any form of medical research consumes time and resources, which need to be used in a way which benefits either patients, institutions or the scientific community. Studies are to be designed so that the research questions are relevant, and the methods are chosen so that the study may eventually answer those questions. All investigators need to have sufficient expertise for conducting a scientific study. Studies should be sufficiently powered to answer the study hypotheses yet not too large in order to avoid over-consuming resources.

Patient consent is generally required when conducting medical research, although there are exceptions to this rule regarding large registry-based studies. The processing of patient data for research purposes without consent is not entirely unproblematic. However, requiring patient consent in large registry-based studies would render many projects impossible as it would reduce participation rates, introduce selection bias and be resource-consuming (Ludvigsson et al., 2015). Therefore, certain kinds of studies may be performed without patient consent as long as permissions are obtained from respective registry holders and the integrity of the participants' data is protected. Data controllers are responsible for the secure and lawful processing of sensitive data. To protect patients' data, the use of methods such as anonymization is encouraged.

To avoid publication bias, results should be published regardless of whether or not they support the study hypotheses or previous literature. When writing manuscripts, previous publications should be credited via appropriate citing of their work, and the study results need to be presented so that individual patients cannot be recognized from the published manuscript. Finally, funding and potential conflicts of interest should be disclosed.

In the studies included in this thesis, personal data was processed on the basis of public interest. Patient consent was not required. The use of time and recourses for the conduction of these studies were justified by the need for real-world data on the safety of DMTs for HARRMS, which have been commercially available for only a relatively short time. Current data protection regulations were complied with when processing patient data, and anonymization or pseudo-anonymization was used during the statistical analyses. Although the published articles also contained some descriptions of individual cases with rare or fatal AEs, these cases were presented in a way which prevented individuals from being recognized. Studies III

and IV were published as "Open Access" articles under a Creative Commons Attribution 4.0 International license to make the results available to a wider audience (Creative Commons, n.d.). Potential conflicts of interest were presented in detail in each published article.

6.6 Clinical implications and suggestions for future research

Numerous DMTs have become available for the treatment of RRMS. The safety aspects of these DMTs need special attention in order to reduce medication-related harm. Clearly, the field of MS therapies needs to evolve in the direction of personalized medicine as there are now different approaches to treating MS. Real-world data can now be utilized when discussing the risks of DMTs. Ideally, choosing a DMT for a person with MS should be a process involving shared decision-making based on the most up-to-date information on the efficacy and safety of different treatment strategies.

Our studies support the findings of earlier research stating that disease reactivation may occur after the discontinuation of anti-trafficking therapies NTZ and FNG (Mustonen et al., 2020; Rauma, Viitala, et al., 2022). We identified a new risk factor for post-NTZ disease reactivation: high EDSS. Our results may be utilized when conceptualizing exit strategies for anti-trafficking therapies. It is the opinion of the author that NTZ should be initiated with the intention of using treatment for long periods of time in order to reduce the relative impact of a potential reactivation. The risk of disease reactivation or rebound should not be ignored when discontinuing NTZ in patients with high level of disability even after long periods of remission, as the number of NTZ infusions did not predict the risk of reactivation. Ideally, each patient's status in the pre-NTZ state should be evaluated when deciding what kind of therapy to recommend after NTZ, taking into account whether that period of time reflected a treatment effect of a preceding DMT or an untreated state, and whether the level of disability during that time was accumulated through relapse-associated worsening or progression independent of relapse activity.

In patients who are – based on clinical judgement – considered eligible for initiating a new DMT after having discontinued NTZ, the subsequent therapy should be initiated within three months (Mustonen et al., 2020). CLAD tablets may present a viable option after NTZ, but larger studies are needed to assess the benefits and risks of this transition (Rauma, Viitala, et al., 2022). Patients switching

from FNG to CLAD tablets were susceptible to relapses in Study IV. Whether a shorter washout period would reduce the risk of relapses in this scenario remains to be determined.

Patients discontinuing anti-trafficking therapies due to family planning require special consideration and should be counselled about the risk of reactivation. In high-risk patients, continuing NTZ until conception or early pregnancy using extended interval dosing may need to be considered as suggested recently (Canibaño et al., 2020; Wiendl et al., 2021). Considering that none of the currently available DMTs for HARRMS are officially recommended during pregnancy, one option would be to switch these patients first to an IRT and then discontinue contraception after the recommended four or six months after the full course of ALEM or CLAD tablets (European Medicines Agency, 2013b, 2017). However, the use of ALEM in this scenario is complicated by the risk of autoimmune AEs which may occur after a long delay. For example, autoimmune thyroiditis manifesting during pregnancy could have adverse outcomes. Also, the use of some of the newer anti-CD20 therapies should be investigated in this setting.

The safety observations from Study III corroborate the understanding that treatment with ALEM is risky and should be initiated only in a scenario where the indication is met with no contraindications and a patient is informed about the expected risks and benefits of the therapy (European Medicines Agency, 2013b; Rauma, Mustonen, et al., 2022). However, our results did not support the notion that pre-existing autoimmune disease would increase the risk of secondary autoimmunity after ALEM. If further studies replicate this finding, the contraindication regarding pre-existing autoimmunity may need to be re-assessed. Given that some Finnish hospitals had used ALEM in only a few patients and the use of ALEM has further decreased after the updated recommendations by EMA, some form of centralization may be considered regarding the future use of this therapy in Finland. As AEs may present with various manifestations after long periods of time, HCPs who encounter pwMS should regularly be reminded about the potential long-term effects of ALEM.

Study II revealed that concentrations of total cholesterol and HDL increased in patients treated with FNG (Rauma et al., 2020). Due to the size of the effect, the clinical relevance of these findings is likely moderate at most. However, this study did raise some potential research questions for future studies. Atherosclerosis is a major cause of disease burden in both Finland and globally and there is interest in developing new ways to prevent cardiovascular comorbidity. More basic research is needed to establish how S1PR modulation might affect lipoproteins in humans.

Whether the more recently approved S1PR modulators affect lipid concentrations or the risk of atherosclerosis in pwMS likewise remains to be studied.

There are still several questions which remain to be answered regarding the safety of DMTs for HARRMS. As people tend to live longer and DMTs are often used sequentially for years or decades, more research is needed on the safety of DMTs in older adults and in patients with history of multiple DMTs. Also, more data is needed about the safety of DMTs during pregnancy and breastfeeding. Finally, clinicians will need tools for detecting individuals who no longer benefit from DMTs so that patients who do not can be relieved of the risks of continuous DMT use.

7 SUMMARY AND CONCLUSIONS

In this thesis, we investigated the use of four different DMTs used to treat HARRMS in Finland. According to the results of this thesis, the following conclusions can be drawn:

1. After discontinuation of NTZ, the risk of clinical disease reactivation at six months was predicted by the number of relapses and an EDSS score of 5.5 or higher before the initiation of NTZ. When initiating a subsequent DMT after NTZ, the washout period should not exceed three months.

2. Minor elevation of total cholesterol and HDL was observed during treatment with FNG. Due to the clinically small coefficients, potential effects on the overall risk for atherosclerosis are likely minimal.

3. Various AEs were reported in patients who had received ALEM for MS. SAEs occurred in altogether 32% of patients during a median follow-up of 30 months. Vigorous monitoring and a low consultation-threshold are advised due to the risk of rare but potentially dangerous AEs.

4. Patients who had received at least two previous DMTs before initiating CLAD tablets had shorter time to first relapse than did patients with 0-1 previous DMTs. This finding may be explained by a subgroup of patients who had switched from FNG to CLAD tablets.

5. The safety profile of CLAD tablets was consistent with the literature, the most common AEs being headache, *Herpes simplex* and nausea. In light of our data, the current monitoring requirements appear justified.

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10 ORIGINAL COMMUNICATIONS

PUBLICATION

I

Risk factors for reactivation of clinical disease activity in multiple sclerosis after natalizumab cessation

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Risk factors for reactivation of clinical disease activity in multiple sclerosis after natalizumab cessation

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ABSTRACT

Background: Natalizumab (NTZ) is widely used for highly active relapsing-remitting multiple sclerosis (MS). Inflammatory disease activity often returns after NTZ treatment discontinuation. We aimed to identify predictive factors for such reactivation in a real-life setting.

Methods: We conducted a retrospective survey in four Finnish hospitals. A computer-based search was used to identify all patients who had received NTZ for multiple sclerosis. Patients were included if they had received at least six NTZ infusions, had discontinued treatment for at least three months, and follow-up data was available for at least 12 months after discontinuation. Altogether 89 patients were analyzed with Cox regression model to identify risk factors for reactivation, defined as having a corticosteroid-treated relapse.

Results: At 6 and 12 months after discontinuation of NTZ, a relapse was documented in 27.0% and 35.6% of patients, whereas corticosteroid-treated relapses were documented in 20.2% and 30.3% of patients, respectively. A higher number of relapses during the year prior to the introduction of NTZ was associated with a significantly higher risk for reactivation at 6 months (Hazard Ratio [HR] 1.65, $p < 0.001$) and at 12 months (HR 1.53, $p < 0.001$). Expanded Disability Status Scale (EDSS) of 5.5 or higher before NTZ initiation was associated with a higher reactivation risk at 6 months (HR 3.70, $p = 0.020$). Subsequent disease-modifying drugs (DMDs) failed to prevent reactivation of MS in this cohort. However, when subsequent DMDs were used, a washout time longer than 3 months was associated with a higher reactivation risk at 6 months regardless of whether patients were switched to first-line (HR 7.69, $p = 0.019$) or second-line therapies (HR 3.94, $p = 0.035$). Gender, age, time since diagnosis, and the number of NTZ infusions were not associated with an increased risk for reactivation.

Conclusion: High disease activity and a high level of disability prior to NTZ treatment seem to predict disease reactivation after treatment cessation. When switching to subsequent DMDs, the washout time should not exceed 3 months. However, subsequent DMDs failed to prevent the reactivation of MS in this cohort.

1. Introduction

Natalizumab (NTZ) is a humanized monoclonal antibody used in the treatment of relapsing-remitting multiple sclerosis (RRMS) (Clerico et al., 2017). It is administered intravenously in every four weeks. NTZ has been proven effective in reducing the recurrence of relapses in multiple sclerosis (MS), and it is generally used in patients with a highly active course of disease or a poor response to the first-line therapies of MS (Kappos et al., 2011; Tramacere et al., 2015). By binding to the α4 subunit on α4β1 integrin, NTZ blocks the binding of these integrins to the vascular-cell adhesion molecule 1 (VCAM-1), which is expressed on the endothelial cells of blood vessels in the

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central nervous system (CNS) (Léger et al., 1997; Yednock et al., 1992). As a result, the migration of T-lymphocytes from the circulation to the CNS is prevented. The therapeutic effect of NTZ is mostly explained by this regulation of T-lymphocyte adhesion and migration across the blood-brain barrier, but other α4-mediated effects of NTZ have also been suggested (Rice et al., 2005).

The use of NTZ is limited due to the risk of progressive multifocal leukoencephalopathy (PML) (Tan and Koralnik, 2010). The risk of PML is increased in patients with long treatment periods, prior immunosuppressive treatment, and positive status with respect to anti-JC virus antibodies (Bloomgren et al., 2012). If the risk is considered too high, switching to an alternative disease-modifying drug (DMD) should be considered.

In Finland, national treatment guidelines are followed when selecting DMDs for MS (Multiple Sclerosis: Current Care Guidelines, 2019). In the Finnish national guidelines, NTZ is positioned either as a first-line or second-line therapy for highly active RRMS with no anti-JC virus antibodies. Cessation of treatment is advised if seroconversion occurs. NTZ is officially licensed only for RRMS in Finland, but it is sometimes used in secondary-progressive multiple sclerosis (SPMS) patients who experience clinical relapses (Multiple Sclerosis: Current Care Guidelines, 2019).

NTZ is cleared from circulation in approximately two months after discontinuation of treatment, but some residual effects may persist for up to 6 months (O'Connor et al., 2011; Stüve et al., 2006). As expected, reactivation of MS has been shown to occur during the first year after discontinuation of NTZ in some patients (Fox et al., 2014; Gueguen et al., 2014; Havla et al., 2011; Iaffaldano et al., 2015; Kerbrat et al., 2011; Lo Re et al., 2015; O'Connor et al., 2011; Salhofer-Polanyi et al., 2014; West and Cree, 2010). A recent systematic review and meta-analysis of six studies demonstrated that younger age, higher number of relapses, and gadolinium-enhancing lesions before initiation of treatment as well as fewer NTZ infusions were associated with an increased risk for disease reactivation after cessation of treatment (Prosperini et al., 2019).

There are no established guidelines on how to treat MS patients discontinuing NTZ therapy, but recent reports have suggested that subsequent treatment with other DMDs should be initiated within 3 months after discontinuation in order to prevent disease reactivation (Iaffaldano et al., 2015; Jokubaitis et al., 2014; Kappos et al., 2015; Lo Re et al., 2015; Salhofer-Polanyi et al., 2014). However, most of the current evidence comes from observatory studies with heterogeneous study settings, and only few randomized trials have been published (Fox et al., 2014; Kappos et al., 2015; O'Connor et al., 2011). Our purpose was to evaluate the predictive factors for post-NTZ disease reactivation in an unselected clinical cohort of MS patients in a real-life setting.

2. Material and methods

2.1. Study population

This retrospective study was carried out using data from four Finnish hospitals covering a catchment area of 1.3 million residents. Three university hospitals (Kuopio University Hospital, Tampere University Hospital and Oulu University Hospital) from different parts of Finland and one medium-sized central hospital (Mikkeli Central Hospital) were chosen to represent MS treatment in Finland. The study was approved by the Research Ethics Committee of the Northern Savo Hospital District, Kuopio, Finland, and had an institutional approval from each participating hospital.

MS patients were identified by a computer-based search using the ICD-8, −9 or −10 diagnosis of MS and treatment with NTZ as search criteria. Patients were included in the study if they had received at least six consecutive infusions of NTZ before the treatment was discontinued and follow-up data was available for at least 12 months after the last infusion. A discontinuation was defined as a three-month period without any NTZ infusions. Shorter gaps between infusions were not considered relevant. We identified a total of 101 MS patients who had discontinued NTZ treatment in years 2009–2016, and 89 of them met the inclusion criteria. A flowchart displaying the selection of the study cohort is shown in Fig. 1.

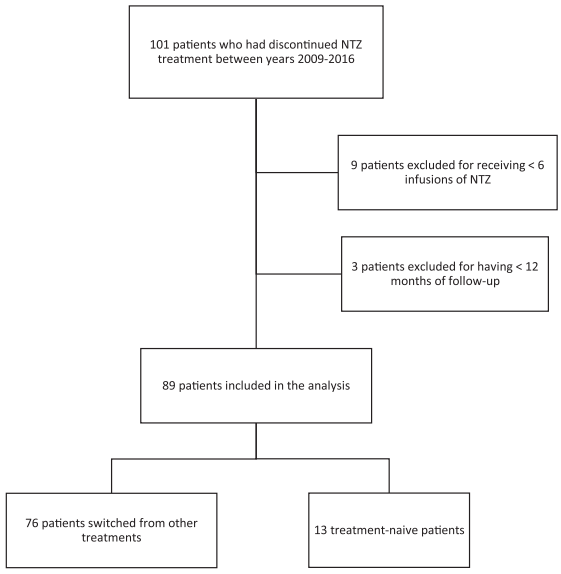

Fig. 1. The selection of the study cohort. NTZ = natalizumab.

2.2. Methods

The patient records were systematically reviewed from the time of the first symptom to the latest available contact with the hospital. Data was collected from both paper archives and the hospital districts' electronic patient information systems. The following variables were collected: gender; onset symptom of MS; time from diagnosis to the initiation of NTZ treatment; existence of gadolinium-enhancing lesions in the pre-NTZ magnetic resonance imaging (MRI) scan; number of NTZ infusions; adverse events during NTZ treatment; primary reason for the discontinuation of NTZ; prior and subsequent DMDs; washout time between DMDs; and all courses of corticosteroid treatment. Age was collected both at the time of diagnosis and at NTZ initiation. Expanded Disability Status Scale (EDSS) was collected at diagnosis, at NTZ initiation, and at NTZ discontinuation. The number of relapses during the year before NTZ initiation and the year after NTZ discontinuation were collected. In our analysis, the onset symptom of MS was also regarded as a relapse. Relapses were collected in two categories. First, all reported relapses were collected regardless of whether they required corticosteroid treatment or not. Second, only relapses which required corticosteroid treatment were collected. The latter were used to define reactivation in the statistical analysis.

Reactivation was defined as having experienced at least one corticosteroid-treated relapse after NTZ discontinuation. Rebound was defined as an increase in the yearly number of all relapses after the discontinuation of NTZ treatment when compared to the year before the initiation of NTZ. Washout time was defined as the time between the last infusion of NTZ and the initiation of the following treatment. In the analysis, EDSS was categorized into two groups with a cut point of 5.5.

Table 1
Clinical characteristics of the study cohort (n = 89). SD = standard deviation, NTZ = natalizumab, EDSS = Expanded Disability Status Scale, PML = progressive multifocal leukoencephalopathy. *Positive anti-JC virus antibodies, long treatment period and/or prior immunosuppressive treatment.

	Patients with at least one relapse at 6 months after cessation (n = 24)	Patients with no relapses at 6 months after cessation (n = 65)	All patients (n = 89)
Female gender, n (%)	17 (70.8%)	46 (70.8%)	63 (70.8%)
Age at the time of diagnosis, years, range (mean ± SD)	17–50 (27.9 ± 9.2)	15–55 (31.4 ± 8.9)	15–55 (30.4 ± 9.0)
Age at NTZ initiation, years, range (mean ± SD)	21–63 (34.5 ± 11.2)	20–56 (36.6 ± 9.6)	20–63 (36.0 ± 10.1)
Time from diagnosis at NTZ initiation, years, range (mean ± SD)	0–21 (6.2 ± 5.8)	0–25 (5.3 ± 5.6)	0–25 (5.5 ± 5.6)
EDSS at the time of diagnosis, range (mean ± SD)	1.0–6.0 (2.6 ± 1.6)	0–5.0 (2.0 ± 1.3)	0–6.0 (2.2 ± 1.4)
EDSS at NTZ initiation, range (mean ± SD)	1.0–7.5 (4.0 ± 1.8)	0–7.0 (3.4 ± 1.8)	0–7.5 (3.6 ± 1.8)
Duration of NTZ treatment			
< 12 months, n (%)	4 (16.7%)	9 (13.8%)	13 (14.6%)
12–36 months, n (%)	15 (62.5%)	38 (58.5%)	53 (59.6%)
> 36 months, n (%)	5 (20.8%)	18 (27.7%)	23 (25.8%)
Primary reason for the cessation of NTZ treatment,			
Risk of PML considered too high*, n (%)	11 (45.8%)	50 (76.9%)	61 (68.5%)
Inefficacy of treatment, n (%)	7 (29.2%)	9 (13.8%)	16 (18.0%)
Pregnancy plans or pregnancy, n (%)	4 (16.7%)	3 (4.6%)	7 (7.9%)
Adverse events, n (%)	1 (4.2%)	2 (3.1%)	3 (3.4%)
Difficulties with peripheral venous cannulation, n (%)	1 (4.2%)	0 (0%)	1 (1.1%)
Patient's own wish to discontinue treatment, n (%)	0 (0%)	1 (1.5%)	1 (1.1%)

2.3. Statistical analysis

Univariate Cox regression model was first used to identify individual variables associated with the risk of reactivation at 6 and 12 months of follow-up. Variables with statistically significant associations in the univariate model were then re-analyzed with multivariate Cox regression. The effect of subsequent DMDs administered after the cessation of NTZ treatment was analyzed using univariate Cox regression with patient as a random effect, and subgroup analysis was performed to determine whether a washout of 0–3 months or longer than 3 months was associated with the risk of reactivation. Results of the Cox regression analyses are shown as hazard ratios (HR) with 95% confidence intervals (CI). Statistical analysis for the risk of rebound was not performed, as there were only few cases representing possible rebound in the cohort. Data was expressed as means with standard deviations (SD) or frequencies with percentages. Statistical analysis was performed using SPSS Statistics 24.0 and R version 3.5.1. Statistical significance was defined as two-tailed $p < 0.05$.

3. Results

3.1. Patient characteristics

A total of 89 patients were included in the study. At the time of NTZ initiation, 95.5% (n = 85) had RRMS and 4.5% (n = 4) had SPMS. The patients received a mean number of 26.9 (range 6–85, SD ± 15.7) infusions of NTZ. Clinical characteristics of the study cohort are shown in Table 1.

Other DMDs were used before NTZ in 85.4% (n = 76) of the patients (Table 2). The most common preceding DMD immediately prior to the initiation of NTZ was glatiramer acetate (39.3%, n = 35). NTZ was used as a first-line therapy in 14.6% (n = 13) patients. Of these 13 treatment-naive patients, five had been observed without treatment for more than 12 months after the diagnosis of MS. For the rest of the treatment-naive patients (n = 8), NTZ was initiated within 12 months after the first symptom.

In the pre-NTZ MRI scan, 32.6% of the patients (n = 29) in the study cohort had gadolinium-enhancing lesions, while 67.4% (n = 60) had no gadolinium-enhancing lesions.

Altogether 15 patients (16.9%) had EDSS of 5.5 or higher at the initiation of NTZ treatment, indicating a high level of disability before the initiation of NTZ. All of these patients had RRMS and eight of them had gadolinium-enhancing lesions at the pre-NTZ MRI scan. In 13 of these patients, the primary reason for initiating NTZ was aggressive course of disease, a poor response to earlier DMDs, or both. For the final two patients, the reason for initiating NTZ was marked radiological activity without clinical relapses.

NTZ was mostly well-tolerated. The following adverse events were reported during treatment: fatigue (7.9%, n = 7); skin symptoms (4.5%, n = 4); nausea (3.4%, n = 3); arrhythmia (3.4%, n = 3); headache (2.2%, n = 2); and fever (1.1%, n = 1). However, only three patients (3.4%) discontinued treatment primarily due to adverse events. Skin symptoms reported in the study cohort included two cases of urticaria, one case of unspecified dermatitis, and one case of exacerbation of pre-existing atopic dermatitis. Of these four cases, only the exacerbation of atopic dermatitis led to discontinuation of NTZ treatment. The other two adverse events leading to treatment discontinuation were fatigue and fever. No cases of PML were reported in the study cohort. As expected, positive status with respect to anti-JC virus antibodies was by far the most common reason for discontinuing treatment (57.7%, n = 51). All reasons for treatment discontinuation are shown in Table 1.

After discontinuation of NTZ, subsequent DMDs were initiated for 68.5% (n = 61) and 77.5% (n = 69) of the patients by the time of 6 and 12 months, respectively. Altogether 21.3% (n = 19) of the patients continued without treatment through the first 12 months of follow-up. Table 3 demonstrates the distribution of patients according to the use of subsequent DMDs and the length of washout time. The most common subsequent treatment was fingolimod (52.8%, n = 47). Other subsequent DMDs included subcutaneous interferons (9.0%, n = 8), glatiramer acetate (8.9%, n = 8), dimethyl fumarate (4.5%, n = 4), and alemtuzumab (2.2%, n = 2). In addition to DMDs, nine patients (10.1%) received preventive high-dose corticosteroids without a clinical relapse within three months after the discontinuation of NTZ. Five of these courses of corticosteroids were given during the first month after discontinuation.

Table 2
The distribution of disease-modifying drugs used immediately prior to the initiation of natalizumab.

	n	%
Glatiramer acetate	35	39.3
Subcutaneous interferons	28	31.5
Mitoxantrone	8	9.0
Azathioprine	4	4.5
Fingolimod	1	1.1
No disease-modifying drugs before natalizumab	13	14.6

Table 3
The distribution of patients according to use of subsequent disease-modifying drugs (DMDs) and length of washout. The proportions of patients with corticosteroid-treated relapses at 6 and 12 months are presented within each group.

	No. of patients in group	Corticosteroid-treated relapse at 0–6 months, n (%)	Corticosteroid-treated relapse at 0–12 months, n (%)
No subsequent DMDs	20	4 (20.0%)	8 (40.0%)
DMD initiated after 0–3 months of washout	13	1 (7.7%)	4 (30.8%)
DMD initiated after >3 months of washout	56	13 (23.2%)	15 (26.8%)
All patients	89	18 (20.2%)	27 (30.3%)

3.2. Reactivation of MS after discontinuation of NTZ

During the first 6 and 12 months of follow-up after the discontinuation of NTZ, a relapse was documented in 27.0% ($n = 24$, Table 1) and 35.6% ($n = 32$) of the patients, respectively. By the time of 6 and 12 months, 20.2% ($n = 18$) and 30.3% ($n = 27$) of patients had experienced a relapse which required corticosteroid treatment, thus meeting our definition for reactivation. The proportions of patients experiencing corticosteroid-treated relapses according to the use of subsequent DMDs and the length of washout are demonstrated in Table 3.

The results of the Cox regression analyses are shown in Table 4. A higher number of relapses during the year before NTZ initiation was associated with a significantly higher risk for reactivation at 6 months (HR 1.65, 95% CI 1.26–2.15, $p < 0.001$) and 12 months (HR 1.54, 95% CI 1.21–1.96, $p < 0.001$) of follow-up. EDSS of 5.5 or higher at the time of NTZ initiation was associated with a significantly higher risk for reactivation at 6 months (HR 3.70, 95% CI 1.23–11.15, $p = 0.020$) but not at 12 months of follow-up when compared to patients with less disability (EDSS 0–5.0). Conversely, EDSS of 5.5 or higher at the time of NTZ discontinuation was associated with a significantly higher risk for reactivation at 12 months (HR 2.63, 95% CI 1.12–6.20, $p = 0.027$) but not at 6 months of follow-up.

According to the univariate analysis, the following variables were not associated with the risk of clinical reactivation after discontinuation of NTZ: gender; age at the initiation of NTZ treatment; time from diagnosis; number of NTZ infusions; and multifocal onset symptoms when compared to other forms of disease onset. These non-significant variables were not included in the multivariate model.

Univariate Cox regression model with patient as a random effect was used to analyze the effect of subsequent DMDs after NTZ cessation on the risk of reactivation. In this analysis, the patients' status with respect to DMDs was classified into three groups, which were compared with each other: first-line therapies (dimethyl fumarate, glatiramer acetate, and interferons); second-line therapies (alemtuzumab and fingolimod); and no DMDs. Subgroup analysis was done separately to compare patients with a washout time of 0–3 months to patients with a washout time longer than 3 months. According to the analysis, the use of subsequent DMDs was not significantly associated with the risk of reactivation at 6 or 12 months of follow-up. The results were the same in the first-line and second-line therapy group. However, the subgroup analysis showed that patients switching to subsequent DMDs after a washout longer than 3 months were in fact at higher risk for reactivation (Table 4) when compared to patients without treatment.

Altogether eight patients (9.0%) showed clinical signs of rebound activity according to the number of relapses they experienced during the year after NTZ discontinuation. These patients experienced their first clinical relapse 1–4 months after discontinuation of NTZ with a median of 3 months. Data of all patients who experienced rebound are shown in Table 5.

4. Discussion

In our study, we demonstrated that 20.2% of patients experienced clinical reactivation of MS within 6 months after discontinuation of NTZ. In previous studies, the proportion of patients experiencing relapses after NTZ discontinuation has ranged from 13.5 to 58% (Jokubaitis et al., 2014; Sangalli et al., 2014; West and Cree, 2010). This variability reflects differences in study populations and the difficulty of defining what constitutes as a relapse. We avoided this problem by using only corticosteroid-treated relapses to define reactivation.

Table 4
The results of the statistical analysis. Hazard rations (HR) and 95% confidential intervals (95% CI) are displayed for the statistically significant findings. For clarity, results with $p > 0.05$ have been removed. NTZ = natalizumab, EDSS = Expanded Disability Status Scale, DMD = disease-modifying drug. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

	6 months HR (95% CI)	12 months HR (95% CI)
Univariate analysis		
No. of relapses during the year before initiation of NTZ	1.71 (1.29–2.27)***	1.66 (1.29–2.14)***
EDSS 5.5 or higher at the initiation of NTZ	2.84 (1.05–7.69)*	–
EDSS 5.5 or higher at the discontinuation of NTZ	3.64 (1.38–9.57)**	2.94 (1.28–6.72)*
Gender	–	–
Age at the initiation of NTZ	–	–
Time from diagnosis	–	–
Number of NTZ infusions	–	–
Multifocal onset symptoms	–	–
Multivariate analysis		
No. of relapses during the year before initiation of NTZ	1.65 (1.26–2.15)***	1.54 (1.21–1.96)***
EDSS 5.5 or higher at the initiation of NTZ	3.7 (1.23–11.15)*	–
EDSS 5.5 or higher at the discontinuation of NTZ	–	2.63 (1.12–6.20)*
The effect of subsequent DMDs (univariate Cox regression with patient as a random effect)		
First-line DMD 0–3 months of washout	–	–
First-line DMD >3 months of washout	7.69 (1.40–42.19)*	–
Second-line DMD 0–3 months of washout	–	–
Second-line DMD >3 months of washout	3.94 (1.11–14.08)*	–

Table 5
Descriptive data of all eight patients who had experienced rebound. DMD = disease-modifying drug, NTZ = natalizumab, EDSS = Expanded Disability Status Scale.

Patient	No. of previous DMDs	No. of NTZ infusions before discontinuation	No. of relapses during the year before NTZ	No. of relapses during the year after NTZ	EDSS at initiation of NTZ	EDSS at discontinuation of NTZ
1	2	6	2	3	4.0	4.0
2	2	33	2	3	2.0	1.5
3	3	38	5	7	2.0	2.0
4	2	11	2	3	6.0	6.5
5	2	12	2	3	4.0	4.0
6	3	41	0	2	3.0	4.0
7	2	12	1	3	6.5	7.0
8	2	6	2	3	6.0	6.5

We demonstrated that a high number of relapses during the year prior to NTZ initiation was significantly associated with an increased risk for reactivation. Our findings are in line with the majority of previous reports. (Jokubaitis et al., 2014; Lo Re et al., 2015; Papeix et al., 2016). However, there is one retrospective study reporting an opposite finding where lower annual relapse rate was associated with an increased risk for reactivation (Salhofer-Polanyi et al., 2014).

We also found that EDSS of 5.5 or higher at the initiation of NTZ was a risk factor for the reactivation of MS at 6 months, and EDSS of 5.5 or higher at the discontinuation of NTZ at 12 months of follow-up. To our knowledge, there are no previous reports on marked disability being a risk factor for reactivation of MS after NTZ cessation. We find this to be of interest, since high EDSS is often associated with SPMS, in which peripheral inflammation has been considered to be minimal. In our cohort, none of the patients with EDSS of 5.5 or higher at the initiation of NTZ were defined as having SPMS. Since this is a retrospective study, it must be taken into consideration that this might be false and the group could represent SPMS patients with activity (Lublin, 2014). The pathogenesis of SPMS is still partially unknown, but it seems that there is compartmentalized smoldering inflammation in the CNS (Correale et al., 2017), which might activate under certain conditions. On the other hand, the patients in our cohort may have had high EDSS due to residual symptoms from previous severe relapses and therefore they were true RRMS patients despite the high EDSS.

Controversial findings have been reported about the association between the number of NTZ infusions and the risk for reactivation, as some studies have reported higher reactivation rates in patients with shorter exposure to NTZ (Lo Re et al., 2015; Miravalle et al., 2011; Prosperini et al., 2019; Vellinga et al., 2008). In our analysis, we did not detect any correlation between the number of NTZ infusions and the risk for reactivation. Furthermore, there was no correlation between age and reactivation risk, which is in line with a majority of earlier studies (Jokubaitis et al., 2014; Lo Re et al., 2015; Salhofer-Polanyi et al., 2014).

A previous study by Iaffaldano et al. has demonstrated the superiority of fingolimod in comparison to interferon beta and glatiramer acetate in controlling post-NTZ disease reactivation (Iaffaldano et al., 2015). However, in our study none of the treatment strategies used after NTZ were able to significantly control reactivation of MS. Furthermore, previous reports suggest that subsequent DMDs should be initiated as soon as possible after discontinuation of NTZ to prevent reactivation (Iaffaldano et al., 2015; Kappos et al., 2015). In the present study, a short washout time did not reduce reactivation, but a washout time longer than 3 months was significantly associated with an increased risk of reactivation. However, the number of patients switching to subsequent DMDs within 3 months after NTZ was very small in our study, which may explain why the selected treatment strategies did not reduce reactivation risk in the analysis.

Some of the previous studies have also identified a so-called rebound phenomenon or rebound effect after discontinuation of NTZ (Gueguen et al., 2014; Kerbrat et al., 2011; Lo Re et al., 2015; Salhofer-Polanyi et al., 2014; Sangalli et al., 2014; Vellinga et al., 2008). However, literature lacks a precise definition of the rebound effect, and not all studies have confirmed its existence (O'Connor et al., 2011). We defined rebound as an increase in the number of yearly relapses after discontinuation of NTZ when compared to the year before NTZ initiation and discovered that 9% of our patients had experienced rebound activity. This is somewhat lower than what has been reported in the majority of earlier reports (Gueguen et al., 2014; Kerbrat et al., 2011; Lo Re et al., 2015), but almost similar to what was reported in two earlier studies (Salhofer-Polanyi et al., 2014; Sangalli et al., 2014).

The study has limitations that should be noted. Due to the retrospective setting, some data were missing. Although it is a common custom in Finland for patients using DMDs to attend regular follow-up visits with neurological examinations, EDSS was not always documented. Furthermore, a substantial part of MRI data was missing. Therefore, we could not use it in the regression analysis. When describing pre-NTZ MRI, we only reported whether gadolinium-enhancing lesions were present, as the exact number of lesions was not reported for every patient.

We find the strength of this study to be its coverage of a large catchment area, the inclusion of four hospitals from different parts of Finland, and the use of an unselected real-life case series. The existence of our national treatment guidelines makes our data uniform and well representative of the actual treatment that Finnish MS patients received in years 2009–2016. Due to our national treatment guidelines, the indications for using different DMDs for reducing disease activity and prescribing corticosteroids for relapses in MS are concordant between different study centers.

Since approximately a fifth of MS patients discontinuing NTZ seem to suffer from reactivation regardless of the duration of their treatment, we suggest that NTZ should only be initiated with the purpose of using the treatment for long periods. None of the therapeutic strategies used in this cohort were able to control the return of disease activity. In the future, the efficacy of the more recently approved DMDs in preventing post-NTZ disease reactivation should be evaluated. Until then, close attention should be paid on patient selection, regarding fertile women with family plans in particular. Washout times should be kept as short as possible after NTZ cessation.

5. Conclusions

Discontinuation of NTZ treatment may lead to a marked reactivation of MS. High disease activity and a high level of disability prior to NTZ initiation seem to predict such reactivation, which could not be prevented with subsequent DMDs. A washout time longer than 3 months was a risk factor for post-NTZ disease reactivation.

Declaration of Competing Interest

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article. IR has received research grants (The Finnish Medical Foundation, Orion Research Foundation sr) and reimbursement of travel expenses (Novartis). PH has received research grants (Biogen,

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Update

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Corrigendum

Corrigendum to "Risk factors for reactivation of clinical disease activity in multiple sclerosis after natalizumab cessation" [Multiple Sclerosis and Related Disorders 38 (2020) 101498]

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The authors regret to inform that the name of the author Sakari Simula was misspelled in the published version of the article and wish for it to be corrected.

The authors would like to apologise for any inconvenience caused.

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PUBLICATION

II

Lipid Profile Alterations during Fingolimod Treatment in Multiple Sclerosis Patients

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LETTER TO THE EDITOR

Lipid Profile Alterations during Fingolimod Treatment in Multiple Sclerosis Patients

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Abstract
Fingolimod reduces inflammatory activity in multiple sclerosis (MS) by acting as a functional antagonist of sphingosine 1-phosphate (S1P) receptors. It has been suggested that S1P might also contribute to the antiatherogenic effect of high-density lipoprotein (HDL). We conducted a retrospective observational study using data of 72 MS patients from two Finnish hospital districts to find out whether lipid profiles change during treatment with fingolimod. A mixed-effects model with patient as a random effect was used to analyze lipid profile alterations. We found a statistically significant elevation in both total cholesterol (0.12 mmol/L per year) and HDL (0.04 mmol/L per year) during a median follow-up of 12 months, while low-density lipoprotein (LDL) and triglycerides remained unchanged. Since the mean elevation observed in both lipid values seems to be modest, we suggest that routine lipid profile monitoring is unnecessary during fingolimod treatment in MS patients without pre-existing cardiovascular comorbidities.

Keywords Cholesterol · Fingolimod hydrochloride · Lipoproteins · Multiple sclerosis · Sphingosine 1-phosphate receptors · Triglycerides

To the Editor;

Minor elevation of total cholesterol and high-density lipoprotein (HDL) can be detected in multiple sclerosis (MS) patients during treatment with fingolimod – an oral immunomodulatory drug for relapsing-remitting multiple sclerosis (RRMS). Based on our results, we suggest that routine lipid profile monitoring during fingolimod treatment is unnecessary in MS patients without pre-existing cardiovascular risk factors or comorbidities.

Fingolimod is a disease-modifying therapy (DMT) used in the treatment of RRMS. It prevents lymphocytes from leaving the lymphoid tissue and from reaching sites of inflammation in the central nervous system (Chun and Hartung 2010). The efficacy of fingolimod in reducing inflammatory activity of RRMS has been shown in randomized controlled trials (Cohen et al. 2010; Kappos et al. 2010).

The mechanism of action of fingolimod is mediated via functional antagonism on sphingosine 1-phosphate (S1P) receptors in lymphocytes (Chun and Hartung 2010; Matloubian et al. 2004). Our current study spawns from the hypothesis that S1P might also contribute to the antiatherogenic effect of HDL (Nofer and Assmann 2005). In fact, a few animal studies have already examined whether fingolimod could reduce the formation of atherosclerotic lesions (Klingenberg et al. 2007; Nofer et al. 2007; Poti et al. 2012).

Since the introduction of high-efficacy DMTs for MS, investigators have become increasingly interested in the long-term safety of these drugs. In order to find out whether alterations of lipid profiles could be seen during fingolimod treatment, we conducted a retrospective observational study using data from two Finnish hospital districts (The Hospital District of Southwest Finland and Kanta-Häme Hospital District)

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covering a catchment area of 650,000 inhabitants. The study was registered and approved by the Turku Clinical Research Center, Finland (T204/2013), and had an institutional approval from Kanta-Häme Central Hospital.

In the two hospital districts, lipid profiles of fingolimod-treated MS patients had been routinely monitored since the introduction of fingolimod until 2015. Total cholesterol, LDL (low-density lipoprotein), HDL, and triglycerides had been measured at baseline and at 1, 3, and 6 months after initiation of treatment, and thereafter on a clinical basis. Enzymatic colorimetric tests had been used to determine lipid profiles in both hospital districts with one exception: Friedewald method (Friedewald et al. 1972) had been used in the Hospital District of Southwest Finland to determine LDL.

We used a computer-based search to identify altogether 72 out of 151 MS patients who had initiated fingolimod between 2011 and 2015 and undergone at least two lipid profile measurements during fingolimod treatment. At least one measurement had to be performed during the first year of treatment. Data was collected from the national Finnish MS register (Laakso et al. 2019) and from the hospitals' patient information archives. 74 patients were excluded due to insufficient lipid profile monitoring, three patients for using lipid-lowering medication at the time of fingolimod initiation, and two patients for having already received fingolimod during the last 12 months. The reasons for individual patients' non-adherence to lipid profile monitoring were not specified in the study data. All patients had used fingolimod at a standard dose of 0.5 mg daily.

Statistical analysis was performed with Stata 15.1. A linear mixed-effects model with patient as a random effect was used to study alterations in plasma lipid profiles. A mixed-effects model is a statistical method which can analyze longitudinal data even when the number of measurements differs between cases or when intervals of repeated measurements are irregular. Statistical significance was defined as $p < 0.05$.

Mean age at treatment initiation was 40 years (standard deviation 8 years) and 56 patients (77.8%) were female. Median follow-up time was 12 months, and ranged from 1 to 50 months for LDL and 1 to 60 months for all other outcome variables. One patient's LDL data was missing for an unknown reason. Follow-up of lipid profiles was terminated due to initiation of lipid-lowering medication during fingolimod treatment in three patients who had not been using such medication at fingolimod initiation.

In the mixed-effects model, we found a small but statistically significant increase in both total cholesterol (coefficient = 0.0101 mmol/L per month, $p = 0.001$) and HDL (coefficient = 0.0034 mmol/L per month, $p = 0.002$). The size of the coefficient was small in both variables, however, translating to a yearly mean elevation of 0.12 mmol/L for total cholesterol and 0.04 mmol/L for HDL, which seems clinically insignificant. A separate analysis of the first 12 months during treatment demonstrated a similar increase in both total cholesterol (coefficient = 0.0332 mmol/L per month, $p = 0.004$) and HDL (coefficient = 0.0138 mmol/L per month, $p < 0.001$). The higher coefficients in the second analysis might indicate that most of the elevation observed in this study occurred during the patients' first year of treatment. LDL and triglyceride levels remained unchanged in both analyses.

The major limitations of this study are the retrospective setting and the relatively small number of subjects used in the analysis. It would have been intriguing to include potential confounding variables such as body mass index or diet changes for more accurate results. These potential confounding variables might have explained some of the inter-individual variance in the lipid profile response. Also, due to the retrospective setting of the study, we could not choose the methods used to determine lipid profiles.

To our knowledge, this is the first study reporting elevation of total cholesterol and HDL in MS patients during treatment with fingolimod using contemporary statistical methods in a real-life setting. Our results are in line with the initial study hypothesis and supported by some of the findings from a previous animal study, in which fingolimod induced a more proatherogenic hypercholesterolemia (Klingenberg et al. 2007). The results of this study are most likely generalizable to MS patients with a Nordic diet.

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Authors' Contributions MS-H and HK contributed to the study conception and design. Material preparation and data collection were performed by IR, MS-H, and HK. Data analysis was designed and performed by IR and HH. The first draft of the manuscript was written by IR, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Research data are not shared due to privacy restrictions.

Compliance with Ethical Standards

Conflict of Interest The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article. IR has received reimbursement of travel expenses (Novartis, Sanofi Genzyme, Teva). HH has no conflicts of interest to declare. MS-H has received honoraria for lectures (Biogen, Merck, Novartis, Roche, Sanofi Genzyme, Teva), reimbursement of congress expenses (Biogen, Merck, Roche, Sanofi Genzyme, Teva), and has served on scientific advisory boards (Biogen, Merck, Novartis, Roche, Sanofi Genzyme, Teva). HK has received honoraria for lectures/consultations (Biogen, Merck, Novartis, Roche, Sanofi Genzyme, Teva), reimbursement of congress expenses (Merck, Sanofi Genzyme,

Teva, Zambon), and has served on scientific advisory boards (Biogen, Merck, Novartis, Roche, Sanofi Genzyme, Teva).

Ethics Approval This is an observational study. According to local guidelines, ethics approval is not required.

Consent to Participate Not applicable.

Consent for Publications Not applicable.

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PUBLICATION

III

Safety of alemtuzumab in a nationwide cohort of Finnish multiple sclerosis patients

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ORIGINAL COMMUNICATION

Safety of alemtuzumab in a nationwide cohort of Finnish multiple sclerosis patients

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Abstract

Background Alemtuzumab is an effective disease-modifying therapy (DMT) for highly active multiple sclerosis (MS). However, safety concerns limit its use in clinical practice.

Objectives To evaluate the safety of alemtuzumab in a nationwide cohort of Finnish MS patients.

Methods In this retrospective case series study, we analyzed the data of all but two MS patients who had received alemtuzumab in Finland until 2019. Data were systematically collected from patient files.

Results Altogether 121 patients were identified, most of whom had received previous DMTs (82.6%). Median follow-up time after treatment initiation was 30.3 months and exceeded 24 months in 78 patients. Infusion-associated reactions (IARs) were observed in 84.3%, 57.3%, and 57.1% of patients during alemtuzumab courses 1–3, respectively. Serious adverse events (SAEs) were observed in 32.2% of patients, serious IARs in 12.4% of patients, and SAEs other than IARs in 23.1% of patients. Autoimmune adverse events were observed in 30.6% of patients. One patient died of hemophagocytic lymphohistiocytosis, and one patient died of pneumonia. A previously unreported case of thrombotic thrombocytopenic purpura was documented.

Conclusions SAEs were more frequent in the present cohort than in previous studies. Even though alemtuzumab is a highly effective therapy for MS, vigorous monitoring with a long enough follow-up time is advised.

Keywords Alemtuzumab · Autoimmunity · Drug-related side effects and adverse reactions · Incidence · Multiple sclerosis · Safety

Introduction

Alemtuzumab (Lemtrada) is a humanized monoclonal antibody against the cell surface antigen CD52. The efficacy of alemtuzumab when compared to subcutaneous interferon β-1a for relapsing–remitting multiple sclerosis (RRMS) was initially demonstrated in three randomized clinical trials [1–3]. The extension studies of these core trials are currently providing safety and efficacy data from 5 to 12 years of follow-up [4–7].

The therapeutic effect of alemtuzumab is thought to be mediated by depletion of circulating T- and B-lymphocytes, followed by a distinct pattern of lymphocyte repopulation [1, 8–10]. There is also evidence that alemtuzumab may have remodeling effects on the innate immune compartment [11]. Alemtuzumab is considered to be the first immune reconstitution therapy for RRMS.

Unfortunately, the use of alemtuzumab is limited by various safety concerns. As with most infused biological therapies, infusion-associated reactions (IARs) may occur. The most common signs or symptoms of an IAR after alemtuzumab are headache, rash, pyrexia, nausea, and flushing [12]. The symptoms are generally manageable with a pretreatment protocol consisting of intravenous steroid infusions on the first three days of any course of alemtuzumab, and additional antihistamine or antipyretic treatment administered at the physician's discretion [12, 13].

As alemtuzumab profoundly affects the immune system, opportunistic infections may occur [1–3]. However, the incidence of infections declines after the first year and does

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not increase with successive courses of alemtuzumab [4, 5]. In addition to the commonly occurring herpetic infections, less frequent pathogens such as *Listeria monocytogenes* have been reported [14]. To prevent herpetic infections, prophylactic oral acyclovir is used daily during the infusions and for one month thereafter [13].

A special safety concern of alemtuzumab revolves around its association with the development of secondary autoimmune diseases, such as thyroid diseases, immune thrombocytopenic purpura (ITP), and autoimmune nephropathy [2, 3]. The mechanism of secondary autoimmunity is not entirely understood. It has been suggested that the B-lymphocyte depletion and repopulation in the absence of T-lymphocyte regulation is a key factor in the development of secondary autoimmunity in genetically susceptible individuals [8]. Furthermore, an overproduction of IL-21 due to genetic factors has been suspected to predispose to alemtuzumab-induced autoimmunity [15]. However, repopulation kinetics of the evaluated peripheral lymphocyte subsets do not predict autoimmune adverse event (AE) occurrence, and biomarkers that would predict risk for autoimmune events have not been identified [16].

Reports of various new AEs have been published in recent years, including acute acalculous cholecystitis (AAC), acute coronary syndrome, autoimmune hepatitis, and hemophagocytic lymphohistiocytosis (HLH) [17–20]. In 2019, the European Medicines Agency (EMA) restricted the use of alemtuzumab for RRMS and initiated a review due to serious autoimmune and cardiovascular AEs [21]. The final decision of the European Commission was issued in 2020 [22]. According to the new recommendations, the drug should only be used for RRMS if the disease is highly active despite treatment with at least one other disease-modifying therapy (DMT), or if the disease is worsening rapidly. Furthermore, new contraindications were introduced, including concomitant autoimmune diseases other than multiple sclerosis (MS) [22].

Finland is a genetically isolated Nordic country with a high incidence of MS as well as other autoimmune diseases such as type 1 diabetes (T1D) and coeliac disease [23–27]. Therefore, we hypothesized that the safety profile of alemtuzumab for MS might differ from previous reports. Remarkably, the World Health Organization (WHO) initiated the third Global Patient Safety Challenge in 2017 aiming to reduce the global level of severe, avoidable medication-related harm by 50% in the next five years [28]. Our nationwide study of Finnish MS patients, working towards the

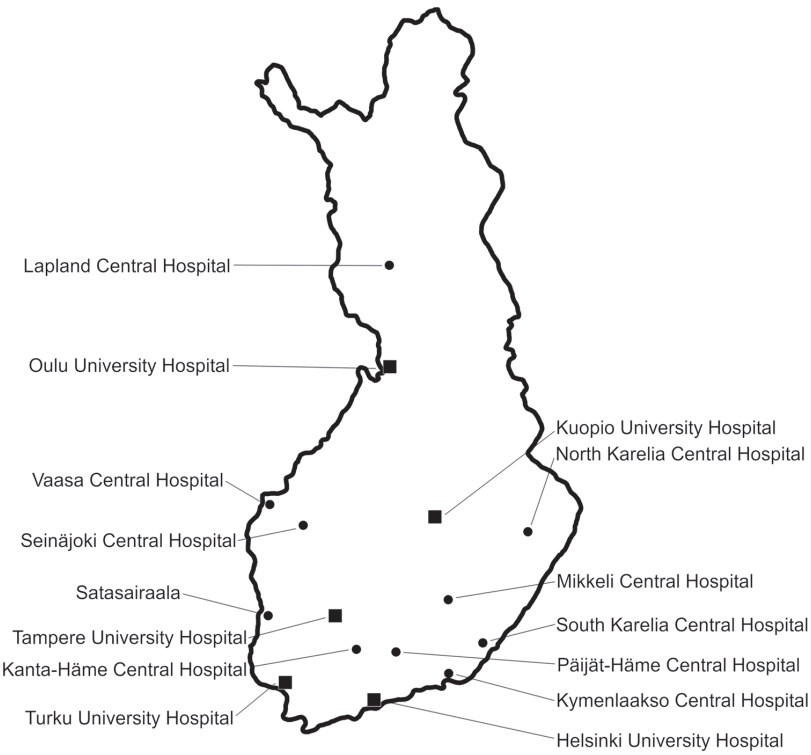

Fig. 1 The geographic distribution of participating hospitals

same goal, evaluated the safety of alemtuzumab treatment in a genetically isolated population.

Materials and methods

Patients

A retrospective non-interventional case series study using real-world data was conducted. All but one Finnish hospital where alemtuzumab had been administered to MS patients participated. The non-participating hospital had two alemtuzumab-treated patients. The participating hospitals included all five university hospitals of Finland and ten central hospitals (Fig. 1), making the data virtually nationwide. An institutional approval was obtained from each organization. A Research Ethics Committee approval or patient consent was not required.

All patients with a diagnosis of MS (ICD-8, -9, or -10) who had received alemtuzumab were included. Data were systematically acquired from electronic patient files during 2018–2019. This was done by the actual treating physician in a majority of hospitals. The timing of data cutoff varied between hospitals due to different schedules in approval processes. Whenever available, the following variables were collected: age at diagnosis and at treatment initiation; sex category; time of diagnosis; pre-existing comorbidities; smoking status; use of vitamin D, course of the disease at treatment initiation; Expanded Disability Status Scale (EDSS) at treatment initiation; DMTs before and after alemtuzumab; number and timing of alemtuzumab infusions; AEs; and outcomes of AEs. When calculating the total number of previous DMTs, subcutaneous interferons were grouped as one therapy. Efficacy was not assessed in this study.

Adverse events

AEs were classified as either IARs or other AEs. Lymphopenia was not considered an AE, as it represents the therapeutic effect of alemtuzumab [10]. Lower urinary tract and upper respiratory tract infections were not considered AEs, as significant underreporting can be expected in these potentially self-limiting conditions. Relapses during follow-up were not considered as AEs, but neurological symptoms during IARs were documented. Serious adverse events (SAEs) were defined the same way as in the alemtuzumab clinical trials [29] as life-threatening, resulting in death, requiring or prolonging hospitalization, disabling, resulting in a congenital anomaly, or requiring intervention to prevent one of these outcomes.

The timing of an AE was defined as the day of first symptom manifestation, or when the AE was first recognized by a health care professional. An IAR was defined as a new symptom or finding presenting within 24 h after an infusion of alemtuzumab. However, urticaria or rash was classified as an IAR as long as it manifested within 48 h after the last dose. If it was obvious that a condition had developed over a long period of time, it was not classified as an IAR even if its first manifestation occurred after an infusion of alemtuzumab (e.g. corticosteroid-induced hyperglycemia in a patient with previously unidentified type 2 diabetes). To avoid fragmentation of study data, all infections and cases of AAC were analyzed as other AE whether or not they presented within 24 h after an infusion.

Statistics

Descriptive analysis was performed on pseudo-anonymized data using IBM SPSS Statistics 25. Incidence rates of AEs were calculated using Stata 16.0. Numerical variables were expressed as means with standard deviations (SDs) or medians with interquartile ranges (IQRs). Categorical variables were expressed as frequencies and proportions. Missing months were imputed as July, and missing days were imputed as the 15th day of the month. Follow-up time was calculated from the first infusion of alemtuzumab to data acquisition, death, or patient being lost to follow-up (i.e. moving to a location wherefrom data was inaccessible). When calculating incidences of IARs, multiple symptoms or findings documented during the same course were calculated as one IAR. A Mantel–Haenszel-type method was used to determine whether pre-existing autoimmune diseases were associated with a higher incidence of secondary autoimmunity.

Results

Study sample

The study sample included data of 121 MS patients who had received alemtuzumab during 2013–2019 (Table 1). Median follow-up time after treatment initiation was 30.3 months (IQR 20.9–42.5 months). Follow-up exceeded 12 months in 108 patients (89.3%), and 24 months in 78 patients (64.5%). A majority of patients had received previous DMTs prior to alemtuzumab ($n = 100$, 82.6%). The most common last preceding therapies were fingolimod ($n = 49$, 40.5%) and natalizumab ($n = 20$, 16.5%). Although alemtuzumab is only indicated for the treatment of RRMS, we identified two patients whose course of the disease was reported to be secondary-progressive multiple sclerosis (SPMS) at the time

Table 1 Demographic details and baseline characteristics of the study sample

	n	%	
Sex category			
Female	90	74.4	
Male	31	25.6	
Course of disease			
Relapsive-remitting MS	119	98.3	
Secondary-progressive MS	2	1.7	
Number of previous DMTs			
0	21	17.4	
1	19	15.7	
2	22	18.2	
3	27	22.3	
4	23	19.0	
5	9	7.4	
Previously treated malignancy	1	0.8	
Reported use of vitamin D during alemtuzumab	89	73.6	
Reported (any amount of) smoking during alemtuzumab	27	22.3	
	Median	IQR	Range
Age at diagnosis of MS, years	26.6	22.2–32.3	13.8–59.2
Age at initiation of alemtuzumab, years	32.0	28.3–37.8	18.2–59.3
EDSS at initiation of alemtuzumab	3.0	2.0–5.0	0–8.0
Time from diagnosis to initiation of alemtuzumab, years	5.3	1.1–8.5	0.1–23.5
Time from discontinuation of previous DMT, months	2.1	1.6–3.4	0–38.2

MS multiple sclerosis, *DMT* disease-modifying therapy, *IQR* interquartile range, *EDSS* Expanded Disability Status Scale

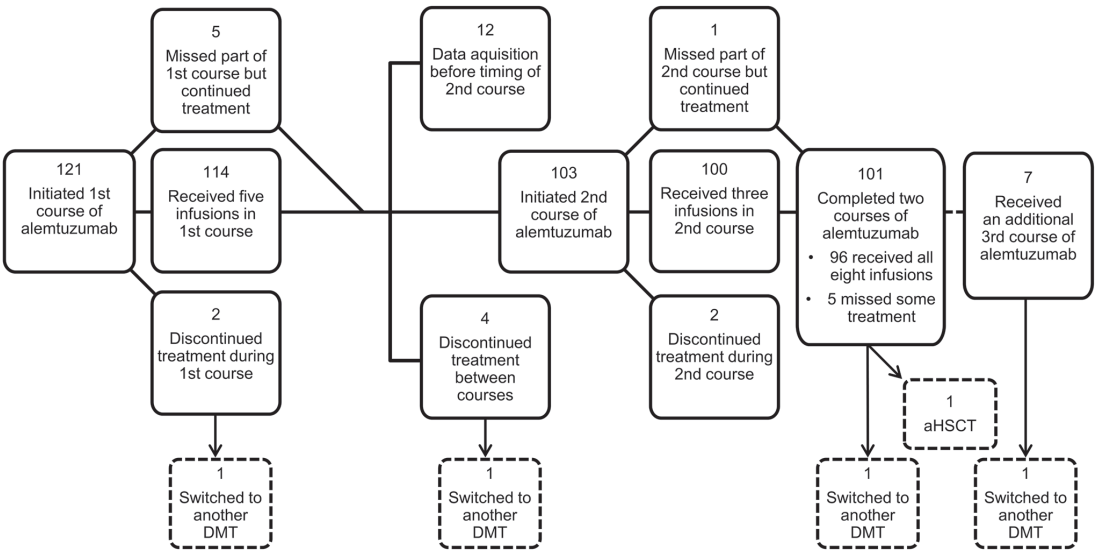

Fig. 2 A flow chart displaying patients receiving treatment during each course of alemtuzumab. *aHSCT* autologous hematopoietic stem-cell transplantation, *DMT* disease-modifying therapy

Table 2 Incidences of infusion-associated reactions according to treatment course

	1st course		2nd course		3rd course	
	n	%	*n*	%	*n*	%
Any infusion-associated reaction	102	84.3	59	57.3	4	57.1
Urticaria or rash	63	52.1	22	21.3	1	14.3
Headache	22	18.2	20	19.4	1	14.3
Hyperthermia or fever	18	14.9	13	12.6	2	28.6
Alterations in heart rate or palpitations	21	17.4	9	8.7	0	
Neurological symptoms	19	15.7	8	7.8	0	
Serious infusion-associated reaction	13	10.7	3	2.9	0	
Patients receiving alemtuzumab in each course	121		103		7	

Only the most frequently observed symptoms or findings are presented separately

Table 3 Incidence rates of adverse events of interest

	Patients with event		Incidence rate
	n	%	Number of events per 100 patient-years
Any AE	116	95.9	
Any IAR	109	90.1	
Any AE excluding IARs	65	53.7	33.1
Any AE leading to treatment discontinuation	6[a]	5.0	
Any serious AE	39	32.2	
Any serious IAR	15	12.4	
Any SAE excluding IARs	28	23.1	10.2
Death	2	1.7	0.6
Any infection event	30	24.8	11.8
Serious infection event	10	8.3	3.4
Any autoimmune AE	37	30.6	13.8
Autoimmune thyroid event	32	26.4	11.8
Serious autoimmune thyroid event	5	4.1	1.6
ITP	2	1.7	0.6
Acute acalculous cholecystitis	3	2.5	1.0
Malignant disease	4	3.3	1.3

AE adverse event, *IAR* infusion-associated reaction, *SAE* serious adverse event, *ITP* immune thrombocytopenic purpura

[a]In addition, two patients (1.7%) discontinued due to lymphopenia, which we did not regard as an adverse event

Table 4 Reasons for discontinuation of treatment

	n
Adverse events resulting in discontinuation	
Acute acalculous cholecystitis	2
Hepatic or hepatobiliary reaction	2
Pulmonary reaction with edema	1
Pyelonephritis	1
Other reasons for discontinuation	
Acute lymphopenia	1
Prolonged lymphopenia	1

of alemtuzumab initiation. However, these patients still had relapses at treatment initiation, and therefore, were progressing with activity.

All treatment courses were administered according to the label using the standard dose of 12 mg daily. At data acquisition, two complete courses of alemtuzumab had been administered to altogether 96 patients (79.3%, Fig. 2). In addition, 5 patients (4.1%) had received alemtuzumab during two courses, but missed part of their treatment due to AEs. A third course had been administered to 7 patients (5.8%) due to disease activity. Other subsequent DMTs were initiated

in 4 patients (3.3%) after alemtuzumab (cladribine, daclizumab, dimethyl fumarate, and ocrelizumab). In addition, 1 patient underwent autologous hematopoietic stem-cell transplantation after receiving two courses of alemtuzumab.

Adverse events

The incidences of AEs are presented by category in Tables 2 and 3. Discontinuation occurred in six patients due to AEs (5.0%), and two patients due to lymphopenia (1.7%), as presented in Table 4. One patient died of HLH, as also described in a case report published earlier [17]. One patient with severe disability died of pneumonia three years after the last infusion of alemtuzumab with MS being the underlying cause of death. Five patients (4.1%) had no AEs.

Infusion-associated reactions

IARs were documented in 84.3%, 57.3%, and 57.1% of patients during treatment courses 1–3, respectively (Table 2). The most frequently observed symptoms or findings of an IAR were urticaria or rash, headache, and hyperthermia or fever. Most of the serious IARs occurred during the first course. One patient experienced a serious IAR in both courses. No discontinuations were reported due to IARs, although four patients discontinued treatment during a course of alemtuzumab due to events other than IARs (AAC, acute lymphopenia, and pyelonephritis).

Of the 15 patients who continued to receive a second course after not having an IAR in their first course, 8 patients (53.3%) did not have an IAR on the second course either. It is noteworthy that although an IAR was documented in altogether 165 treatment courses (out of 231 treatment courses administered), 39% of them did not require any extra interventions, examinations, prolonged hospitalization, or re-hospitalization.

Neurological symptoms were documented during 27 IARs, occurring in 11.7% of all courses administered. They were mostly exacerbations of pre-existing neurological symptoms but also new neurological symptoms were documented. Of the 16 patients who experienced a neurological symptom during their first course and continued to receive a second course, only 4 patients (25%) had a neurological symptom during the second course as well.

Other adverse events

Infections

Infections were observed in 30 patients (24.8%; Table 3). *Herpes zoster* reactivation was the most frequent infection (10 patients, 3.4/100 patient-years). Serious infections occurred in 10 patients, with pneumonia being the most common serious infection (4 patients, 1.3/100 patient-years). Other serious infections included cases of *herpes zoster*, pyelonephritis, dental infection, and unspecified infection with a strong suspicion of bacterial etiology. One patient discontinued treatment due to pyelonephritis, which interrupted

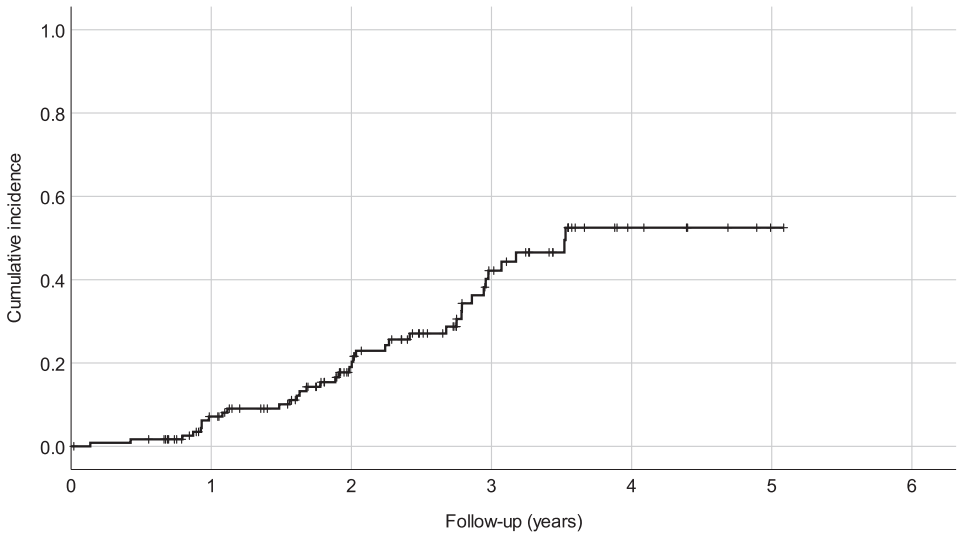

Fig. 3 A survival curve displaying the cumulative incidence of first autoimmune adverse event

the second treatment course. No cases of *Listeria monocytogenes* were observed.

Secondary autoimmunity

Autoimmune AEs were observed in altogether 37 patients (30.6%; Table 3; Fig. 3), most of whom developed autoimmune thyroid AEs. The most frequent thyroid AE was hyperthyroidism. Serious autoimmune thyroid AEs included four cases of hyperthyroidism resulting in thyroidectomy and one case of thyroiditis resulting in hospitalization.

Two cases of ITP were observed, one of which was serious. In addition, single cases of the following autoimmune AEs were observed: asthma; HLH, psoriasis; thrombotic thrombocytopenic purpura (TTP); T1D; and vitiligo. Of these, the cases with HLH, TTP, and T1D were classified as serious. The patient with TTP required intensive care and plasmapheresis.

Of the 37 patients who developed autoimmune AEs, 5 had a pre-existing autoimmune disease in addition to MS. However, having a pre-existing autoimmune disease was not associated with an increased incidence of secondary autoimmunity ($p = 0.95$). Three patients developed more than one new autoimmune disease after alemtuzumab initiation (multiple thyroid AEs in single patients were only counted as one). One patient suffered from recurrent arthralgia and myalgia which was not classified as an autoimmune disease even though the patient recovered after steroid treatment, as no evidence of an underlying rheumatic disease could be found. No cases of acquired hemophilia A, anti-glomerular basement membrane nephropathy, or autoimmune hepatitis were observed.

Hepatobiliary adverse events

AAC was observed in three patients (2.5%). In two of the cases, AAC manifested during or after the third infusion of the first course of treatment, and resulted in discontinuation of treatment. The third case manifested one year after the second course of treatment in a patient who soon after developed HLH. In addition, one case of acute calculous cholecystitis resulting in cholecystectomy was observed. All cases of cholecystitis were serious.

Two patients developed unspecified reactions with either hepatic or hepatobiliary involvement. The first case had elevated alanine aminotransferase (ALT), and was suspected for autoimmune hepatitis after responding to steroid treatment. The second case had both elevated ALT and amylase together with mild dilatation of bile ducts in an ultrasonography and eosinophilic granulocytes in a liver biopsy. Drug reaction or cholangitis was suspected. A definitive diagnosis could not be made in either case. Both AEs were classified as serious, and resulted in discontinuation of treatment, as they manifested between treatment courses.

Neoplasms

Malignancies were observed in four patients (3.3%). They included two cases of breast cancer, one cervical cancer, and one cervical carcinoma in situ. Additionally, one patient underwent a gynecological intervention due to cervical dysplasia, which was classified as a precancerous condition. No cancer-related deaths were observed during the studied period.

Unclassified adverse events

Various other AEs were also observed in the study data, mostly in single patients. A few of these AEs were considered to be of particular interest. One patient discontinued treatment after developing a pulmonary reaction with edema four days after completing the first course of treatment. The condition was diagnosed as a drug reaction to alemtuzumab, and treated with steroids by a pulmonologist. One patient developed sarcoidosis with both pulmonary and renal manifestations almost three years after the second course of alemtuzumab. The patient presented with acute symptoms and required urgent hospitalization due to hypercalcemia.

No cases of myocardial infarction were observed. One ischemic stroke was observed in a patient previously treated with plasmapheresis. In addition, one patient with ITP developed recurrent thrombophlebitis. No cases of autoimmune nephropathy were observed, but one patient with pre-existing type 2 diabetes developed mild nephropathy. Asymptomatic microalbuminuria and microhematuria were observed in one patient.

Two patients suffered from transient diarrhea requiring treatment in the emergency room 1 and 4 months after receiving alemtuzumab. In one of the cases, infectious colitis was suspected. A third patient with diarrhea and loss of appetite was evaluated in an outpatient setting. Despite investigations, an underlying pathology could not be identified.

Discussion

In this large nationwide real-world cohort, we report the safety of alemtuzumab with a special focus on SAEs, secondary autoimmunity, and previously unreported AEs. The present study can be compared with five previous real-world studies evaluating the use of the currently recommended dose of alemtuzumab, as well as the 12 mg treatment arm of the pivotal CARE-MS II core trial, in which study patients had received previous DMTs (Table 5) [3, 30–34]. However, comparison between studies is not straightforward due to

Table 5 Our results compared with previous findings from real-world studies evaluating the use of the currently recommended dose of alemtuzumab, as well as the 12 mg treatment arm of the pivotal CARE-MS II core trial

Reference	Present study	Huhn et al	Prosperini et al	Frau et al	Zmira et al	Brecl Jakob et al	CARE-MS II (12 mg group)
		[30]	[31]	[32]	[33]	[34]	[3, 5]
N	121	50	40	90	35	71	426
Follow-up time, months	30 (median)	15 (mean)	36	27 (mean)	24	38 (mean)	24 (core trial)
Females, %	74.4	60.0	82.5	74.4	54.3	71.8	66
Previous DMT use, %	82.6	100	97.5	92.2	100	71.8	100
EDSS at treatment initiation, median	3.0	3.0	4.0	2.5	4.0	3.0	2.5
Any AE, %	95.9	NA	NA	NA	100	84.1	98
Any IAR	90.1	NA	95	95.5	100	59.2	90
Any non-IAR AE *(per 100 PY)*	53.7 *(33.1)*	NA	37.5	NA	37.1	NA	NA *(255.8)*
Any SAE, % *(per 100 PY)*	32.2	NA	9 events[a]	NA	NA	NA	13% *(11.1)*[b]
Any serious IAR	12.4	NA	7 events[a]	NA	NA	NA	3%
Any non-IAR SAE *(per 100 PY)*	23.1 *(10.2)*	NA	2 events[a]	NA	NA	NA	NA *(10.0)*
Autoimmune AE, %	30.6	4	9 events[a]	12.2	8.6	NA	NA
Autoimmune thyroid AE *(per 100 PY)*[c]	26.4 *(11.8)*	2	8 events[a]	11.1	8.6	31.9	16 *(8.8)*[c]
ITP *(per 100 PY)*	1.7 *(0.6)*	0	0	3.3	0	1.4	1 *(0.5)*
Other autoimmune AEs	Asthma, HLH, psoriasis, TTP, T1D, vitiligo	Hemolytic anemia	Acquired hemophilia A	–	–	–	Hemolytic anemia, membranous nephropathy
Serious infection, % *(per 100 PY)*	8.3 *(3.4)*	2	0	NA	NA	NA	4 *(1.9)*
Malignant disease, % *(per 100 PY)*	3.3 *(1.3)*	2	0	0	2.9	0	< 1 *(0.2)*

Values represent the proportions of patients with an AE. Incidence rates are provided for specific categories in parenthesis for the present study and the CARE-MS II trial

AE adverse event, *DMT* disease-modifying therapy, *EDSS* expanded disability status scale, *NA* not available, *IAR* infusion-associated reaction, *PY* patient-years, *SAE* serious adverse event, *ITP* immune thrombocytopenic purpura, *HLH* hemophagocytic lymphohistiocytosis, *TTP* thrombotic thrombocytopenic purpura, *T1D* type 1 diabetes

[a]Only the number of events is provided, and not the proportion of patients with an event

[b]Excluding multiple sclerosis relapses

[c]In the CARE-MS II trial, the reported value represents "any thyroid event", and not "any autoimmune thyroid event"

different study protocols and varying follow-up times. In this report, four additional studies in which at least some patients had received previous or off-label doses of alemtuzumab, as well as two studies not reporting the dose of alemtuzumab, were not included for comparison [35–40].

SAEs—serious IARs in particular—were more frequent in this cohort than in the CARE-MS II core trial (Table 5) [3]. The slightly longer follow-up time in the present study is not likely to explain this difference, as a large amount of SAEs in this cohort were serious IARs. In our cohort, serious IARs most often manifested during the first course of alemtuzumab even though premedication with intravenous steroids was administered. Also, serious infections and malignancies were observed at slightly higher incidence

rates in this cohort when compared to the CARE-MS II core trial [5]. These differences may reflect different study populations, as real-world patients tend to have more comorbidities than patients in clinical trials. In the present study, we used the same definition for SAEs as used in clinical trials. Therefore, our findings should be comparable despite different settings.

It seems that AE severity is seldom assessed in retrospective studies. Only one large Italian cohort study reported seven serious IARs and two other SAEs during a 36-month follow-up of 40 MS patients [31]. Although they did not report incidences, their findings regarding serious IARs are similar to ours. This suggests that serious IARs are more frequent in a real-world setting in contrast to clinical studies, possibly reflecting higher comorbidity in real-world patients.

Autoimmune AEs were observed in 30.6% of patients in our study. The incidence rate of thyroid AEs in the present study was comparable to the CARE-MS II core trial (Table 5), although slightly different AE categories were used: our study reports the incidence rate of autoimmune thyroid AEs, whereas the CARE-MS II trial reported the incidence rate of all thyroid AEs including conditions such as goiter [3, 5]. In a recent real-world study by Brecl Jakob et al., more autoimmune thyroid AEs (31.9%) were observed than in the present study (26.4%), which may be explained by the longer follow-up time [34]. Other previous real-world studies have reported autoimmune thyroid AEs occurring in only 2–11% of patients, but these studies have had shorter follow-up times (Table 5) [30, 32, 33].

In addition to autoimmune thyroid AEs, two cases of ITP and six cases of other autoimmune AEs were observed, including one case of fatal HLH [17] and one case of TTP, which is a previously unreported autoimmune AE after alemtuzumab therapy. We hypothesize that either environmental factors or genetic predisposition to autoimmune diseases in the population may explain why so many different types of autoimmune AEs were observed in this cohort, as Finland is known to be a high-risk region for certain autoimmune diseases such as MS, T1D and coeliac disease [23, 24, 26, 27]. However, we did not observe any association between pre-existing autoimmune diseases and the development of secondary autoimmunity. Another possible explanation may be the previous exposure to different DMTs, which could modify long term immune responses. Only 17.4% of our patients were treatment-naive, whereas 48.7% of patients had received three or more previous DMTs.

We confirmed the findings of previous reports stating that cases of AAC, acute sarcoidosis, and T1D can be observed after alemtuzumab therapy for MS, highlighting the meaningfulness of post-marketing real-world studies [18, 41, 42]. Notably, reports of new AEs led to EMA initiating its review of alemtuzumab in 2019 [21]. In our cohort, one serious pulmonary reaction and two hepatic or hepatobiliary reactions were also observed, all of which led to treatment discontinuation. The various presentations of AEs in the present study emphasize the importance of collaboration with other medical specialties when diagnosing potential AEs after alemtuzumab therapy. Although malignancies were rare in our cohort, the occurrence of one cervical cancer, one cervical carcinoma in situ, and one cervical dysplasia underline the need for cervical screening.

IARs were common in the present study, which is in line with most previous studies (Table 5) [5, 31–33]. Only five patients (4.1%) missed part of their treatment due to an IAR, and the four discontinuations occurring during a course of alemtuzumab were due to events other than IARs. In general, it can be concluded that a majority of IARs are either mild or moderate in severity, and manageable in an inpatient setting. Furthermore, the incidence of IARs declined after the first course of alemtuzumab, and serious IARs were rare in subsequent courses.

The strength of the present study is the large sample size and the inclusion of almost every alemtuzumab-treated MS patient in Finland with both urban and rural areas covered. Finland is considered a genetically isolated nation [25]. Therefore, the present study had the advantage of evaluating the risk of secondary autoimmunity in a unique setting in contrast to other populations. Our cohort is the largest among real-world studies evaluating the use of the currently recommended dose of alemtuzumab for MS, and the first to present incidences of SAEs (Table 5). Data were collected systematically from comprehensive medical records, and in most cases this was done by the actual treating physician. Limitations to the present study include its retrospective setting and the lack of comparison to patients treated with other DMTs. A longer follow-up may reveal additional AEs and confirm the outcomes of some SAEs such as malignancies.

In conclusion, we observed more SAEs—serious IARs in particular—than in the comparable 12 mg treatment arm of the pivotal CARE-MS II trial [3]. In addition, we observed a previously unreported case of TTP. Even though alemtuzumab is a highly effective therapy for MS, vigorous monitoring with a long enough follow-up time is advised. Clinicians must be alert, as AEs are shown to present with various clinical manifestations. More nationwide cohort studies evaluating the safety of alemtuzumab are needed to identify the role of different ethnic and genetic backgrounds in the appearance of AEs.

Author contributions IR, AMR, and HK contributed to the study's conception and design. Data collection was performed by IR, TM, JMS, MU, MM, AV-A, MK, JTS, LL, SS, MR, RA, JOTS, IP, and TT. The manuscript was written by IR and commented on by the other authors. The manuscript was revised after the reviewers' comments by IR, AMR, and HK, while the other authors had the opportunity to provide comments. All authors read and approved the final manuscript.

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Availability of data and material Research data are not shared due to privacy restrictions.

Code availability Not applicable.

Declarations

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Ethics approval This study was conducted retrospectively from data obtained for clinical purposes. According to local guidelines, a Research Ethics Committee approval was not required.

Consent to participate Consent to participate was not required.

Consent for publication Consent for publication was not required.

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Finnish multiple sclerosis patients treated with cladribine tablets: a nationwide registry study

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Finnish multiple sclerosis patients treated with cladribine tablets: a nationwide registry study

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ABSTRACT

Background: Cladribine tablets for adult patients with highly active relapsing multiple sclerosis (MS) have been available in Finland since 2018. Real-world data from different genetic and geographical backgrounds are needed to complement data from clinical trials.

Methods: We investigated the use of cladribine tablets in Finland in a non-interventional cohort study, based on real-world data from the nationwide Finnish MS registry. All eligible patients who had initiated treatment with cladribine tablets in 2018-2020 were included. Descriptive analyses for outcomes were conducted using summary statistics. Time-dependent endpoints were analyzed using cumulated events analysis based on 1-Kaplan–Meier estimates and curves. Subgroups were analyzed separately according to the number of previous disease-modifying therapies (DMTs) and the most common last preceding therapies.

Results: Data of 179 patients were analyzed. Median follow-up time was 19.0 months (interquartile range [IQR] 12.0-26.2). Of the 134 patients who were followed for at least 12 months, 112 patients (83.6%) remained relapse-free during follow-up. Mean annualized relapse rate (ARR) was 1.0 (standard deviation [SD] 0.89) at baseline, and 0.1 (SD 0.30) at follow-up. Patients with two or more previous DMTs had shorter time to first relapse (median 2.5 months, IQR 0.6-9.3) when compared to patients with 0-1 previous DMTs (median 11.4 months, IQR 8.7-13.1) (p=0.013). After excluding patients switching from fingolimod (n=33), a statistically significant difference in time to first relapse was no longer observed between the two groups (p=0.252). Adverse events (AEs) were reported in 30 patients (16.8%). The most frequent AE was headache (n=14, 7.8%). One patient (0.6%) died of cardiac arrest. Discontinuation of cladribine tablets was reported in nine patients (5.0%).

Abbreviations: ALC, absolute lymphocyte count; AE, adverse event; ARR, annualized relapse rate; CD, cluster of differentiation; CTCAE, Common Terminology Criteria for Adverse Events; DMF, dimethyl fumarate; DMT, disease-modifying therapy; EDSS, Expanded Disability Status Scale; IQR, interquartile range; JC virus, John Cunningham virus; mo, months; MRI, magnetic resonance imaging; MS, multiple sclerosis; SF, standard deviation.

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Conclusion: The mean ARR observed in this cohort was similar to what has been reported in clinical trials. Approximately half of the patients had used two or more previous DMTs before cladribine tablets. These patients had a shorter time to first relapse when compared to patients with 0-1 previous DMTs, mostly driven by early relapses in patients switching from fingolimod.

1. Introduction

Cladribine tablets for adult patients with highly active relapsing multiple sclerosis (MS) have been available in Finland since 2018 and fully reimbursed since 2020. Cladribine tablets are administered in two annual courses over two years (MAVENCLAD EU SmPC, 2021). Long-term clinical efficacy is expected to be acquired after full dosing, and can last for at least four years (Giovannoni et al., 2018, 2010). Treatment with cladribine leads to a transient reduction of B and T lymphocytes (Comi et al., 2019). There is a greater reduction of CD19+ B cells than CD4+ and CD8+ T cells (Comi et al., 2019). The subsequent lymphocyte kinetics, recovery of lymphocyte counts, and the reconstitution of immune function have been thought to explain the long-term therapeutic effect of cladribine (Stuve et al., 2019). Preferential reduction of memory B cells has been proposed to be the main driver of the therapeutic effect of cladribine (Ceronie et al., 2018).

Finland is a high-risk region for MS with an age-standardized prevalence ranging from 149/100 000 in Pirkanmaa to 276/100 000 in South Ostrobothnia during 2010-2016 (Pirttisalo et al., 2020). The national Finnish MS registry is used at both university and central hospitals to record attributes of MS patients during clinical practice (Laakso et al., 2019). The collected data can also be used for nationwide real-world studies.

Real-world data on cladribine tablets is still limited to a few cohorts (Bose et al., 2021; Lizak et al., 2021; Möhn et al., 2019; Patti et al., 2020; Pfeuffer et al., 2021; Rolfes et al., 2021). In the pivotal phase III CLARITY trial, patients who had failed two or more previous DMTs were excluded (Giovannoni et al., 2010). As a result, there is a need for real-world studies investigating the use of cladribine tablets especially in patients who have received at least two previous DMTs before cladribine treatment. The present study offers new insights into this subgroup of patients.

Here, we report the demographic details and clinical outcomes of patients treated with cladribine tablets from our non-interventional nationwide real-world cohort study in Finland. We set out to investigate treatment sequencing and the impact of previous disease-modifying therapies (DMTs) on clinical outcomes in patients treated with cladribine tablets.

2. Material and methods

2.1. Study design and outcomes

All patients in the Finnish MS registry who had initiated treatment with cladribine tablets for MS from January 1, 2018, to December 31, 2020, in the clinical setting were included. Data entries to the registry had been made either manually by health care professionals or by integration from hospital administrative data (Laakso et al., 2019). Demographic and clinical data were extracted on May 31, 2021. In the five hospital districts represented by authors IR, HK, SA, JOTS, MR, and MS-H, the registry data had been updated immediately prior to data extraction to minimize missing data. Magnetic resonance imaging (MRI) data were not included, as the national coverage of MRI data in the registry was insufficient for this study. When calculating the number of previous DMTs, all interferon treatments were grouped as one therapy. Discontinuation of cladribine tablets was defined as any of the following: an end date had been recorded (if, for example, the second-year dosing had been withheld); a new DMT had been initiated; or patient died during follow-up. Follow-up time was calculated from the first dose to either death, treatment switch minus one day (if a subsequent DMT had been initiated), or data extraction (if the patient was alive and no other DMT had been initiated). Efficacy outcomes included number of relapses, annualized relapse rate (ARR), time to first relapse, and Expanded Disability Status Scale (EDSS). Safety outcomes included adverse events (AEs) and absolute lymphocyte counts (ALCs). Lymphopenia was stratified according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (U.S. Department of Health and Human Services, National Institutes of Health, 2017): grade I ($<$1.0-0.8 $\times$ 10^9/L); grade II ($<$0.8-0.5 $\times$ 10^9/L); grade III ($<$0.5-0.2 $\times$ 10^9/L); and grade IV ($<$0.2 $\times$ 10^9/L) lymphopenia.

Since this was a registry study where patients were not contacted at any time, a Research Ethics Committee approval or patient consent was not required according to Finnish legislation. Permission from the Finnish National Institute for Health and Welfare was obtained to allow secondary use of patient data for the purpose of this study.

2.2. Statistical analysis

Data analysis and visualization was performed on pseudo-anonymized data using RStudio (Version 1.4.1103). Descriptive analyses were conducted using summary statistics. Numerical variables were expressed as means with standard deviations (SDs) or medians with interquartile ranges (IQRs). Categorical variables were expressed as frequencies and proportions based on non-missing data. Group comparisons for continuous variables were performed using the Wilcoxon rank-sum test or Student's t-test depending on the normality of the groups, and for the categorical variables using Fisher's exact test. For controlling and checking the False Discovery Rate, Benjamini–Hochberg procedure was used as a correction for multiple comparisons in demographic and clinical variables. Time-dependent endpoints were analyzed using cumulated events analysis based on 1-Kaplan–Meier estimates and curves. Log-rank test was utilized to assess differences between overall event probabilities.

Baseline EDSS was defined as the last recorded EDSS within one year before treatment initiation, and baseline ARR was defined as the number of relapses during that year. Two subgroup analyses were performed. The first comparison was between patients with two or more previous DMTs and patients with 0-1 previous DMTs prior to cladribine tablets. Group comparison testing and time-dependent endpoints were based on this subgroup analysis. A sensitivity analysis was also conducted to assess the reason for early relapses in the group of two or more previous DMTs. The second comparison was between patients with specific DMTs before cladribine tablets. In this analysis, glatiramer acetate, interferons and teriflunomide were grouped together as 'platform therapies' and dimethyl fumarate, fingolimod and natalizumab were analyzed separately. The first six months of each patient's washout period were examined for relapses.

3. Results

3.1. Study sample

The study sample included data on 179 patients from 16 of the 21 hospital districts in Finland, who were followed for a median 19.0 months (IQR 12.0-26.2). Follow-up time exceeded 12 months in 134 patients (74.9%) and 24 months in 57 patients (31.8%). Median EDSS at baseline was 2.0 (IQR 1.0-3.0) and mean ARR at baseline was 1.0 (SD 0.89). Demographic details and clinical parameters of the study sample

are presented in Table 1, and comparison between subgroups in Table 2 and Table 3.

A majority of the patients had received previous DMTs prior to cladribine tablets (n=126, 70.4%) (Table 1 and Fig. 1) and approximately half of the cohort had received two or more previous DMTs (n=92, 51.4%). At baseline, patients with two or more previous DMTs were older, had longer disease duration, and slightly higher EDSS when compared to patients with 0-1 previous DMTs prior to cladribine tablets (Table 2). They were also more often relapse-free at baseline and had lower baseline ARR when compared to patients with 0-1 previous DMTs (Table 2).

The most common last preceding DMT was fingolimod (n=33, 26.2%) followed by dimethyl fumarate (DMF) (n=29, 23.0%) and natalizumab (n=20. 15.9%). The reasons for discontinuing last preceding DMTs were inefficacy (n=65), AEs (n=32), anti-John Cunningham virus (anti-JC virus) antibodies (n=9), patient's wish (n=6), pregnancy (n=5), alteration of disease course (n=2), other (n=8), and unknown (n=7). Multiple reasons were reported in some patients. In patients with platform therapies or DMF as their last preceding therapy, the previous therapy was most frequently discontinued due to inefficacy (72.7% and 82.8%, respectively). Fingolimod was most frequently discontinued due to AEs (39.4%) or inefficacy (33.3%), whereas natalizumab was most frequently discontinued due to presence of anti-JC virus antibodies (45.0%). During washout period, relapses were reported in nine patients switching from fingolimod (27.3%), and one patient each switching from platform therapies (3.0%), DMF (3.4%), and natalizumab (5.0%) (Table 3).

3.2. Efficacy

A total of 154 patients (86.0%) remained relapse-free until the end of follow-up. In comparison, 112 of the 134 patients (83.6%) who were followed for at least 12 months remained relapse-free. After initiation of cladribine tablets, 32 relapses were reported in 25 patients (14.0%). Mean ARR was 0.1 during follow-up (SD 0.3) (Table 1). Notably, 49 of the 53 treatment-naive patients (92.5%) remained relapse-free until the end of follow-up, and mean ARR in treatment-naive patients was 0.1 (SD 0.2) during follow-up (Table 3). EDSS assessments were available in 116 patients at baseline (EDSS 2.0, IQR 1.0-3.0) and 55 patients at 12 months (EDSS 2.0, IQR 1.0-3.0, n=55). Subgroup analysis of EDSS scores was not performed due to the small number of patients with sufficient EDSS values during follow-up.

Median time to first relapse was 6.8 months (IQR 1.3-12.2) and was shorter in patients with two or more previous DMTs (median 2.5 months, IQR 0.6-9.3) when compared to patients with 0-1 previous DMTs (median 11.4 months, IQR 8.7-13.1) (Table 2, Fig. 2, log-rank test, p=0.013). Patients who had switched from fingolimod had a particularly short time to first relapse (median 1.3 months, IQR 0.6-2.7, Table 3). Also, of the 12 patients (6.7%) who experienced an early relapse within the first six months of follow-up, eight (66.7%) had switched from fingolimod. In contrast, only one of these patients (8.3%)

Table 1
Demographic details and clinical parameters of the study cohort.

	All patients n=179	
Before cladribine tablets initiation		
Sex category, n (%)		
Female	153	(85.5)
Male	26	(14.5)
Age, years, mean (SD)		
at diagnosis of MS	29.6	(8.46)
at cladribine tablets initiation	35.9	(9.86)
Disease duration, years, median [Q1, Q3]	4.2	[0.3, 11.2]
Course of disease, n (%)		
RRMS	177	(98.9)
SPMS	2	(1.1)
Number of previous DMTs, n (%)		
0	53	(29.6)
1	34	(19.0)
2	34	(19.0)
3 or more	58	(32.4)
EDSS at baseline, median [Q1, Q3]	2.0	[1.0, 3.0]
Relapses at baseline[a], n (%)		
No relapses	59	(33.0)
1 relapse	71	(39.7)
2 or more relapses	49	(27.4)
ARR at baseline[a], mean (SD)	1.0	(0.89)
After cladribine tablets initiation		
Relapses during follow-up, n (%)		
No relapses	154	(86.0)
1 relapse	18	(10.1)
2 relapses	7	(3.9)
ARR at follow-up, mean (SD)		
at entire follow-up	0.1	(0.3)
at 0-12 mo	0.1	(0.4)
at 12-24 mo	0.1	(0.2)
Time to first relapse[b], mo, median [Q1, Q3]	6.8	[1.3, 12.2]
Number of patients discontinuing cladribine tablets, n (%)	9	(5.0)

[a] During the last 12 months before initiation of cladribine tablets.
[b] In patients with relapses during follow-up.

Table 2
Patients stratified into two groups according to the number of previous disease-modifying therapies.

	0-1 previous DMTs n = 87		2 or more previous DMTs n = 92		p
Before cladribine tablets initiation					
Sex category female, n (%)	76	(87.4)	77	(83.7)	0.550
Age at cladribine tablets initiation, years, mean (SD)	33.5	(8.73)	38.2	(10.35)	0.007
Disease duration, years, median [Q1, Q3]	0.3	[0.2, 2.9]	9.5	[5.7, 14.0]	<0.001
Reason for discontinuing last preceding DMT, n (%)					
Inefficacy	26	(76.5)[a]	39	(42.4)	
Adverse events	4	(11.8)[a]	28	(30.4)	
JC virus	2	(5.9)[a]	7	(7.6)	
Patient's wish	1	(2.9)[a]	5	(5.4)	
Pregnancy	2	(5.9)[a]	3	(3.3)	
Alteration of disease course	1	(2.9)[a]	1	(1.1)	
Other or unknown	2	(5.9)[a]	12	(13)	
EDSS at baseline, median [Q1, Q3]	1.5	[1.0, 2.5]	2.0	[1.5, 3.5]	0.028
Relapses at baseline[b], n (%)					<0.001
No relapses	17	(19.5)	42	(45.7)	
1 relapse	36	(41.4)	35	(38.0)	
2 or more relapses	34	(39.1)	15	(16.3)	
ARR at baseline[b], mean (SD)	1.3	(0.89)	0.7	(0.81)	<0.001
After cladribine tablets initiation					
Relapses during follow-up, n (%)					0.031
No relapses	80	(92.0)	74	(80.4)	
1 relapse	7	(8.0)	11	(12.0)	
2 relapses	–		7	(7.6)	
ARR at follow-up, mean (SD)					
at entire follow-up	0.1	(0.18)	0.2	(0.38)	0.063
at 0-12 mo	0.1	(0.2)	0.2	(0.5)	0.063
at 12-24 mo	0.0	(0.2)	0.1	(0.3)	0.550
Time to first relapse[c], mo, median [Q1, Q3]	11.4	[8.7, 13.1]	2.5	[0.6, 9.3]	0.013
Number of patients discontinuing cladribine tablets, n (%)	2	(2.3)	7	(7.6)	0.208

[a] In the group of patients with 0-1 previous DMTs, the proportion displayed here is relative to patients with one previous DMT (n=34) and not the whole group.
[b] During the last 12 months before initiation of cladribine tablets.
[c] In patients with relapses during follow-up.

Table 3
Comparison between patients switching from the most common previous disease-modifying therapies as well as treatment-naive patients. The table does not represent the entire cohort, as patients switching from other disease-modifying therapies (alemtuzumab [n=6], daclizumab [n=1], ocrelizumab [n=3], and rituximab [n=1]) are not shown. For clarity, null values are not shown.

Last preceding DMT before cladribine tablets	Platform therapies[a]		Dimethyl fumarate		Fingolimod		Natalizumab		Treatment-naive	
	n = 33		n = 29		n = 33		n = 20		n = 53	
Before cladribine tablets initiation										
Sex category female, n (%)	27	(81.8)	22	(75.9)	27	(81.8)	17	(85.0)	50	(94.3)
Age at cladribine tablets initiation, years, mean (SD)	37.4	(10.39)	35.3	(8.41)	38.9	(10.48)	37.4	(11.83)	33	(9.17)
Disease duration, years, median [Q1, Q3]	7.2	[3.1, 10.1]	3.5	[2.3, 10.4]	11.2	[5.9, 13.8]	7.9	[4.6, 14.7]	0.2	[0.1, 0.3]
Number of previous DMTs, n (%)										
1	11	(33.3)	14	(48.3)	1	(3.0)	7	(35.0)	–	
2 or more	22	(66.7)	15	(51.7)	32	(96.7)	13	(65.0)	–	
Reason for discontinuing last preceding DMT, n (%)										
Inefficacy	24	(72.7)	24	(82.8)	11	(33.3)	3	(15.0)	–	
Adverse events	8	(24.2)	4	(13.8)	13	(39.4)	3	(15.0	–	
JC virus	–	–	–	–	–		9	(45.0)	–	
Patient's wish	1	(3.0)	1	(3.4)	2	(6.1)	2	(10.0)	–	
Pregnancy	–		–		3	(9.1)	2	(10.0)	–	
Alteration of disease course	–		–		–		2	(10.0)	–	
Other or unknown	1	(3.0)	1	(3.4)	4	(12.1)	5	(25.0)	–	
Washout from previous DMT, mo, median [Q1, Q3]	1.6	[0.7, 3.0]	0.9	[0.2, 2.1]	3.1	[2.2, 4.8]	3.1	[1.3, 6.3]	–	
Patients with relapses during washout[b], n (%)	1	(3.0)	1	(3.4)	9	(27.3)	1	(5.0)	–	
EDSS at baseline, median [Q1, Q3]	1.5	[1.0, 2.2]	2.5	[1.8, 4.0]	2.0	[2.0, 2.5]	2.2	[1.0, 3.8]	1.5	[0.2, 2.0]
Relapses at baseline[c], n (%)										
No relapses	11	(33.3)	12	(41.4)	13	(39.4)	15	(75.0)	2	(3.8)
1 relapse	17	(51.5)	11	(37.9)	13	(39.4)	5	(25.0)	20	(37.7)
2 or more relapses	5	(15.2)	6	(20.7)	7	(21.2)	–		31	(58.5)
ARR at baseline[c], mean (SD)	0.8	(0.76)	0.8	(0.77)	0.9	(0.89)	0.2	(0.44)	1.7	(0.77)
After cladribine tablets initiation										
Relapses during follow-up, n (%)										
No relapses	30	(90.9)	25	(86.2)	23	(69.7)	17	(85.0)	49	(92.5)
1 relapse	3	(9.1)	2	(6.9)	7	(21.2)	1	(5.0)	4	(7.5)
2 relapses	–		2	(6.9)	3	(9.1)	2	(10.0)	–	
ARR at entire follow-up, mean (SD)	0.1	(0.22)	0.2	(0.41)	0.2	(0.37)	0.2	(0.42)	0.1	(0.20)
Time to first relapse[d], mo, median [Q1, Q3]	6.8	[3.6, 15.8]	5.7	[2.4, 10.3]	1.3	[0.6, 2.7]	7.4	[5.5, 16.6]	11.8	[10.8, 12.6]
Patients discontinuing cladribine tablets, n (%)	–		1	(3.4)	6	(18.2)	1	(5.0)	1	(1.9)

[a] Glatiramer acetate (n=7), interferons (n=13) and teriflunomide (n=13).
[b] The first six months of each patient's washout period were examined for relapses.
[c] During the last 12 months before initiation of cladribine tablets.
[d] In patients with relapses during follow-up.

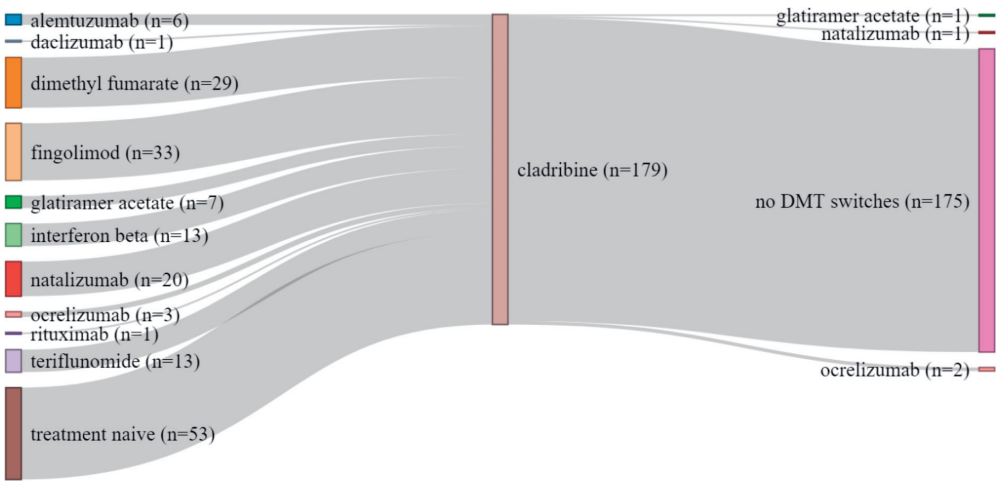

Fig. 1. Treatment sequencing before and after cladribine tablets. Last preceding therapies are presented on the left. Subsequent therapies after a treatment switch are presented on the right.

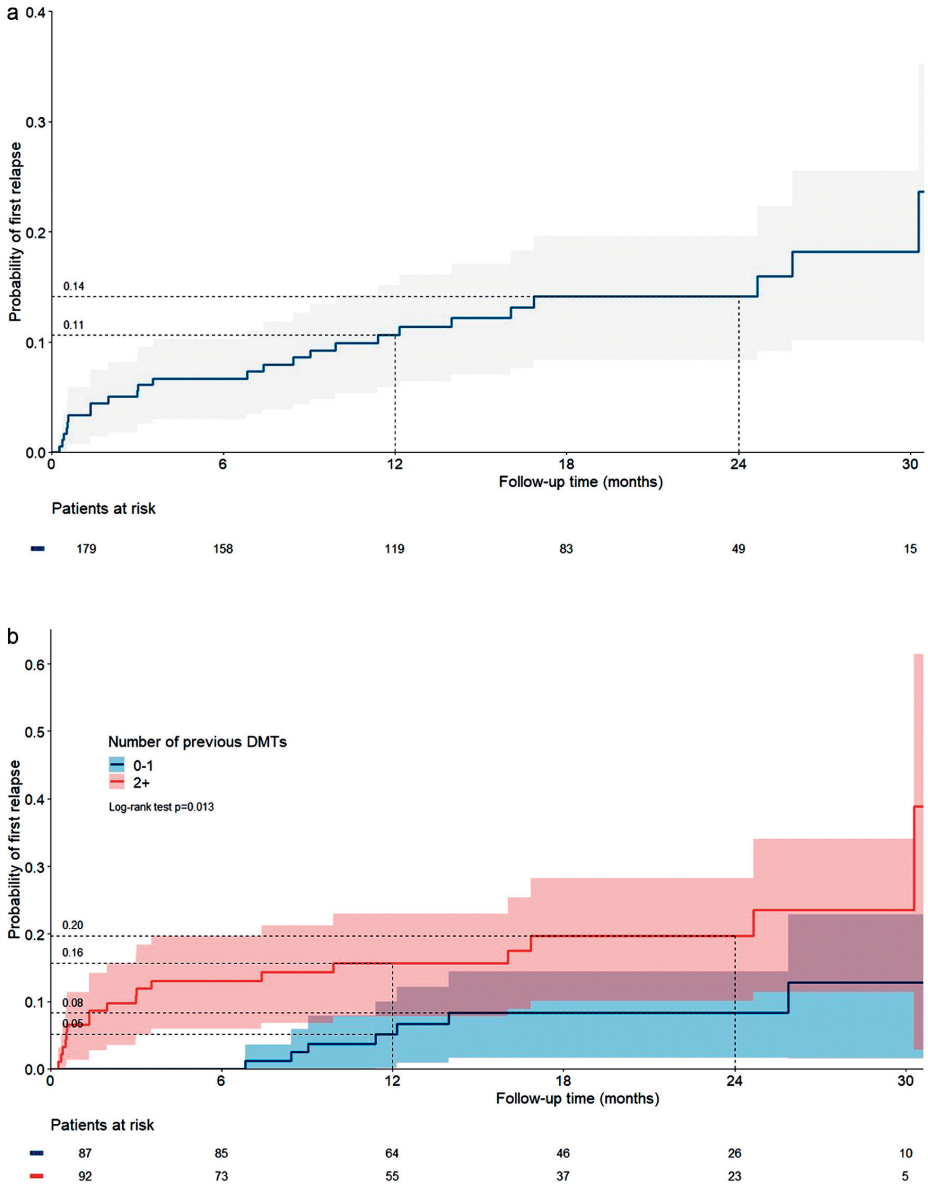

Fig. 2. A cumulative events curve displaying probability of first relapse (and 95% confidence intervals) during follow-up: a) all patients, b) according to the number of previous disease-modifying therapies (DMTs)

Table 4
Adverse events reported during follow-up. For clarity, proportions are not shown in categories with only 1 patient (0.6%).

	Patients with event	
	n	%
Any adverse event	30	16.8
Infections and infestations		
Herpetic skin infection	8	4.5
Herpes simplex	7	3.9
Herpes zoster	1	
Non-herpetic skin or mucocutaneous infection[a]	5	2.8
Upper respiratory infection	5	2.8
Unspecified infection	1	
Urinary tract infection	1	
Vaginal infection	1	
Nervous system disorders		
Headache	14	7.8
Dysesthesia	1	
Gastrointestinal disorders		
Nausea	7	3.9
Abdominal pain	2	1.1
Diarrhea	2	1.1
Dyspepsia	1	
Skin disorders		
Alopecia	2	1.1
Acne	1	
Dermatitis	1	
General disorders		
Malaise	2	1.1
Musculoskeletal disorders		
Back pain	2	1.1
Cardiovascular disorders		
Cardiac arrest	1	
Hepatobiliary disorders		
Hepatitis[b]	1	
Psychiatric disorders		
Insomnia	1	

[a] Non-herpetic skin or mucocutaneous infection includes terms abscess, furunculus, ringworm, and tinea pedis.
[b] Etiology of hepatitis is not reported.

had switched from natalizumab. To test the impact of a possible disease reactivation effect after fingolimod, all patients switching from fingolimod (n=33) were excluded, after which there was no difference in the time to first relapse between patients with 0-1 and two or more previous DMTs (p=0.252, data not shown).

3.3. Safety

3.3.1. Adverse events

A total of 61 AEs were reported in 30 patients (16.8%) (Table 4). The most frequent AEs were headache (n=14, 7.8%), *Herpes simplex* (n=7, 3.9%), and nausea (n=7 patients 3.9%). Only one *Herpes zoster* infection (0.6%) was reported in a patient who switched from natalizumab to cladribine tablets. In addition to herpetic infections, seven other AEs suggestive of representing skin or mucocutaneous infections (abscess, furunculus, ringworm, and tinea pedis) were reported in altogether seven patients (3.9%), although the site of these infections was not specified. Notably, a single case of unspecified hepatitis was reported (0.6%). One patient died of cardiac arrest 1.4 years after the second-year dosing of cladribine tablets.

3.3.2. Lymphocytes

ALCs were available in 166 patients (92.7%). Of these patients, 29 (17.5%) had grade I, 58 (34.9%) grade II, and 37 (22.3%) grade III lymphopenia at any point during follow-up. No grade IV lymphopenia was recorded. Before the second-year dosing at 12 months, 7/95 (7.4%) patients had an ALC lower than 0.8 10^9/L (Fig. 3).

ALCs remained within normal limits in 42 (25.3%) patients. To investigate whether clinical efficacy was altered in this population, an additional descriptive analysis on relapse rates was performed. Five of these patients (11.9%) experienced a relapse during follow-up, and mean ARR in patients without lymphopenia was 0.1 (SD 0.3), thus being in line with the total cohort.

3.4. Treatment discontinuation and subsequent therapies

Cladribine tablets were discontinued in nine patients (5.0%), seven of whom had used two or more previous DMTs before cladribine tablets. Reasons for treatment discontinuation were variable. These included: AEs (n=2); inefficacy (n=2); change of diagnosis (n=1); death (n=1); medication to a comorbidity (n=1); and unknown (n=2). The two AEs resulting in discontinuation included lymphopenia and lymphopenia together with hepatitis. One patient was diagnosed with an autoimmune comorbidity, which required treatment with another immunosuppressive agent, leading to the termination of cladribine tablets after the first year of treatment.

Discontinuation was most common among patients who had switched from fingolimod to cladribine tablets (n=6/33, 18.2%) (Table 3). However, no common cause for discontinuation could be identified among these patients (data not shown). After treatment discontinuation, a subsequent DMT was initiated in four patients (2.2%). These patients had discontinued cladribine tablets during their first (n=2), second (n=1), or third (n=1) treatment year. The subsequent DMTs included ocrelizumab (n=2), glatiramer acetate (n=1), and natalizumab (n=1) (Fig. 1).

4. Discussion

In this nationwide real-world study, we used data from the Finnish MS registry to describe the demographic details and clinical outcomes of 179 patients treated with cladribine tablets in Finland. After the initiation of cladribine tablets, mean ARR was 0.1 during a median follow-up of 19.0 months. For comparison, in the clinical CLARITY trial, ARR was 0.14 during a 96-week study, whereas in a previous real-world study, ARR was 0.31 during a median follow-up of 3.5 years (Giovannoni et al., 2010; Lizak et al., 2021). The higher ARR observed in the real-world study by Lizak et al. may reflect differences in follow-up times and baseline characteristics between studies. Also, the majority of patients had received only their first year dose of cladribine, which may influence efficacy outcomes (Lizak et al., 2021).

In the present study, patients initiating cladribine tablets for MS were characterized by a high female to male ratio in contrast to previous cohorts (Bose et al., 2021; Lizak et al., 2021; Pfeuffer et al., 2021; Rolfes et al., 2021). The reason for the relative paucity of male individuals especially among treatment naive patients in this cohort is unknown. Cultural or family planning preferences may attribute to this finding. Two distinct subgroups could be identified according to previous DMT use. Approximately half of the patients were either naive to treatment or had used one previous DMT, while the rest had used two or more previous DMTs before switching to cladribine tablets. Patients with two or more previous DMTs had a more advanced disease in contrast to patients with 0-1 previous DMTs, characterized by longer disease duration, less relapses, and slightly higher EDSS before switching to cladribine tablets. These findings are not unexpected, as they reflect not only the natural course of MS, but also current treatment guidelines and reimbursement criteria.

In Finland, reimbursement criteria for the use of cladribine tablets are in line with the therapeutic indication evaluated by the European Medicines Agency (European Medicines Agency, 2021). Treatment-naive patients are entitled to full reimbursement of cladribine tablets if they have experienced at least two relapses within one year and have inflammatory findings in an MRI, whereas patients switching from other therapies may be entitled to full reimbursement despite being relapse-free in case of an inflammatory MRI finding while

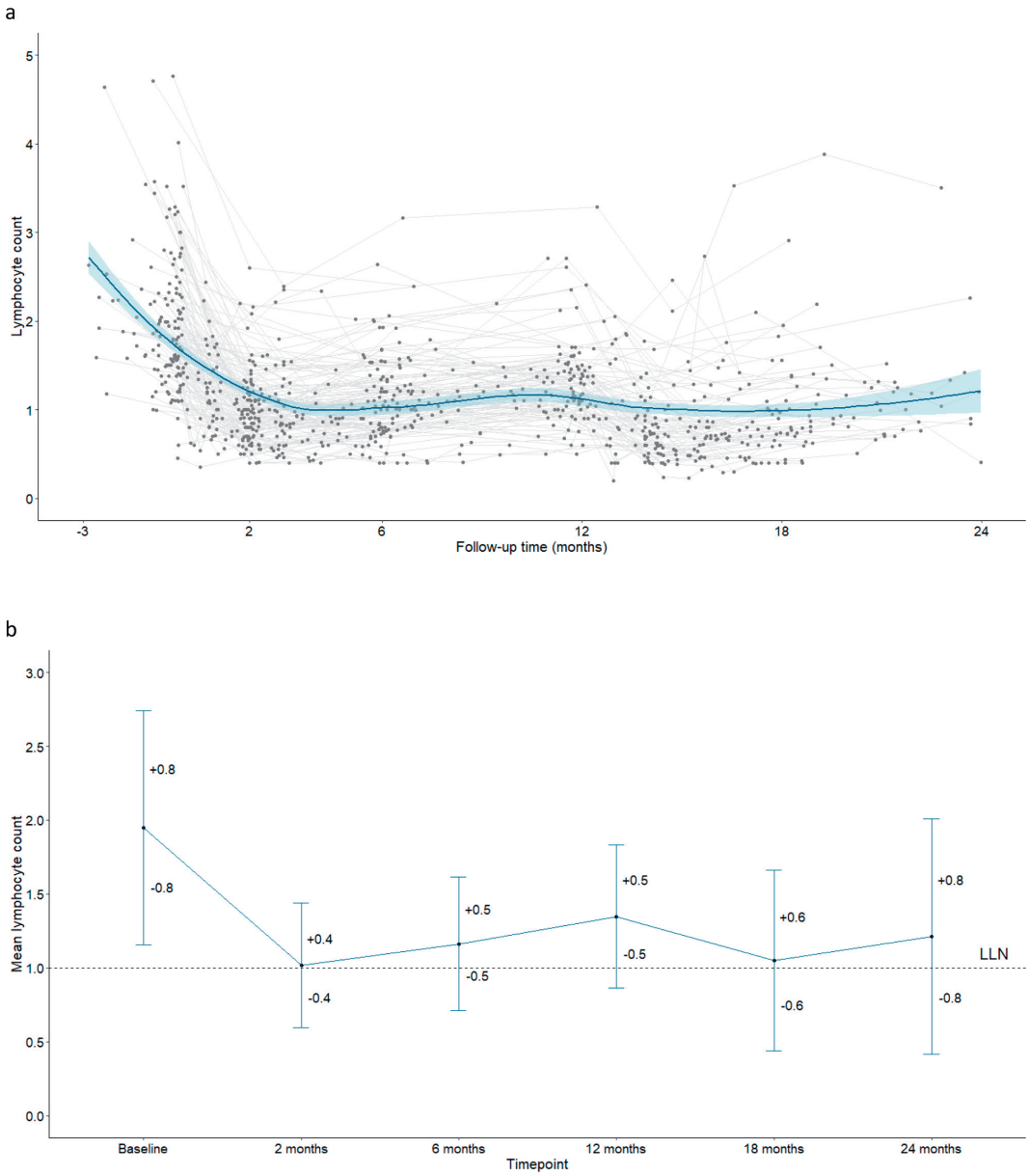

Fig. 3. Mean absolute lymphocyte counts (10^9/L) before and after initiation of cladribine tablets: a) absolute values, b) means and standard deviations at time points of special interest. LLN = Lower Limit of Normal

on previous DMT. In light of these stringent reimbursement criteria, it is surprising that 33% of the patients in our cohort were relapse-free during the year before treatment initiation. This may be explained by clinically stable patients switching therapies due to tolerability issues, family planning, presence of anti-JC virus antibodies, or radiological disease activity.

Patients with two or more previous DMTs had a shorter time to first relapse when compared to patients who were treatment-naive or had used one previous DMT. The former group has not been investigated in clinical trials, therefore giving new information on the outcomes of cladribine tablet use in a real-world setting. In patients with two or more previous DMTs, time to first relapse was very short (median 2.5 months). This effect was shown to be driven by early relapses in patients switching from fingolimod to cladribine tablets, likely representing disease reactivation or rebound after fingolimod. This is not entirely unexpected, as disease reactivation after fingolimod discontinuation has been reported (Barry et al., 2019). There are few previous reports on patients switching from fingolimod to cladribine tablets, including

description of cases with disease reactivation (Coss-Rovirosa et al., 2020; Prosperini et al., 2019).

Interestingly, while patients switching from fingolimod seemed to be at risk for disease reactivation both during washout and after the switch, few patients switching from natalizumab showed signs of clinical disease activity despite similar washout period lengths. The number of patients in each subgroup was small and follow-up time was limited, and therefore, any conclusions should be made with caution. Although discontinuation of natalizumab has been associated with an increased risk for relapses or disease reactivation (Mustonen et al., 2020; Prosperini et al., 2019), conflicting results have been published on the occurrence of disease reactivation when switching from natalizumab to cladribine tablets (Möhn et al., 2019; Pfeuffer et al., 2021). In our cohort, the most common reason for discontinuing natalizumab was the presence of anti-JC virus antibodies. Nevertheless, disease reactivation has also been shown to occur in patients discontinuing natalizumab due to presence of anti-JC virus antibodies, as shown in an observational study comparing rituximab and fingolimod after natalizumab (Alping et al., 2016).

The most frequently reported AE was headache, which is in line with the findings from the clinical trial program (Cook et al., 2019; Giovannoni et al., 2010). A recent prospective bicentric cohort study reported skin reactions to be common after cladribine tablets (Rolfes et al., 2021). In our study, skin disorders including alopecia, acne, and dermatitis were reported in five patients (2.8%). Furthermore, seven patients (3.9%) experienced AEs which could be categorized as non-herpetic skin or mucocutaneous infections, although the site of infection in these AEs was not reported. One case of hepatitis was reported, which is interesting, as hepatobiliary disorders have also been reported in the clinical trials (Giovannoni et al., 2010; Montalban et al., 2018). We also report one fatality in a patient who died of cardiac arrest 1.4 years after the last dose of cladribine tablets.

Most patients experienced mild or moderate lymphopenia. Grade III lymphopenia was detected in 22.3% of the patients, which is similar to the 25% observed at any time during the clinical trials (Cook et al., 2019). Also, no grade IV lymphopenia was recorded, in contrast to the <1% of recorded cases in the clinical trial program (Cook et al., 2019). Most patients' lymphocytes recovered before the second-year dose of cladribine tablets. A small subgroup of patients had no reduction in ALCs. Nevertheless, clinical outcomes of these patients were comparable to the total cohort. This is not unexpected, as the mode of action of cladribine has been linked to the kinetics of specific subtypes of lymphocytes rather than total lymphocyte numbers (Baker et al., 2017; Comi et al., 2019; Stuve et al., 2019).

The strengths of the present study include the relatively large sample size and the good national coverage of the Finnish MS registry. Our cohort represents real-world MS patients treated at both secondary and tertiary centers in Finland. These types of cohort studies provide unique insights into diverse populations, including patients with different ethnic or genetic backgrounds, variable comorbidities, and previous exposure to multiple DMTs – in contrast to clinical trials – where stringent inclusion and exclusion criteria may limit generalizability. However, due to its non-randomized setting, the present study is not optimal for assessing efficacy outcomes, and regression to mean may affect relapse outcomes. EDSS data during follow-up were limited, and therefore, conclusions about long-term disability outcomes could not be made. Other limitations include the possibility of missing data due to the voluntary nature in which data entries to the Finnish MS registry are made, as well as the lack of MRI data.

In conclusion, this registry study demonstrated that approximately half of the patients initiating cladribine tablets in Finland had used two or more previous DMTs before switching to cladribine. These patients had a particularly short time to first relapse, driven mostly by early relapses experienced by patients switching from fingolimod, and likely representing rebound after fingolimod. However, ARR during follow-up was low in the total cohort. Headache was the most frequent AE. Overall, these data support the findings from clinical trials on the clinical outcomes and safety of cladribine tablets in a real-life setting.

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CRediT authorship contribution statement

Ilkka Rauma: Methodology, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Matias Viitala:** Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Hanna Kuusisto:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Sari Atula:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Jussi O T Sipilä:** Methodology, Investigation, Writing – review & editing. **Mervi Ryytty:** Methodology, Investigation, Writing – review & editing. **Merja Soilu-Hänninen:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Elina Järvinen:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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