JUSTUS-LIEBIG-UNIVERSITÄT GIEßEN

Fachbereich Medizin

Neurologische Klinik
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Die Sicherheit und Wirksamkeit der Immunrekonstitutionstherapie in der Regelversorgung der Multiplen Sklerose

Habilitationsschrift
zur Erlangung der Lehrbefähigung für das Fach Neurologie
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vorgelegt von

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Die kumulative Habilitationsschrift beschäftigt sich mit der Bedeutung der Therapiehistorie und –Sequenz bei Patienten, die eine sog. Immunrekonstitutionstherapie zur Behandlung der schubförmig verlaufenden Multiplen Sklerose erhalten, auseinander. Dieser Arbeit liegen 13 eigene Veröffentlichungen zugrunde. Im Einzelnen setzen sich diese auseinander mit:

- Dem unmittelbaren Einfluss der immunmodulatorischen Vortherapie auf die Sicherheit und Wirksamkeit einer Therapie mit dem Medikament Alemtuzumab

Pfeuffer S*, Ruck T*, Pul R, Rolfes L, Korsukewitz C, Pawlitzki M, Wildemann B, Klotz L, Kleinschnitz C, Scalfari A, Wiendl H, Meuth SG. Impact of previous disease-modifying treatment on effectiveness and safety outcomes, among patients with multiple sclerosis treated with alemtuzumab. J Neurol Neurosurg Psychiatry. 2021 Sep;92(9):1007-1013. doi: 10.1136/jnnp-2020-325304.

- Den Veränderungen des immunologischen Netzwerkes durch das Medikament Alemtuzumab und hieraus ableitbare Implikationen für die Entwicklung sekundärer Autoimmunität

Ruck T*, Barman S*, Schulte-Mecklenbeck A*, **Pfeuffer S***, Steffen F, Nelke C, Schroeter CB, Willison A, Heming M, Müntefering T, Melzer N, Krämer J, Lindner M, Riepenhausen M, Gross CC, Klotz L, Bittner S, Muraro PA, Schneider-Hohendorf T, Schwab N, Meyer Zu Hörste G, Goebels N, Meuth SG, Wiendl H. Alemtuzumab-induced immune phenotype and repertoire changes: implications for secondary autoimmunity. Brain. 2022 Jun 3;145(5):1711-1725. doi: 10.1093/brain/awac064.

- Den Veränderungen der peripheren Immunglobulin-Spiegel nach Therapie mit Alemtuzumab und die Assoziation zu bakteriellen Infekten

Möhn N, **Pfeuffer S**, Ruck T, Gross CC, Skripuletz T, Klotz L, Wiendl H, Stangel M, Meuth SG. Alemtuzumab therapy changes immunoglobulin levels in peripheral blood and CSF. Neurol Neuroimmunol Neuroinflamm. 2019 Dec 11;7(2):e654. doi: 10.1212/NXI.0000000000000654.

- Dem Nutzen einer Therapie mit Alemtuzumab gegenüber einer Therapie mit Fingolimod nach Vorbehandlung von Patienten mit Natalizumab.

Pfeuffer S, Schmidt R, Straeten FA, Pul R, Kleinschnitz C, Wieshuber M, Lee DH, Linker RA, Doerck S, Straeten V, Windhagen S, Pawlitzki M, Aufenberg C, Lang M, Eienbroeker C, Tackenberg B, Limmroth V, Wildemann B, Haas J, Klotz L, Wiendl H, Ruck T, Meuth SG. Efficacy and safety of alemtuzumab versus fingolimod in RRMS after natalizumab cessation. J Neurol. 2019 Jan;266(1):165-173. doi: 10.1007/s00415-018-9117-z.

- Der Bedeutung des Schilddrüsen-Autoantikörper-Status für die Vorhersage von sekundärer Autoimmunität bei Patienten vor Behandlung mit Alemtuzumab

Ruck T, Schulte-Mecklenbeck A, **Pfeuffer S**, Heming M, Klotz L, Windhagen S, Kleinschnitz C, Gross CC, Wiendl H, Meuth SG. Pretreatment anti-thyroid autoantibodies indicate increased risk for thyroid autoimmunity secondary to alemtuzumab: A prospective cohort study. EBioMedicine. 2019 Aug;46:381-386. doi: 10.1016/j.ebiom.2019.07.062.

- Dem Auftreten einer Vitiligo oder einer Sarkoidose als neuartiges sekundäres Autoimmunphänomen nach Behandlung mit Alemtuzumab.

Ruck T, **Pfeuffer S**, Schulte-Mecklenbeck A, Gross CC, Lindner M, Metze D, Ehrchen J, Sondermann W, Pul R, Kleinschnitz C, Wiendl H, Meuth SG, Klotz L. Vitiligo after alemtuzumab treatment: Secondary autoimmunity is not all about B cells. Neurology. 2018 Dec 11;91(24):e2233-e2237. doi: 10.1212/WNL.0000000000006648.

Pfeuffer S. Sarcoidosis following alemtuzumab treatment: Autoimmunity mediated by T cells and interferon-γ. Mult Scler. 2018 Nov;24(13):1783-1784. doi: 10.1177/1352458518804124.

- Dem unmittelbaren Einfluss der immunmodulatorischen Vortherapie auf die Sicherheit und Wirksamkeit einer Therapie mit dem Medikament Ocrelizumab

Pfeuffer S, Rolfes L, Ingwersen J, Pul R, Kleinschnitz K, Korsen M, Räuber S, Ruck T, Schieferdecker S, Willison AG, Aktas O, Kleinschnitz C, Hartung HP, Kappos L, Meuth SG. Effect of Previous Disease-Modifying Therapy on Treatment Effectiveness for Patients Treated With Ocrelizumab. Neurol Neuroimmunol Neuroinflamm. 2023 Apr 11;10(3):e200104. doi: 10.1212/NXI.0000000000200104.

- Der Bedeutung eines variablen Dosisintervalls einer Immuntherapie mit Ocrelizumab in Zeiten der COVID-19-Pandemie

Rolfes L, Pawlitzki M, **Pfeuffer S**, Nelke C, Lux A, Pul R, Kleinschnitz C, Kleinschnitz K, Rogall R, Pape K, Bittner S, Zipp F, Warnke C, Goereci Y, Schroeter M, Ingwersen J, Aktas O, Klotz L, Ruck T, Wiendl H, Meuth SG. Ocrelizumab Extended Interval Dosing in Multiple Sclerosis in Times of COVID-19. Neurol Neuroimmunol Neuroinflamm. 2021 Jul 14;8(5):e1035. doi: 10.1212/NXI.0000000000001035.

- Dem unmittelbaren Einfluss der immunmodulatorischen Vortherapie auf die Sicherheit und Wirksamkeit einer Therapie mit dem Medikament Cladribin

Pfeuffer S, Rolfes L, Hackert J, Kleinschnitz K, Ruck T, Wiendl H, Klotz L, Kleinschnitz C, Meuth SG, Pul R. Effectiveness and safety of cladribine in MS: Real-world experience from two tertiary centres. Mult Scler. 2022 Feb;28(2):257-268. doi: 10.1177/13524585211012227.

- Den langfristigen laborchemisch in der klinischen Routine fassbaren Veränderungen nach Therapie mit Cladribin

Rolfes L, **Pfeuffer S**, Huntemann N, Schmidt M, Su C, Skuljec J, Aslan D, Hackert J, Kleinschnitz K, Hagenacker T, Pawlitzki M, Ruck T, Kleinschnitz C, Meuth SG, Pul R. Immunological consequences of cladribine treatment in multiple sclerosis: A real-world study. Mult Scler Relat Disord. 2022 Aug;64:103931. doi: 10.1016/j.msard.2022.103931.

- Der Wirksamkeit der saisonalen Influenza-Impfung bei Patienten, die eine Therapie mit Cladribin erhielten

Rolfes L*, **Pfeuffer S***, Skuljec J, He X, Su C, Oezalp SH, Pawlitzki M, Ruck T, Korsen M, Kleinschnitz K, Aslan D, Hagenacker T, Kleinschnitz C, Meuth SG, Pul R. Immune Response to Seasonal Influenza Vaccination in Multiple Sclerosis Patients Receiving Cladribine. Cells. 2023 Apr 25;12(9):1243. doi: 10.3390/cells12091243.

- Dem Auftreten seltener Hautreaktionen bei Patienten unter Therapie mit Cladribin

Rolfes L, **Pfeuffer S**, Hackert J, Pawlitzki M, Ruck T, Sondermann W, Korsen M, Wiendl H, Meuth SG, Kleinschnitz C, Pul R. Skin Reactions in Patients With Multiple Sclerosis Receiving Cladribine Treatment. Neurol Neuroimmunol Neuroinflamm. 2021 Apr 9;8(3):e990. doi: 10.1212/NXI.0000000000000990.

INHALTSVERZEICHNIS

1. EINFÜHRUNG

Die Multiple Sklerose (MS) betrifft weltweit aktuell rund 2,9 Millionen Menschen. In Deutschland wird die Zahl der Erkrankten auf knapp 280.000-300.000 Einwohner geschätzt. Damit ist sie eine der häufigsten chronisch-entzündlichen Erkrankungen in Deutschland.[1] Zahlreiche Registerstudien belegen den erheblichen sozioökonomischen Schaden der Erkrankung, welcher allein die durchschnittlichen medizinischen Behandlungskosten pro Jahr um den Faktor 14 gegenüber der Normalbevölkerung steigert.[2] Das wissenschaftliche Verständnis der Erkrankung ist weiterhin allenfalls lückenhaft und trotz der zahlreichen neu zugelassenen Medikamente leidet ein substanzieller Bestandteil der Patienten an zunehmender Behinderung durch die Erkrankung. Die Lebenserwartung wird durch die Erkrankung um etwa sieben Jahre im Vergleich zur Durchschnittsbevölkerung reduziert.[3]

1.1 Kursorische Einführung in den Erkrankungsverlauf und Begrifflichkeiten

Die pathophysiologischen Grundlagen der Erkrankung sollen hier nur soweit, wie es für das Verständnis der nachfolgenden Habilitationsschrift erforderlich ist, eingeführt werden. Ausführlichere Übersichten für den Kliniker finden sich unter anderem bei REICH und Kollegen.[4]

Im Rahmen der Erkrankung kommt es, bereits Jahre vor dem ersten Auftreten von klinischen Symptomen, zu einer Aktivierung von T- und B-Lymphozyten, welche sich gegen Bestandteile des zentralen Nervensystems (ZNS) richten. Die Determinanten dieses Prozesses sind nicht abschließend verstanden, jedoch scheinen sowohl genetische als auch erworbene Risikofaktoren eine Rolle zu spielen.[5]

Diese autoreaktiven Immunzellen besitzen ohne weiteres nicht die Fähigkeit, die Blut-Hirn-Schranke, welche durch das Endothel der hirnversorgenden Blutgefäße sowie der daran angrenzenden Glia des Hirnparenchyms gebildet wird, zu überwinden. Unter bestimmten, aktuell nicht näher verstandenen Bedingungen exprimiert die Blut-Hirn-Schranke jedoch Adhäsions-Moleküle, über welche die Lymphozyten in das Hirngewebe einwandern und eine Entzündungsreaktion hervorrufen können.[6] Im Rahmen dieser akuten Entzündung kommt es vorwiegend zu einer Schädigung der Myelinscheiden der Nervenfasern (Demyelinisierung) sowie, in variablem Ausmaß, auch zu einem neuroaxonalen Schaden selbst.[7]

Die akute Entzündungsreaktion kann z.B. mittels Kernspin-Tomographie (MRT) durch die Affinität zu Gadolinium-haltigem Kontrastmittel sichtbar gemacht werden. Die akute Entzündung dauert etwa sechs Wochen an und hiernach schließt sich eine Phase der Regeneration des betroffenen Gewebes an. Diese Regeneration verläuft in variablem

Ausmaß, jedoch nie vollständig. Abgelaufene Demyelinisierungen können zeitlebens in der kranialen MRT nachgewiesen werden und werden als „Läsionen“ oder umgangssprachlich auch „Herde“ bezeichnet.[8]

Prinzipiell kann eine solche fokale Entzündungsreaktion an jeder Stelle des zentralen Nervensystems (ZNS) auftreten, auch wenn gewisse Prädilektionsstellen existieren (z.B. die Balkenregion, das zervikale Myelon oder der Sehnerv). Tritt eine solche fokale Entzündung an eloquenter Stelle des Nervensystems auf, wird sie durch entsprechende neurologische Defizite manifest (dies wird als „Schub“ bezeichnet). Statistisch betrachtet ist dies nur bei jeder fünften bis zehnten Läsion der Fall, sodass das MRT entsprechend deutlich sensitiver bezüglich der Krankheitsaktivität ist.[9]

Analog den oben genannten Regenerationsprozessen bilden sich die Symptome eines Schubes in variabler Ausprägung zurück. Vereinfacht gesprochen definiert also das Produkt aus der Anzahl erlittener Schübe und der jeweiligen Residuen das Gesamtmaß der klinischen Behinderung. Die Phase der Erkrankung, in welcher Schübe das klinische Bild dominieren, wird „(vorherrschend) schubförmige MS“ (*relapsing MS* (RMS)) bezeichnet. Dies ist das Patientenkollektiv, welches in dieser Habilitationsschrift vorwiegend thematisiert wird.[5]

Unabhängig hiervon existiert bei der MS von Beginn an eine chronische entzündliche Neurodegeneration, welche sich unter anderem durch eine im Vergleich zur gesunden Durchschnittsbevölkerung stark beschleunigte Hirnatrophie zeigt (0,3-0,4%/Jahr vs. 0,1-0,2%/Jahr).[10] Zudem leiden Patienten von Beginn an (tatsächlich ist dies bereits bei pädiatrischen MS-Patienten nachweisbar) an einer schleichenden Verschlechterung der neurologischen Funktion. Diese ist zwar mittels der aktuell in der Regelversorgung etablierten Assessments teils schwer und oft nur verzögert

nachweisbar, macht über die gesamte Länge des Krankheitsverlaufes letztlich jedoch einen großen Teil der Gesamtbehinderung aus.[11]

Aus historischen Gründen ist diese „Progression“ der Erkrankung vorwiegend bei Patienten, die keine nennenswerte klassisch-entzündliche „Aktivität“ mehr aufweisen, nachweisbar. Da bei dem Großteil der Patienten die Aktivität im Laufe des Lebens respektive mit zunehmender Erkrankungsdauer nachlässt, wird in diesem Kontext oft noch von „sekundär-progredienter MS“ (sPMS) gesprochen, auch wenn dieser Begriff zunehmend überholt erscheint und durch den allgemeineren Begriff der „progredienten MS“ (PMS) verdrängt wird.[12]

Ein geringer Anteil der Patienten leidet an einer „primär progredienten MS“ (pPMS), die in ihrem Krankheitsverlauf zu keinem Zeitpunkt schubförmige Verschlechterungen aufweist.[13] Hierauf soll im Kontext dieser Arbeit nicht weiter eingegangen werden, da sich diagnostische Kriterien und insbesondere therapeutische Strategien teils erheblich unterscheiden.

Das Gesamtmaß der Behinderung eines Patienten wird angesichts des Dilemmas, dass die MS durch ihre variable Ausprägung in unterschiedlichen Funktionsbereichen des zentralen Nervensystems klinisch sehr unterschiedlich manifest sein kann, durch einen Komposit-Score beschrieben. Der „*expanded disability status scale*“ (EDSS)-Score (kurz als „der EDSS“ bezeichnet) bildet acht Funktionsbereiche des Nervensystems ab (‚visuelles System‘, ‚Hirnstammfunktion‘, ‚Pyramidenbahn‘, ‚Kleinhirnfunktion‘, ‚sensibles System‘, ‚autonomes System‘, ‚neurokognitive Funktion‘ und ‚Gehfähigkeit‘) und kann Werte von 0 (kein nachweisbares Defizit in der formalen Untersuchung) bis 10 (Tod durch MS) annehmen.[14] Als „Meilensteine“ werden oft die EDSS-Werte 3,0 (moderate singuläre Funktionseinschränkung oder multiple milde

Funktionseinschränkungen; hier besteht regelhaft Alltagsrelevanz) und 6,0 (Verlust des freien Gehens ohne Hilfsmittel) angesehen.[15]

Die Zunahme des EDSS-Wertes wird, in Abhängigkeit des Ausgangswertes, bei Überschreiten bestimmter Grenzen als „signifikant“ angesehen (z.B. bis zu einem Ausgangswert von 5,0 werden Anstiege um 1,0 Punkte als klinisch relevant gewertet, danach Anstiege bereits um 0,5 Punkte). Da sich nach einem Schub eine längere Regenerationsphase mit möglichem Wiedererlangen zeitweilig verloren gegangener Funktion anschließen kann, wird eine Zunahme der Behinderung im klinischen und wissenschaftlichen Kontext meist nach drei bis sechs Monaten erneut evaluiert, bevor sie als „bestätigte Zunahme der Behinderung“ („*confirmed disability worsening*“ (CDW)) angesehen wird.[16,17]

1.2 Notwendigkeit und Nutzen der Immuntherapie der Multiplen Sklerose

Grundsätzlich existieren drei Säulen der medikamentösen Behandlung der Multiplen Sklerose: (1) Die Behandlung akuter Krankheitsschübe, (2) die symptomatische Behandlung neurologischer Dysfunktion, (3) die immunmodulatorische oder „verlaufsmodifizierende“ Behandlung („*disease-modifying treatment*“ (DMT)), welche das Auftreten einer neuen Krankheitsaktivität und möglichst auch -Progression verhindern soll. Diese letzte Therapie wird in dieser Habilitationsschrift kurz als „Immuntherapie“ bezeichnet.

Ziel der Behandlung akuter Krankheitsschübe ist es, das neu aufgetretene Defizit möglichst rasch zurückzubilden, beziehungsweise eine weitere Verschlechterung zu verhindern. Erstlinientherapie ist die intravenöse, hochdosierte Kortikosteroid-Stoßtherapie, wobei sich Methylprednisolon in einer Dosis von 500-1000mg pro Tag über 3-5 Tage als Goldstandard etabliert hat. Sollte sich im Rahmen dieser Behandlung keine optimale Krankheitskontrolle erreichen lassen, stehen extrakorporale Apherese-Verfahren (Plasmapherese oder Immunadsorption) in der Zweitlinien-Behandlung zu Verfügung.[18] Im besten Fall ist eine (fast) vollständige Rückbildung der Symptome wünschenswert, die jedoch nur etwa 10-20% der Patienten erreichen. Das nach Schubtherapie persistierende Defizit scheint prädiktiv für künftige Behinderungsprogression.[19] Ob, und wenn ja, inwiefern die Schubtherapie selbst jedoch einen eigenen, anhaltend verlaufsmodifizierenden Charakter besitzt, ist unklar.[20,21]

Die symptomatische Therapie der Erkrankung dient der Linderung von persistierenden Defiziten und umfasst zum Beispiel die antispastische Therapie oder die Behandlung

von Miktions- oder Defäkationsstörungen. Obgleich der EDSS-Wert hierdurch positiv beeinflusst werden kann (z.B. durch Reduktion der Spastik wieder zunehmende Gehstrecke), hat diese Medikation keinen unmittelbaren Einfluss auf den Langzeitverlauf der Erkrankung selbst.[22]

Die Immuntherapie der Erkrankung ist im Gegensatz dazu designiert und geeignet, das Auftreten entzündlicher Krankheitsaktivität (in Form von klinisch manifesten Schüben oder neuer MRT-Läsionen) zu reduzieren und dadurch auch die Entstehung neuer Behinderungen zu verzögern.[23] Die Wirkmechanismen, über die dies bewerkstelligt wird, sind je nach verwendeter Substanz unterschiedlich gut verstanden und teils erheblich verschieden. Für die weitere Abhandlung relevante Aspekte werden an entsprechender Stelle näher beschrieben.

Nachdem bereits seit den 1970er-Jahren verschiedene konventionelle Immunsuppressiva wie Azathioprin genutzt wurden, um die MS zu behandeln[24], wurden in Deutschland ab 1998 die ersten Beta-Interferon-Formulierungen und ab 2001 auch Glatirameracetat regelhaft eingesetzt. Diese subkutan oder intramuskulär zu applizierenden Substanzen reduzierten in ihren Placebo-kontrollierten Zulassungsstudien das Auftreten neuer Schübe um etwa 30%.[25-27] 2002 wurde mit dem Chemotherapeutikum Mitoxantron eine hochwirksame Substanz zugelassen, die jedoch aufgrund ihrer schweren Nebenwirkungen (Kardiotoxizität, Fruchtschädigung, Entwicklung sekundärer Malignome) und eines kumulativen Dosislimits nur eingeschränkt eingesetzt wurde.[28] Der Begriff „*pre-treatment era of MS*“ wird heute häufig auch auf die Phase bis 2006 ausgedehnt und bezeichnet den Zustand insgesamt mangelhafter therapeutischer Möglichkeiten.[29]

2006 wurde mit Natalizumab eines der auch heute noch wirksamsten Immuntherapeutika zugelassen, nachdem es in seinen Zulassungsstudien eine Reduktion der Schubrate um mindestens 60% zeigte.[30,31] Die Anwendung wird aber durch das Risiko der Entwicklung einer progressiven multifokalen Leukoenzephalopathie (PML) limitiert.[32,33] Die PML ist eine nicht kausal zu behandelnde opportunistische Infektion des ZNS, die durch das Humane Polyomavirus 2 (HPyV-2; JC-Virus (JCV)) verursacht wird. Das Risiko der PML korreliert mit der Therapiedauer von Natalizumab sowie dem Auftreten von JCV-Antikörpern im Patientenserum sowie der relativen Höhe des Antikörperspiegels (im Extremfall beträgt das vorhergesagte Risiko für Patienten bis zu 1:30).[34] Aufgrund des zahlreichen Auftretens dieser Erkrankung erfolgte eine Aussetzung der Zulassung, bis 2008 eine Risikostratifizierung ermöglicht wurde. Auch trotz eines optimierten Risikomanagements steht die Therapie aufgrund der Risiken weiterhin nur einem Teil der MS-Patienten offen und dies meist zeitlich limitiert.[35]

Seit 2011 kam es zu einer Zulassung zahlreicher Präparate zur Behandlung der RMS, beginnend mit Fingolimod 2011[36] und vorläufig abgeschlossen mit Ublituximab 2022[37], sodass aktuell etwa 20 unterschiedliche Präparate in der Therapie der MS eingesetzt werden können. Diese verteilen sich auf neun unterschiedliche Wirkstoffklassen mit jeweils vergleichbarem Wirkmechanismus.[38]

In der Literatur wird der in der vergangenen Dekade eingeläutete Zeitraum, in welchem diese zahlreichen Substanzen zugelassen und verfügbar wurden, gegenüber der davorliegenden Zeit als „*treatment-era of MS*“ bezeichnet.[39]

Im Folgenden sollen einzelne in der Therapiegestaltung grundsätzliche Paradigmen entsprechend der derzeit gültigen nationalen und internationalen Empfehlungen dargestellt werden.

Entsprechend ihrer relativen Wirksamkeit im Vergleich zu Placebo oder Komparator in den Zulassungsstudien werden die Immuntherapeutika in unterschiedliche Wirkungsklassen gruppiert[40], wobei sich in „Wirkstoffklasse 1“ befindliche Substanzen in den Studien durch etwa 30% Schubratenreduktion zu Placebo auszeichneten. Diese Substanzen werden heute oft auch als „Plattform-Therapien“ bezeichnet; in dieser Arbeit umfasst dieser Begriff die Substanzen Glatirameracetat und beta-Interferone während die weiteren Substanzen der „Wirkstoffklasse 1“ separat benannt werden, wo erforderlich.

„Wirkstoffklasse 2“ zeichnete sich in den Studien durch etwa 50% Schubratenreduktion aus und umfasst nach heutiger Definition die Substanzen Cladribin sowie die Familie der S1P-Rezeptormodulatoren (S1PRM; Fingolimod, Siponimod, Ozanimod und Ponesimod).

Die „Wirkstoffkategorie 3“ umfasst sämtlich monoklonale Antikörper (Alemtuzumab, Ocrelizumab, Ofatumumab, Ublituximab und Natalizumab). Diese stellen die wirksamsten zugelassenen Substanzen mit einer Schubratenreduktion von mehr als 60% dar. Diese Einteilung findet sich in virtuellen Analysen, welche auf Basis der Zulassungsstudien die Wirksamkeit gegen eine Placebo-Behandlung simulieren, wieder.[41]

Angesichts der Verfügbarkeit dieser zahlreichen Substanzen und der Verfügbarkeit hochwirksamer Therapien ist das grundsätzliche Therapieziel nun die Freiheit von jeglicher Krankheitsaktivität („*no evidence of disease activity*“ (NEDA)).

Dies umfasst in seiner klinisch meist angewandten Form die vollständige Abwesenheit von Krankheitsschüben, neu auftretenden MRT-Läsionen und dem Ausbleiben einer Behinderungszunahme.[42]

Das Erreichen dieses Therapieziels ist langfristig mit sehr vorteilhaften Krankheitsverläufen assoziiert. Vor allem wurde wiederholt gezeigt, dass die konsequente Immuntherapie das Risiko für das Erleiden eines sekundär chronisch-progredienten Krankheitsverlaufes substanziell senkt.[43-45]

1.3 Therapiestrategien in der Behandlung der Multiplen Sklerose

Die Auswahl eines Immuntherapeutikums wird durch multiple Faktoren beeinflusst, wobei diese vom Patienten vorgegeben werden (Präferenzen der Anwendungsform respektive des Dosisintervalls, Komorbiditäten, Kinderwunsch, …), aber auch durch regulatorische Vorgaben bedingt sind (Zulassungstext, Erstattungsfähigkeit, erforderliche Ressourcen im Rahmen der Therapie (z. B. Monitorüberwachung während der Infusion)).[46]

Grundsätzlich besteht eine Proportionalität zwischen Wirkung und Nebenwirkung eines Immuntherapeutikums, da ein umso effektiverer Eingriff in das Immunsystem potenziell dessen erwünschte Funktion desto mehr beeinflussen kann (wie zum Beispiel das oben bereits genannte Risiko einer PML unter Therapie mit Natalizumab).[47] Unter Berücksichtigung solcher Aspekte konkurrieren heutzutage zwei grundsätzliche Therapiestrategien.

1.3.1 Eskalationstherapie

Grundsätzlich kann zunächst eine Therapie mit einer Plattform-Substanz eingeleitet werden unter der Annahme, dass ein substanzieller Teil der Patienten hierunter das definierte Therapieziel erreicht und hierfür eine potenziell gut verträgliche Substanz mit potenziell weniger (schwerwiegenden) Nebenwirkungen ausreicht.[48] Die Patienten mit fortgesetzter Krankheitsaktivität benötigen dahingegen im Verlauf der Therapie eine Eskalation auf eine Substanz höherer Wirksamkeit. Hierbei besteht jedoch das Risiko, durch die fortgesetzte Krankheitsaktivität eine bereits irreversible Behinderung erlitten zu haben.[49,50]

„Primär-aktive“ Krankheitsverläufe können auch heute noch nur unzureichend vorhergesagt werden, auch wenn verschiedene Parameter (Ausmaß der intrakraniellen Läsionslast, schlechte Rückbildungstendenz eines zur Diagnose führenden Schubes, polysymptomatische Manifestation, spinale oder infratentorielle Läsionslast) herangezogen werden können.[51]

Angesichts der möglichen Interaktionen verschiedener Immuntherapeutika existieren für den Wechsel zwischen jeweiligen Substanzen Empfehlungen bezüglich Sicherheitsabständen, diese werden, sofern relevant, in den jeweiligen Abschnitten separat erläutert.[52]

1.3.2 Induktionstherapie

Der „Eskalationstherapie“ gegenüber ist auch das Konzept der „Induktionstherapie“ möglich, wobei in der Literatur sich jüngst Synonyme wie „*flipping the pyramid*“ oder „*hit hard and early*“ etabliert haben.[53,54] Dieses Konzept ist grundsätzlich nicht neu und wurde z.B. im Bereich der chronisch-entzündlichen Darmerkrankungen bereits vor etwa 10 Jahren durch den frühzeitigen Einsatz monoklonaler Antikörper etabliert.[55]

Der Grundgedanke ist hier der frühzeitige Einsatz hochwirksamer Substanzen unter der Inkaufnahme einer möglichen Übertherapie einzelner Patienten. Im Wesentlichen wird dieses Therapieregime erst durch die Verfügbarkeit zahlreicher hochwirksamer Substanzen mit unterschiedlichen Wirk- und Nebenwirkungsprofilen ermöglicht, da das individuelle Therapierisiko durch die Auswahl einer geeigneten Substanz deutlich reduziert werden kann und dies das „Übertherapie-Risiko“ des Patienten akzeptabler erscheinen lässt.[56]

Die Gegenüberstellung der beiden oben genannten Therapiestrategien ist Gegenstand zahlreicher Studien. 2021 zeigte ein Vergleich der nationalen MS-Register Dänemarks (primär Eskalationstherapie) und Schwedens (primär Induktionstherapie) ein 29% geringeres Risiko, eine relevante Behinderungsprogression über eine mittlere Beobachtungsdauer von 4,1 Jahren zu erleiden.[54] Auch andere Arbeiten, insbesondere aus der MSBase-Studiengruppe zeigten, dass der Einsatz der hochwirksamen Substanzen mit einem etwa 34% geringeren Risiko sekundärer Krankheitsprogression verbunden war im Vergleich zum Einsatz der Plattform-Therapien in der Langzeit-Therapie.[57]

1.4 Therapieformen in der Behandlung der Multiplen Sklerose

1.4.1 Erhaltungstherapie

Neben ihrer anzunehmenden Wirkstärke können Immuntherapeutika anhand ihres Wirkmechanismus in zwei Familien eingeteilt werden: Erhaltungstherapeutika und Immunrekonstitutionstherapeutika.[58]

Das Konzept des Erhaltungstherapeutikums entspricht dem unserer „klassischen" Vorstellung eines Medikaments. Die angewendete Substanz wirkt über ihre pharmakodynamische Interaktion an entsprechenden Zielstrukturen und vermittelt darüber ihren Effekt. Zum Beispiel blockiert Natalizumab das *very late antigen-4* (VLA4) auf Lymphozyten und verhindert dadurch die Überwindung der Blut-Hirn-Schranke. Die Wirkdauer wird im Wesentlich durch klassische pharmakokinetische Parameter beschrieben (Bindungskonstante, Eliminationshalbwertszeit) und nach ausreichender Entsättigung der Zielstruktur verliert sich die Wirkung des Medikaments. Im Falle von Natalizumab entsättigt VLA4 ab einem Zeitraum von etwa vier bis sechs Wochen, hieraus resultiert das Dosisintervall der Behandlung.[59]

Zu den Erhaltungstherapeutika gehören heute die Plattform-Therapien (Beta-Interferone, Glatirameracetat), die Fumarate, Teriflunomid sowie die hochwirksamen Substanzen Natalizumab und die S1PRM.[58] Das Verständnis bezüglich der potenziellen Nebenwirkungen der Medikamente soll erleichtert werden durch die Einteilung ihrer biologischen Wirkung in drei Kategorien:

1. Erwünschter *on target*-Effekt: Derjenige Effekt, auf welchem die Medikamentenwirkung beruht, z.B. die Bindung der S1PRM an den Sphingosin-1-Phosphat-Rezeptor 1, wodurch Lymphozyten in lymphatischen Organen sequestriert und dadurch an der Zirkulation und letztlich der Überwindung der Blut-Hirn-Schranke gehindert werden.[60]

2. Unerwünschter *on target*-Effekt: Derjenige unerwünschte Effekt, der auf der Medikamentenwirkung an der Zielstruktur resultiert. Beispielsweise ist S1PR1 auch auf atrialen Kardiomyozyten exprimiert und die Einnahme von S1PRM führt zu einer Reduktion der Herzfrequenz, welcher durch Überwachung bei Erstgabe (Fingolimod) oder durch sukzessive Eindosierung (Ozanimod, Ponesimod) begegnet werden muss.[61]

3. (Häufig unerwünschter) *off target*-Effekt: Neben seiner Bindung an S1P-Rezeptoren hemmt Fingolimod zahlreiche weitere Stoffwechselwege, u.a. der Ceramid-Synthese und anderer Sphingolipide. Eventuell ist dies, gepaart mit seiner extensiven Beanspruchung des Cytochrom-P450-Systems, ursächlich für die in bis zu 10% der Fälle auftretende Leberschädigung.[62,63]

Erhaltungstherapeutika bedienen diese drei Domänen von erwünschten und unerwünschten Nebenwirkungen für die Dauer ihrer Anwendung (z.B. normalisiert sich die unter Therapie mit Fingolimod auftretende Lymphopenie über einen Zeitraum einiger Wochen nach Absetzen).

Interessanterweise führen jedoch auch verschiedene Erhaltungstherapeutika zu immunologischen Effekten, die weit über den Zeitpunkt der letzten erwarteten Wirksamkeit hinausgehen. Hierauf wird später an relevanter Stelle weiter eingegangen werden.

1.4.2 Immunrekonstitutionstherapie

Entgegen der oben beschriebenen Erhaltungstherapie weisen Alemtuzumab, Cladribin und die CD20-Antikörper Ocrelizumab, Ofatumumab und Ublituximab in ihrem Wirkmechanismus einen erheblichen Unterschied auf. Diese Medikamente (bis auf Cladribin handelt es sich um monoklonale Antikörper) lösen nach ihrer Anwendung recht unmittelbar den Untergang bestimmter Lymphozyten-Subpopulationen aus.[64]

Cladribin vermittelt als Nukleosid-Analogon nach seiner Aktivierung hin zu Cladribin-Phosphat die Apoptose durch Interferenz mit der DNA-Synthese und -Reparatur. Die hohe Selektivität der Substanz wird dadurch erreicht, dass in Lymphozyten ein günstiges Verhältnis aus aktivierenden und inaktivierenden Enzymen vorliegt, sodass eine kritische Akkumulation des aktiven Metaboliten gelingt.[65] Die Einnahme von Cladribin erfolgt in Form von Zyklen, wobei im ersten und zweiten Therapiejahr je in den Wochen 1 und 5 eine fünftägige, gewichtsabhängige Dosierung erfolgt.[66] Ein solcher Therapiezyklus beträgt nach aktueller Zulassung vier Jahre, d.h. ab dem fünften Jahr kann eine erneute Gabe erwogen werden, sollte eine fortgesetzte Krankheitsaktivität bestehen.[67]

Die monoklonalen Antikörper Alemtuzumab, Ocrelizumab, Ofatumumab und Ublituximab lösen den Untergang von Lymphozyten-Subpopulationen durch Bindung an Oberflächenantigene und anschließende Aktivierung von Komplementsystem und Phagozyten aus. Während Ocrelizumab, Ofatumumab und Ublituximab als anti-CD20-Antikörper im Wesentlichen B-Zell-Subpopulationen depletieren[68], bindet Alemtuzumab an CD52, das auf T- und B-Zellen exprimiert wird, und löst dadurch eine noch weitreichendere Depletion von Immunzellen aus.[69] Alemtuzumab wird ebenfalls

in jährlichen Zyklen appliziert (im Standard-Protokoll in Jahr eins über fünf Tage und in Jahr zwei über drei Tage, ergänzende Zyklen sind in den Jahren drei und vier entsprechend der Zulassung im Falle fortgesetzter Aktivität möglich). Ocrelizumab wird alle sechs Monate intravenös verabreicht, während Ofatumumab im Rahmen einer subkutanen Injektion alle vier Wochen verabreicht wird (auf die letztere Substanz soll hier angesichts der noch fehlenden Erfahrung in der Regelversorgung („*real world-evidence*“) nicht weiter eingegangen werden). Ublituximab wird wie Ocrelizumab halbjährlich infundiert, auch hierauf soll angesichts der erst jüngst erfolgten Zulassung in der Abhandlung nicht weiter eingegangen werden. Auf die mechanistischen Einzelheiten und langfristigen Effekte wird in detaillierterer Form später eingegangen werden.

Die Dauer des therapeutischen Effekts eines Immunrekonstitutionstherapeutikums (IRT) wird nun durch die Repopulationskinetik der vormals entsprechend depletierten Zellen definiert. B-Zellen neigen zu einer Repletion im peripheren Blut binnen drei bis sechs Monaten. T-Zellen, insbesondere $CD4^+$ T-Helferzellen benötigen bis zu fünf Jahre zu einer Repletion, wobei Zellzahlen wie vor Therapie im peripheren Blut meist nicht mehr erreicht werden.[70] Da die IRT selbst nur wenige Wochen im Körper nachweisbar sind, besteht prinzipiell der große Vorteil, dass eine langfristige Krankheitsstabilität außerhalb laufender Therapie („*drug-free remission“)* erreicht werden kann.[71]

Das Nebenwirkungsprofil dieser Medikamente weicht dadurch erheblich von den Erhaltungstherapeutika ab, beispielsweise bestehen deutlich weniger Probleme in der Medikamenteninteraktion oder der Elimination (im Sinne von deutlich reduzierten *off target*-Effekten), auch wenn durch die Therapiezyklen akute Nebenwirkungen möglich

sind (Infusionsreaktionen unter Antikörpertherapie, gastrointestinale Nebenwirkungen unter Cladribin-Einnahme).

Nebenwirkungen von Immunrekonstitutionstherapeutika sind im Wesentlichen unerwünschte *on target*-Effekte, die aus der Immunmodulation resultieren und potenziell dauerhaft anhalten können, beispielsweise das Risiko zur Entwicklung sekundärer Autoimmunphänomene nach Therapie mit Alemtuzumab.[72]

1.5 Zusammenfassung, offene Fragen und Zielsetzung

Die Immuntherapie der Multiplen Sklerose hat sich in der vergangenen Dekade erheblich gewandelt. Eine vormals nur unzureichend behandelbare Erkrankung wird nunmehr mit dem Therapieziel „NEDA“ bedacht, nachdem zahlreiche Medikamente in randomisierten kontrollierten Studien eine erhebliche Reduktion der Krankheitsaktivität bewirkt haben. Einen besonderen Stellenwert nehmen hier die IRT ein, da sie ihre hohe Wirksamkeit mit dem Vorteil langfristiger Therapiefreiheit verbinden können. Allerdings verbleiben auch nach den Zulassungsstudien relevante Fragen ungeklärt.

Zunächst sind Zulassungsstudien dadurch charakterisiert, dass sie ein möglichst homogenes, junges, gesundes und auch therapienaives Patientenkollektiv umfassen sollten. Dies dient neben einer Minimierung unerwünschter Ereignisse auch der Selektion eines Kollektivs von Patienten, das möglichst gut auf eine Immuntherapie anspricht und entsprechend große Effektstärken zulässt.[73]

Dieses bildet formal ein wünschenswertes Kollektiv an Patienten für die Strategie des „*hit hard and early*“ ab, entspricht aber nicht unbedingt dem Patientenkollektiv, welchem in der klinischen Routine begegnet wird (insbesondere in Hinblick auf vorbehandelte Patienten).

Beispielsweise weist die Zulassungsstudie für Cladribin (CLARITY) einen Anteil von 71% therapienaiven Patienten aus.[66] In den Zulassungsstudien von Ocrelizumab (OPERA I&II-Studien, veröffentlicht 2017) waren 73% der Patienten therapienaiv.[16] Alemtuzumab wurde in zwei separaten Studien untersucht (CARE-MS I für naive Patienten und CARE-MS II für Patienten nach Vortherapie; jeweils 2012 veröffentlicht).[74, 75] Hier kommt allerdings ein weiteres Dilemma zum Vorschein. Sofern

jenseits von Glatirameracetat und Beta-Interferonen weitere Vortherapeutika nicht bereits per Protokoll von der Studienteilnahme exkludiert wurden, waren sie zum Zeitpunkt der Rekrutierung gegebenenfalls noch nicht oder nicht lange genug verfügbar. In der CARE-MS II-Studie erhielten z.B. nur 15 Patienten Natalizumab, während die restlichen Patienten im Wesentlichen Glatirameracetat und Beta-Interferone erhielten. Teriflunomid, Dimethylfumarat oder Fingolimod waren schlicht noch nicht zugelassen. Ein ähnliches Bild ergibt sich für Cladribin (unter den Vorbehandlungen ausschließlich Glatirameracetat und Beta-Interferone) und für Ocrelizumab (von insgesamt 827 Ocrelizumab-exponierten Patienten erhielten von den 220 vorbehandelten Patienten nur sieben Patienten andere Medikamente als Glatirameracetat oder Beta-Interferone).

Analysen der Abrechnungsdaten der gesetzlichen Krankenversicherungen in Deutschland untermauern jedoch den weltweit anhaltenden Trend, dass Interferone und Glatirameracetat zunehmend seltener verschrieben werden zugunsten oraler Substanzen oder aber monoklonaler Antikörper, welche gemeinsam inzwischen einen Marktanteil von über zwei Dritteln aufweisen.[76] Die Datenlage zur Wirksamkeit und Sicherheit hochaktiver Therapien bei diesen Patienten ist formal ungenügend in Hinblick auf Sicherheit und Wirksamkeit.

Da Zulassungsinhaber verständlicherweise kein Interesse haben, ihre einmal erfolgreich untersuchten und zugelassenen Medikamente in Patientenkollektiven mit potenziell schlechterer Erfolgsaussicht (mehrere Vortherapien, vorheriges Versagen hochaktiver Medikamente) zu evaluieren, stellt die Untersuchung von *real world*-Kohorten mit ausreichend hohen Fallzahlen die vorrangige Möglichkeit dar, Sicherheit und Effektivität bestimmter Therapiesequenzen näher zu beleuchten.[77] Auch wenn der frühe Einsatz von IRT zusehends im Rahmen der oben genannten Konzepte zur

Induktionstherapie propagiert wird, machen solche Patienten selbst in sehr progressiv ausgerichteten Ländern nur etwa ein Fünftel aller Patienten aus[54], sodass die Eskalationstherapie in der Breite weiterhin der Standard ist.

Die oft unzureichende externe Validität der Ergebnisse randomisierter Studien erstreckt sich auch auf Sicherheitsaspekte dahingehend, dass sich das Nebenwirkungsprofil von Immuntherapeutika unter *real world*-Bedingungen durchaus ändern kann. Ein Beispiel hierfür ist wohl die Entwicklung der PML als Komplikation einer Natalizumab-Therapie, welche zwar in der Kohorte der Zulassungsstudien, aber erst deutlich nach der initialen Zulassung erkannt wurde.[78,79]

Die vorliegende Habilitationsschrift fasst die wissenschaftliche Tätigkeit des Autors/der Arbeitsgruppe auf dem Feld der Therapiesequenzen im Hinblick auf eine IRT zusammen und stellt die eigenen Arbeiten in den aktuellen wissenschaftlichen Kontext. Hierzu werden im Wesentlichen die Daten herangezogen, die im Rahmen langjähriger Erhebungen multizentrisch erfasst wurden. Als Rahmen wurden folgende Fragestellungen formuliert, wobei diese Fragen in Hinblick auf die einzelnen Substanzen an entsprechender Stelle präzisiert werden:

1. Inwiefern divergiert das in der Regelversorgung beobachtete Sicherheits- und Nebenwirkungsprofil von Immunrekonstitutionstherapeutika von den in randomisierten Studien dokumentierten Profilen?
2. Beeinflusst eine immunmodulatorische Vorbehandlung die Sicherheit und/oder Wirksamkeit einer IRT in der Regelversorgung?
3. Lässt sich aus den durchgeführten Untersuchungen eine Empfehlung für einen Therapiealgorithmus ableiten im Sinne einer „wenn Vorbehandlung mit X, dann IRT vorzugsweise mit Y“?

2. DISKUSSION IM KONTEXT EIGENER ARBEITEN

Die Diskussion der eigenen wissenschaftlichen Arbeiten wird der besseren Übersicht halber an den zugelassenen Immunrekonstitutions-Therapeutika ausgerichtet.

Es werden die hinführenden Aspekte der jeweiligen Substanzen in diesem Kapitel vorab nochmals dargestellt. Diese stellen die Evidenz (2.X.1) und die offenen Fragen (2.X.2 & 2.X.4) dar, die zu den jeweiligen eigenen Arbeiten geführt haben. Abgeschlossen werden die Abschnitte mit „offenen Fragen“, welche im Verlauf dann durch die eigenen Arbeiten beantwortet werden (2.X.3 & 2.X.5). Die Diskussion wird in Aspekte zur Wirksamkeit und zur Sicherheit aufgeteilt, wobei in diese Habilitationsschrift keine isolierten Arbeiten zur Sicherheit von Ocrelizumab eingeflossen sind.

Diese Darstellung dient der Übersicht über den absoluten Erkenntnisgewinn, vernachlässigt jedoch die historische Entwicklung („*work-in-progress*“). Diesbezüglich sei an dieser Stelle darauf verwiesen, dass die entsprechenden Arbeiten stets ein Produkt ihrer Zeit waren und die Erkenntnisse oft aufeinander aufbauten.

2.1 Alemtuzumab

2.1.1 Zulassungsdaten

Alemtuzumab führt nach Infusion mittels Bindung an das CD52-Oberflächenantigen zu einer raschen Zerstörung von Lymphozyten im peripheren Blut und teilweise auch in den lymphatischen Organen. Die Behandlung erfolgt an fünf aufeinander folgenden Tagen in Jahr eins und an drei aufeinander folgenden Tagen in Jahr zwei.[69] Die Immunrekonstitution der B-Zellen setzt im peripheren Blut binnen etwa drei bis sechs Monaten ein, während die peripheren T-Zellen über etwa fünf Jahre hinweg langsam wieder repopulieren, meist aber die Ausgangswerte nicht wieder erreichen.[70]

Das zur Zulassung der Substanz zur Behandlung der schubförmigen Multiplen Sklerose führende Studienprogramm umfasste insbesondere eine Phase-II-Studie (CAMMS223)[80] sowie zwei Phase-III-Studien (CARE-MS I & CARE-MS II)[74,75], wobei letztere therapienaive Patienten (CARE-MS I) und vorbehandelte Patienten (CARE-MS II) separat evaluierten.

Innerhalb der Phase-III-Studien zeigte sich im Vergleich zum Komparator jeweils eine etwa 50%ige Reduktion der jährlichen Schubrate mit entsprechender statistischer Signifikanz. Der primäre Endpunkt der Studien, das Erreichen einer über sechs Monate hinweg bestätigten Behinderungsprogression, war in CARE-MS I mit etwa 30%iger Reduktion nicht statistisch signifikant. In CARE-MS II wurde dieser Endpunkt mit einer 42%igen Reduktion erreicht. Die Abwesenheit statistischer Signifikanz bezüglich des primären Endpunktes wurde in der CARE-MS I-Studie meist mit einer

geringer als erwartet ausfallenden Ereignisrate im Komparator-Arm erklärt. Die 5-Jahres-Daten zur Wirksamkeit zeigten die Abwesenheit von bestätigter Behinderungsprogression von knapp 80% in der CARE-MS I-Extensionsstudie und von knapp 75% in der CARE-MS II-Extensionsstudie.[81,82]

Von den 426 mit Alemtuzumab im Rahmen der CARE-MS II-Studie behandelten Patienten, die eine immunmodulatorische Vortherapie erhielten, wurden nur 15 Patienten mit Natalizumab behandelt, während der Rest (abgesehen von 17 Patienten, die intravenöse Immunglobuline oder Azathioprin als historische Substanzen erhielten) entweder beta-Interferone oder Glatirameracetat erhielt.[75]

2.1.2 Offene Fragen zur Wirksamkeit in der Regelversorgung

Jenseits dieser 15 Studienpatienten, die vormals Natalizumab erhielten, lagen keine Daten zur Wirksamkeit von Alemtuzumab bei Natalizumab-vorbehandelten Patienten vor. Die Natalizumab-Therapie ist bei einem Großteil der Patienten aufgrund des Risikos, eine progressive multifokale Leukoenzephalopathie (PML) zu entwickeln, jedoch zeitlich limitiert.[34] Daten zur Wirksamkeit einer wirksamen Folgetherapie lagen nur für Fingolimod vor. Hier zeigte sich jedoch bei 50% der Patienten nach 24 Wochen eine anhaltende Krankheitsaktivität.[83] Rituximab hätte zwar auch eine wirkungsvolle Option dargestellt[84], wurde von den Krankenkassen seinerzeit, wenn überhaupt, nur in Ausnahmefällen erstattet.

Fingolimod war zum Zeitpunkt der CARE-MS-Studien noch nicht zugelassen und tauchte deshalb bei den Vortherapien nicht auf. Es gab jedoch früh erste Berichte über eine möglicherweise eingeschränkte Wirksamkeit von Alemtuzumab bei Patienten, die eine Vorbehandlung mit dem S1PRM Fingolimod erhielten. WILLIS und Kollegen berichteten exemplarisch über neun Patienten, die unter Alemtuzumab eine Krankheitsaktivität erlitten, welche das aus den Studien bekannte Maß deutlich überschritt.[85] Eine retrospektive Analyse von 77 Patienten, die von Fingolimod zu Alemtuzumab wechselten, zeigte in der Mehrzahl der Patienten eine Reduktion der Schubrate unter Alemtuzumab, litt jedoch ebenso wie eine multizentrische retrospektive Analyse aus Deutschland an relevanten Limitationen des Studiendesigns (retrospektives Design, Fehlen von Vergleichsarmen).[86,87]

Es stellte sich also die Frage: „Ist Alemtuzumab bei Patienten, die eine Vorbehandlung mit Natalizumab oder Fingolimod erhielten, eine sinnvolle Option im Falle eines Therapiewechsels?“

2.1.3 Eigene Arbeiten zur Wirksamkeit in der Regelversorgung

In dieses Kapitel eingeflossene eigene Arbeiten:

A1 **Pfeuffer S**, Schmidt R, Straeten FA, Pul R, Kleinschnitz C, Wieshuber M, Lee DH, Linker RA, Doerck S, Straeten V, Windhagen S, Pawlitzki M, Aufenberg C, Lang M, Eienbroeker C, Tackenberg B, Limmroth V, Wildemann B, Haas J, Klotz L, Wiendl H, Ruck T, Meuth SG. Efficacy and safety of alemtuzumab versus fingolimod in RRMS after natalizumab cessation. J Neurol. 2019 Jan;266(1):165-173. doi: 10.1007/s00415-018-9117-z.
A2 **Pfeuffer S***, Ruck T*, Pul R, Rolfes L, Korsukewitz C, Pawlitzki M, Wildemann B, Klotz L, Kleinschnitz C, Scalfari A, Wiendl H, Meuth SG. Impact of previous disease-modifying treatment on effectiveness and safety outcomes, among patients with multiple sclerosis treated with alemtuzumab. J Neurol Neurosurg Psychiatry. 2021 Sep;92(9):1007-1013. doi: 10.1136/jnnp-2020-325304.

Um zu klären, ob Alemtuzumab eine sinnvolle Therapieoption bei Patienten, die Natalizumab beenden mussten, ist, evaluierten wir in einer retrospektiven Analyse 144 Patienten, die nach Beendigung von Natalizumab entweder Alemtuzumab (42 Patienten) oder Fingolimod (102 Patienten) an 12 deutschen Versorgungszentren erhielten.[88] **(A1)**

Hier zeigten wir, dass im Vergleich zu einer Therapie mit Fingolimod das Risiko klinischer Schübe binnen 18 Monaten nach Umstellung 2,2fach niedriger unter Alemtuzumab war als unter Fingolimod. Fingolimod-Patienten erlitten zudem 4,8fach häufiger eine Zunahme der Behinderung und setzten darüber hinaus in 39,6% Fingolimod während der Nachbeobachtung aufgrund schlechter Wirksamkeit oder unerwünschter Arzneimittelwirkungen ab, während nur zwei Patienten auf eine erneute Infusion von Alemtuzumab verzichteten. 17 zuvor mit Fingolimod behandelte Patienten entschieden sich im weiteren Verlauf dann für eine Therapie mit Alemtuzumab. Insgesamt konnten wir damit zeigen, dass die Wirksamkeit von Alemtuzumab auch bei Patienten, die mit Natalizumab vorbehandelt waren, konsistent zu den bisherigen Daten war.

Um eine mögliche Einschränkung der Wirksamkeit von Alemtuzumab bei Fingolimod-vorbehandelten Patienten weiter einzuordnen, evaluierten wir die von uns aufgebaute, prospektive PROGRAM^{MS}-Kohorte von 170 Patienten unter Alemtuzumab.[89] **(A2)** Hier sahen wir in Bezug auf die Wirksamkeit der Substanz bei einem medianen *follow-up* von 44 Monaten bei vormals therapienaiven Patienten und solchen, die vorher Plattform-Substanzen, Fumarate oder Teriflunomid erhielten, eine den Zulassungsstudien vergleichbare Wirkung in den ersten beiden Therapiejahren (71,4% Reduktion der Krankheitsschübe bei naiven Patienten und 89,1% Reduktion nach Plattform-Substanzen).

Bei Patienten, die von Fingolimod auf Alemtuzumab umgestellt wurden, fanden wir weiterhin eine signifikante Reduktion der Schubrate im Vergleich zur vorherigen Therapie mit Fingolimod (38,4%ige Reduktion der Krankheitsschübe). Allerdings war das Risiko fortgesetzter Krankheitsaktivität im Vergleich zu vormals naiven Patienten um den Faktor 5,4 erhöht. Im Rahmen dieser Untersuchung zeigten wir zudem, dass mit Fingolimod vorbehandelte Patienten auch ein ungleich höheres Risiko anhaltender Behinderungsprogression aufwiesen (7,7fach im Vergleich zu naiven Patienten). Wir konnten hier eine qualitative Veränderung der Erkrankung dahingehend nachweisen, dass diese Patienten in mehr als der Hälfte der Fälle Läsionen im Rückenmark entwickelten (gegenüber 10-20% innerhalb der verbleibenden Kohorte). Diese Läsionen sind erfahrungsgemäß mit deutlicher klinischer Behinderung assoziiert.[90]

Zusammenfassend konnten wir bezüglich der Therapiesequenz die aus den Zulassungsstudien im Hinblick auf nicht vorbehandelte Patienten und Patienten bestätigen, die vormals Plattform-Therapien, Fumarate oder Teriflunomid erhielten. Bezüglich der hochwirksamen Therapien Natalizumab und Fingolimod zeigten wir in systematischen Erhebungen, dass die Sequenz „Natalizumab→Alemtuzumab“ wirksam ist, während in der Sequenz „Fingolimod→Alemtuzumab“ mit einem suboptimalen Ansprechen zu rechnen ist.

2.1.4 Offene Fragen zur Sicherheit in der Regelversorgung

Die bereits aus früheren Anwendungsgebieten bekannte, schwerwiegende Komplikation der Therapie mit Alemtuzumab ist die Entwicklung sekundärer Autoimmunphänomene. Diese wurde in den Phase-III-Studien entsprechend auch bei MS-Patienten beobachtet. Etwa 20% der Patienten entwickelten eine Autoimmunität gegen die Schilddrüse und etwa 1-3% der Patienten gegen das hämatopoietische System während der Kernstudien. Einzelfälle von autoimmuner Nephropathie wurden ebenfalls dokumentiert. Stand 2019 zeigte sich in der Nachverfolgung der Zulassungsstudien eine Prävalenz von etwa 40-50% bezüglich Schilddrüsen-Autoimmunität, 4-5% bezüglich Blutbildungsstörungen und 0,3% bezüglich autoimmuner Nephropathien.[91]

Angesichts der Tatsache, dass die häufigen sekundären Autoimmunphänomene unter Alemtuzumab zunächst sämtlich Antikörper-vermittelt erschienen, galt insbesondere die nach Infusion auftretende, teils überschießende, Repopulation der B-Zellen als ursächlich.[69] Entsprechende Phänomene waren vorher bereits bei Patienten mit schwerer Lymphopenie anderer Ätiologie bekannt, beispielsweise bei Patienten nach Stammzelltransplantation.[64,92]

COLES und Kollegen beschrieben initial einen möglichen Zusammenhang zwischen erhöhten IL-21-Spiegeln im Blut und dem Auftreten sekundärer Autoimmunität, was angesichts der Rolle von IL-21 in der Differenzierung von B-Zellen zu Plasmazellen mit der obigen Hypothese in Einklang stünde. Ein Biomarker konnte hieraus jedoch mangels Sensitivität nicht entwickelt werden.[93,94]

Allerdings konnte die Arbeitsgruppe von COLES und Kollegen im Verlauf auch zeigen, dass es im Rahmen der Therapie mit Alemtuzumab und der anschließenden Lymphopenie zu einer Proliferation peripherer T-Zellen kommt (was angesichts der nach der Adoleszenz zunehmend sistierenden Thymopoiese verständlich erscheint) und dass dies mit der Entstehung sekundärer Autoimmunität assoziiert ist.[95]

Ferner wurde das Risiko, ein sekundäres Autoimmunphänomen zu entwickeln, nicht durch zusätzliche Zyklen von Alemtuzumab gesteigert.[96] Da die wiederholte Gabe angesichts der anhaltenden Depletion der T-Zellen vorwiegend eine Wirkung auf die B-Zellen hat, sprach dies auch eher für eine primäre Störung im T-Zell-Kompartiment.

Folgende Fragen stellten sich im Hinblick auf die Sicherheit von Alemtuzumab in der Regelversorgung:

1. Sind die sekundären Autoimmunphänomene nach Therapie mit Alemtuzumab allein durch eine überschießende Repletion von B-Zellen bedingt oder sind diese nur Epiphänomen einer gestörten T-Zell-Repletion?
2. Lässt sich anhand von Biomarkern das Risiko, eine sekundäre Autoimmunerkrankung zu entwickeln, abschätzen?

2.1.5 Eigene Arbeiten zur Sicherheit in der Regelversorgung

In dieses Kapitel eingeflossene eigene Arbeiten:

A2 **Pfeuffer S***, Ruck T*, Pul R, Rolfes L, Korsukewitz C, Pawlitzki M, Wildemann B, Klotz L, Kleinschnitz C, Scalfari A, Wiendl H, Meuth SG. Impact of previous disease-modifying treatment on effectiveness and safety outcomes, among patients with multiple sclerosis treated with alemtuzumab. J Neurol Neurosurg Psychiatry. 2021 Sep;92(9):1007-1013. doi: 10.1136/jnnp-2020-325304.
A3 Ruck T, Schulte-Mecklenbeck A, **Pfeuffer S**, Heming M, Klotz L, Windhagen S, Kleinschnitz C, Gross CC, Wiendl H, Meuth SG. Pretreatment anti-thyroid autoantibodies indicate increased risk for thyroid autoimmunity secondary to alemtuzumab: A prospective cohort study. EBioMedicine. 2019 Aug;46:381-386. doi: 10.1016/j.ebiom.2019.07.062.
A4 Möhn N, **Pfeuffer S**, Ruck T, Gross CC, Skripuletz T, Klotz L, Wiendl H, Stangel M, Meuth SG. Alemtuzumab therapy changes immunoglobulin levels in peripheral blood and CSF. Neurol Neuroimmunol Neuroinflamm. 2019 Dec 11;7(2):e654. doi: 10.1212/NXI.0000000000000654.
A5 **Pfeuffer S.** Sarcoidosis following alemtuzumab treatment: Autoimmunity mediated by T cells and interferon-γ. Mult Scler. 2018 Nov;24(13):1783-1784. doi: 10.1177/1352458518804124.
A6 Ruck T, **Pfeuffer S**, Schulte-Mecklenbeck A, Gross CC, Lindner M, Metze D, Ehrchen J, Sondermann W, Pul R, Kleinschnitz C, Wiendl H, Meuth SG, Klotz L. Vitiligo after alemtuzumab treatment: Secondary autoimmunity is not all about B cells. Neurology. 2018 Dec 11;91(24):e2233-e2237. doi: 10.1212/WNL.0000000000006648.
A7 Ruck T*, Barman S*, Schulte-Mecklenbeck A*, **Pfeuffer S***, Steffen F, Nelke C, Schroeter CB, Willison A, Heming M, Müntefering T, Melzer N, Krämer J, Lindner M, Riepenhausen M, Gross CC, Klotz L, Bittner S, Muraro PA, Schneider-Hohendorf T, Schwab N, Meyer Zu Hörste G, Goebels N, Meuth SG, Wiendl H. Alemtuzumab-induced immune phenotype and repertoire changes: implications for secondary autoimmunity. Brain. 2022 Jun 3;145(5):1711-1725. doi: 10.1093/brain/awac064.

Da Autoimmun-Thyreopathien die mit Abstand häufigsten sekundären Autoimmunphänomene unter einer Therapie mit Alemtuzumab darstellen, evaluierten wir, ob das Vorhandensein asymptomatischer Schilddrüsen-Antikörper bei Therapieeinleitung ein möglicher Risikofaktor ist. Wir analysierten die zu dem Zeitpunkt verfügbaren 106 Patienten, von denen 29 eine Autoimmunerkrankung der Schilddrüse entwickelt hatten. Hier konnten wir zeigen, dass die Anwesenheit von asymptomatischen Schilddrüsen-Antikörpern vor Therapiebeginn das Auftreten einer

Schilddrüsen-Erkrankung nach Einleitung von Alemtuzumab 12,2fach wahrscheinlicher machte gegenüber vormals seronegativen Patienten.[97] **(A3)**

Die viel grundsätzlichere Frage, ob eine sekundäre Autoimmunität primär durch eine Funktionsstörung des B-Zell-Kompartiments entsteht, oder ob doch eine Funktionsstörung des T-Zell-Kompartiments vorliegt, wurde in mehreren Arbeiten behandelt.

Einerseits konnten wir zeigen, dass die Infusion mit Alemtuzumab dosisabhängig in einer Subpopulation unserer Kohorte von 38 Patienten eine Reduktion der Immunglobulin-Spiegel verursachte.[98] **(A4)** Diese Effekte waren in Serum und Liquor gleichermaßen ausgeprägt und führten auch zu einer Abnahme der intrathekalen Immunglobulin-Synthese. Somit widersprachen unsere Ergebnisse der Hypothese einer überschießenden Plasmazell-Entwicklung nach Therapie mit Alemtuzumab und widersprachen den Beobachtungen von COLES und Kollegen bezüglich IL-21.

Zudem beobachteten wir im Laufe der Zeit zunehmend auch Autoimmunphänomene ohne Beteiligung von Autoantikörpern. Ich wurde im Kontext zweier Einzelfallberichte einer neu aufgetretenen Sarkoidose um eine Einordnung gebeten.[99] **(A5)**

Im Verhältnis zu den jeweiligen lokalen Kohorten war die Zahl der Fälle signifikant von der zu erwartenden Fallzahl divergent, sodass dies einen Zusammenhang zur Therapie nahelegte. Die Sarkoidose ist eine zellulär vermittelte Autoimmunerkrankung, charakterisiert durch eine aberrante T-Helfer-Zell-Antwort und eine übermäßige Sekretion von Tumor-Nekrosefaktor-alpha und Interferon-gamma. Autoantikörper beziehungsweise B-Zellen spielen hier keine Rolle.[100]

Ferner dokumentierten wir in unserer eigenen Kohorte ebenfalls „neuartige“ sekundäre Autoimmunphänomene, insbesondere drei Fälle mit ausgedehnter Vitiligo.[101] **(A6)**

Pathophysiologisch liegt dieser Erkrankung der Verlust von Melanozyten infolge einer Zerstörung dieser Zellen durch zytotoxische T-Lymphozyten zugrunde. Autoantikörper sind hier ebenfalls nicht relevant.[102] Im Rahmen weiterführender Untersuchungen konnten wir entsprechende quantitative und qualitative Veränderungen des Kompartiments der zytotoxischen T-Lymphozyten nachweisen. Zudem fanden wir bei den Patienten die typischerweise mit Vitiligo assoziierten HLA (humane Leukozyten-Antigen)-Haplotypen.

Außerdem konnten wir innerhalb der PROGRAM^{MS}-Kohorte die Vortherapie mit Fingolimod als Risikofaktor für die Entstehung einer sekundären Autoimmunität identifizieren. [89] **(A2)** Patienten, die vormals Fingolimod erhielten, zeigten ein 5,9fach höheres Risiko für eine sekundäre Autoimmunität im Vergleich zu vormals naiven Patienten. Ursächlich hierfür schienen durch Fingolimod ausgelöste, quantitative und qualitative Veränderungen im T-Zell-Kompartiment, welche wir beispielsweise in Form gestörter Interferon-gamma-Sekretion im peripheren Blut vormals nachweisen konnten.[103]

Um unsere bisherigen Erkenntnisse und die bestehende Literatur abschließend in Einklang zu bringen, evaluierten wir ausführlich die uns zur Verfügung stehende Kohorte unter Einsatz erheblicher immunologischer Methodik. Wir konnten nach ausführlicher longitudinaler Immunzell-Phänotypisierung sowie entsprechender B- und T-Zell-Rezeptor Sequenzierung nachweisen, dass das Missverhältnis zwischen insuffizienter Thymopoiese und gesteigerter homöostatischer Proliferation von T-Zellen die sekundäre Autoimmunität begünstigt.[70] **(A7)** In den entsprechenden Analysen zeigte sich zudem, dass die Expansion von B-Zellen im Rahmen der sekundären Autoimmunität eine Folge der T-Zell-Expansion ist. Wir fanden zudem bereits bei

Baseline entsprechend pathologische Veränderungen im T-Zell-Kompartiment bei Patienten, die später eine sekundäre Autoimmunität entwickelten.

Insgesamt konnten wir Risikofaktoren für die Entwicklung einer sekundären Autoimmunität identifizieren (Schilddrüsen-Antikörper, Vorbehandlung mit Fingolimod). Außerdem zeigten wir durch die Erweiterung des beschriebenen Spektrums sekundärer Autoimmunphänomene und durch die intensive Immunphänotypisierung, dass eine sekundäre Autoimmunität nach Alemtuzumab-Behandlung durch eine Störung im T-Zell-Kompartiment vermittelt wird.

2.2 Cladribin

2.2.1 Zulassungsdaten

Bei Cladribin handelt es sich um ein Purin-Analogon, welches eine hohe Selektivität für Lymphozyten aufweist. Ursächlich hierfür ist die Überführung des Prodrugs in die pharmakologisch aktive Form, welche dann durch Interferenz mit dem Energiestoffwechsel und der Nukleinsäuresynthese die Apoptose auslöst. Diese Überführung erfolgt durch Polyphosphorylierung mittels Desoxycytidinkinase (DCK), wobei die aktive Form parallel durch eine 5‘-Nukleotidase abgebaut wird. Dass Lymphozyten im Gegensatz zu anderen Zelltypen ein deutliches Übergewicht der DCK aufweisen, erklärt die hohe Selektivität der Substanz. [104]

Die zur Zulassung der Substanz führende CLARITY-Studie wurde bereits 2010 publiziert.[66] Da im Rahmen des Zulassungsverfahrens jedoch weitere Sicherheitsdaten seitens der europäischen und der amerikanischen Zulassungsbehörden gefordert wurden (insgesamt traten bei 10 von 884 mit Cladribin behandelten Patienten Tumorerkrankungen auf, während dies bei keinem der 435 Placebo-behandelten Patienten der Fall war), wurde der Zulassungsantrag seinerzeit jedoch zurückgezogen.

Die Zulassung erfolgte somit erst 2017, nachdem (unter anderem im Rahmen von Extensionsstudien) gezeigt werden konnte, dass die Substanz kein erhöhtes Tumorrisiko barg.[105] Aufgrund dieser Umstände ist Cladribin das letzte zugelassene Medikament, dessen Zulassungsstudie keinen aktiven Komparator enthielt. Darüber hinaus erhielten die 260/889 (29,2%) mit Cladribin-behandelten Patienten, die vorher eine Immuntherapie erhalten hatten, sämtlich Interferone oder Glatirameracetat, während Daten zu anderen Vortherapien bei Zulassung fehlten.

2.2.2 Offene Fragen zur Wirksamkeit in der Regelversorgung

Da die Patienten in der CLARITY-Studie zwischen 2005 und 2007 rekrutiert wurden, wird die externe Validität der Zulassungsstudie vor dem Hintergrund des kontemporären Patientenkollektivs durch einige Faktoren eingeschränkt. Oben wurde bereits darauf verwiesen, dass die therapeutischen Möglichkeiten sich zwischenzeitlich durchgreifend gewandelt haben. Die zahlreichen, seitdem neu zugelassenen Substanzen finden sich als Vortherapien in der Studie nicht. Dementsprechend können keine Aussagen zu Therapiesequenzen gemacht werden.

Außerdem ließ sich über die vergangenen Jahrzehnte eine Veränderung von Schubraten und Behinderungsprogression in den Placebo-/Komparator-Armen klinischer Studien dahingehend nachweisen, dass die durchschnittliche Schubrate von Patienten insgesamt abnahm.[106] Dementsprechend ist auch die Argumentation anhand absoluter Schubraten nicht zielführend.

Es gab dennoch den Versuch, mittels virtueller Komparator-Analysen Cladribin innerhalb der aktuellen Therapielandschaft einzuordnen. Hier erschien Cladribin vergleichbar wirksam zu den S1PRM, wirksamer als die Plattformtherapeutika und weniger wirksam als Natalizumab.[41]

Es stellte sich also die Frage: „Wie ist die Wirksamkeit von Cladribin innerhalb verschiedener Therapiesequenzen einzuordnen?“

2.2.3 Eigene Arbeiten zur Wirksamkeit in der Regelversorgung

In dieses Kapitel eingeflossene eigene Arbeiten:

A8

Pfeuffer S, Rolfes L, Hackert J, Kleinschnitz K, Ruck T, Wiendl H, Klotz L, Kleinschnitz C, Meuth SG, Pul R. Effectiveness and safety of cladribine in MS: Real-world experience from two tertiary centres. Mult Scler. 2022 Feb;28(2):257-268. doi: 10.1177/13524585211012227.

Wir inkludierten unmittelbar nach Zulassung alle mit Cladribin behandelten Patienten in eine prospektive Kohortenstudie. Hier evaluierten wir insgesamt 270 Patienten unter Therapie mit Cladribin von 2017 bis 2020.[107] **(A8)** Insgesamt zeigten wir eine 85%ige Schubratenreduktion innerhalb der Gesamtkohorte nach Einleitung der Therapie mit Cladribin.

Im Vergleich zu therapienaiven Patienten war die Wirkung von Cladribin bei Patienten mit vorheriger Einnahme einer Plattform-Therapie vergleichbar. Dies schloss auch die neueren Therapien (Fumarate, Teriflunomid), welche zum Zeitpunkt der CLARITY-Studie noch nicht verfügbar waren, ein. Bei Patienten, die Fingolimod als letzte Vortherapie erhielten, sahen wir ein tendenziell höheres Risiko von Krankheitsaktivität verglichen mit naiven Patienten. Angesichts zu geringer Fallzahlen in dieser Subgruppe ließen sich aber keine eindeutigen Aussagen zu Über- oder Unterlegenheit treffen.

Außerdem sahen wir bei Patienten mit Natalizumab-Vorbehandlung im Vergleich zu naiven Patienten ein 5fach erhöhtes Risiko für klinische Schübe oder neue T2-hyperintense MRT-Läsionen.

Zusammenfassend konnten wir im Wesentlichen die Ergebnisse der Zulassungsstudie bestätigten, zeigten aber auch, dass die Wirksamkeit von Cladribin bei den Patienten

nicht ausreichend ist, die vormals Natalizumab erhielten. Entsprechend ist der Einsatz von Cladribin bei Patienten, die Natalizumab aufgrund eines erhöhten PML-Risikos absetzen müssen, gut abzuwägen. Wir konnten somit die Vermutungen der bisherigen, virtuellen Analysen bestätigen.

2.2.4 Offene Fragen zur Sicherheit in der Regelversorgung

Cladribin führt, vergleichbar zu Alemtuzumab, zu einer langanhaltenden Depletion von Lymphozyten. Diese ist zwar geringer ausgeprägt, Cladribin weist allerdings als kleines Molekül ein deutlich größeres Verteilungsvolumen auf und scheint sogar in das zentrale Nervensystem selbst einzudringen.[108] Entsprechend signalisiert eine Lymphopenie unter Therapie mit Cladribin im Gegensatz zu einer apparenten (nur das periphere Blut betreffenden) Lymphopenie unter beispielsweise einem S1PRM eine quantitative Funktionsstörung der adaptiven Immunität.

Konsequenterweise erschien die Zahl der Lymphozyten im peripheren Blut und ein Infektionsrisiko auch direkt assoziiert, insbesondere bei Varizella-Zoster-Virus (VZV)-Infektionen.[109] Während in der CLARITY-Studie bei den mit der letztendlich zugelassenen Dosis behandelten Patienten 59.5% im Nadir eine Lymphopenie von <800/mm^3 und 24.2% eine von <500/mm^3 im Nadir zeigten, waren schwere Lymphopenien (<200/mm^3) nur bei 0,6% der Patienten nachweisbar.[66] Neun dieser Patienten erlitten einen Herpes zoster, wobei der Grad der Lymphopenie innerhalb der Studie prädiktiv war.

Real world-Daten lagen hierzu bisher nicht vor. Ferner lagen zwischenzeitlich auch trotz Extensionsstudien keine Berichte über „neue“, jenseits der Kernstudie aufgetretene Nebenwirkungen vor.

Wie bereits im vorangegangenen Kapitel zu Alemtuzumab deutlich ersichtlich, hinterlassen Vortherapien aber häufig langanhaltende Veränderungen im Immunsystem (konkret: Veränderungen im T-Zell-Kompartiment nach Fingolimod).

Inwiefern diese Veränderungen die Therapiesicherheit von Cladribin beeinflussen, war mangels Erfahrung nicht bekannt.

Zuletzt bestand Unklarheit bezüglich der Wirksamkeit von Impfungen unter Therapie mit Cladribin, da bisher keine Impfstudien publiziert wurden. Da die Reduktion von T-Zellen grundsätzlich mit dem Risiko eingeschränkter Impfantworten behaftet ist, sind Lebendimpfstoffe unter Therapie mit Cladribin prinzipiell kontraindiziert und bei Totimpfstoffen sind entsprechend aktuell gültiger internationaler Empfehlungen Nutzen-Risiko-Abwägungen zu treffen. Bis dato lagen diesen Empfehlungen allerdings nur Daten von 17 mit Cladribin behandelten Patienten zugrunde. [110]

Folgende Fragen stellten sich bezüglich der Sicherheit von Cladribin in der Regelversorgung:

1. Ist der Verlauf der Laborparameter, insbesondere der Lymphozyten in der Regelversorgung noch kongruent zu den aus der Zulassungsstudie erhobenen Daten?
2. Wird das Sicherheitsprofil von Cladribin durch eine Vortherapie beeinflusst?
3. Unter welchen Bedingungen sind Impfungen unter Cladribin sicher und wirksam?
4. Sind im Rahmen der Regelversorgung weitere Nebenwirkungen zu erwarten?

2.2.5 Eigene Arbeiten zur Sicherheit in der Regelversorgung

In dieses Kapitel eingeflossene eigene Arbeiten:

A8 **Pfeuffer S**, Rolfes L, Hackert J, Kleinschnitz K, Ruck T, Wiendl H, Klotz L, Kleinschnitz C, Meuth SG, Pul R. Effectiveness and safety of cladribine in MS: Real-world experience from two tertiary centres. Mult Scler. 2022 Feb;28(2):257-268. doi: 10.1177/13524585211012227.
A9 Rolfes L, **Pfeuffer S**, Huntemann N, Schmidt M, Su C, Skuljec J, Aslan D, Hackert J, Kleinschnitz K, Hagenacker T, Pawlitzki M, Ruck T, Kleinschnitz C, Meuth SG, Pul R. Immunological consequences of cladribine treatment in multiple sclerosis: A real-world study. Mult Scler Relat Disord. 2022 Aug;64:103931. doi: 10.1016/j.msard.2022.103931.
A10 Rolfes L*, **Pfeuffer S***, Skuljec J, He X, Su C, Oezalp SH, Pawlitzki M, Ruck T, Korsen M, Kleinschnitz K, Aslan D, Hagenacker T, Kleinschnitz C, Meuth SG, Pul R. Immune Response to Seasonal Influenza Vaccination in Multiple Sclerosis Patients Receiving Cladribine. Cells. 2023 Apr 25;12(9):1243. doi: 10.3390/cells12091243.
A11 Rolfes L, **Pfeuffer S**, Hackert J, Pawlitzki M, Ruck T, Sondermann W, Korsen M, Wiendl H, Meuth SG, Kleinschnitz C, Pul R. Skin Reactions in Patients With Multiple Sclerosis Receiving Cladribine Treatment. Neurol Neuroimmunol Neuroinflamm. 2021 Apr 9;8(3):e990. doi: 10.1212/NXI.0000000000000990.

Um das Sicherheitsprofil von Cladribin in der Regelversorgung klarer zu definieren, analysierten wir zunächst in einer Kohorte von 80 Patienten prospektiv zahlreiche Laborparameter mit einem Schwerpunkt auf dem Blutbild und den Lymphozyten-Subpopulationen.

Grundsätzlich konnten wir die Dynamik der Lymphozyten in einer Kohorte mit 80 Patienten bestätigen, wobei hier 50% der Patienten vormals therapienaiv waren und 50% der Patienten mit Plattform-Therapeutika vorbehandelt wurden.[111] **(A9)** Innerhalb der Studie zeigte sich zudem, dass die Erholung der B-Zellen nach sechs Monaten weitgehend abgeschlossen war, während die Zahl der T-Zellen anhaltend supprimiert blieb. Letzteres betrifft T-Helfer-Zellen deutlicher als zytotoxische T-Zellen. Die

weitere, sehr ausführliche laborchemische Charakterisierung der Patienten innerhalb dieser Kohorte war ansonsten unauffällig.

Innerhalb der gesamten prospektiven Kohorte fanden wir jedoch erhebliche Unterschiede bezüglich des Verlaufs der Lymphozyten unter Therapie sowie des Auftretens von Infektionen.[107] **(A8)** Grundsätzlich zeigten wir unter Therapie mit Cladribin den klassischen Verlauf der Lymphopenie mit Nadiren jeweils in den Monaten 3 und 14 auch bei unterschiedlich vorbehandelten Patienten.

Gemessen an den vormals therapienaiven Patienten als Referenz, sahen wir bei Patienten unter Plattform-Therapie einen vergleichbar ausgeprägten Abfall der Lymphozyten. Selbiges war auch bei Patienten der Fall, die vormals Fingolimod erhielten (wobei sich hier bei Baseline die periphere Lymphozyten-Zahl jeweils ausreichend erholt hatte). Bei Patienten, die vormals Natalizumab erhielten, zeigten sich im Verlauf durchgängig erhöhte Lymphozyten-Werte. Dies war bereits bei Baseline der Fall, da eine Natalizumab-Therapie zum Anstieg der Lymphozyten im peripheren Blut führt.[116] Bei Patienten, die vormals Dimethylfumarat (DMF) als Therapie erhielten, sahen wir jedoch unter Therapie mit Cladribin eine deutlich stärker ausgeprägte Lymphopenie. Diese erreichte im Nadir bei etwa 50% der Patienten einen Wert <500/mm^3. Insgesamt war das Risiko, eine Lymphopenie<500/mm^3 zu entwickeln, nach DMF-Vortherapie fünffach erhöht. Direkt hiermit assoziiert war bei diesen Patienten auch ein deutlich erhöhtes Risiko, eine VZV-assoziierte Erkrankung zu entwickeln (13 von 22 Patienten mit VZV-assoziierter Erkrankung erhielten als Vortherapie DMF).

Die pathophysiologischen Zusammenhänge hinter der Entwicklung einer Lymphopenie in dieser Therapiesequenz verbleiben letztlich unklar, allerdings ist die

Entwicklung teils langanhaltender Lymphopenien unter Therapie mit Fumaraten bereits langjährig bekannt[112,113] und zudem mit teils schwerwiegenden Nebenwirkungen assoziiert, so auch der Entwicklung einer PML.[114]

Das Ansprechen auf eine Impfung haben wir in einer prospektiven Phase-IV-Impfstudie getestet. (*Immune Response to Seasonal Influenza Vaccination in Multiple Sclerosis Patients Receiving Cladribine* (CIRMS); NCT05019248). Aufgrund der COVID-19-Pandemie wurde die Rekrutierung nach insgesamt 90 Patienten gestoppt. Die Patienten wurden hierbei in Gruppen stratifiziert, abhängig vom zeitlichen Abstand der letzten Cladribin-Exposition zur Impfung.[115] **(A10)** Insgesamt zeigte sich, dass die Impfantwort unabhängig vom zeitlichen Abstand zur letzten Einnahme von Cladribin suffizient war, sich also keine relevanten Unterschiede zu der mittels Plattform-Therapie behandelten Kontrollgruppe zeigten. Wir konnten dementsprechend nachweisen, dass eine Totimpfung unter Therapie relativ unabhängig vom Zeitpunkt der letzten Einnahme erfolgen kann, was insbesondere die Planung saisonaler Impfungen deutlich erleichtert.

Zuletzt haben wir bezüglich des Auftretens neuer Nebenwirkungen die in unserer Kohorte beobachteten Hautreaktionen systematisch ausgewertet. Insgesamt fanden wir 77 Patienten, die im Zusammenhang mit der Einnahme von Cladribin solche Reaktionen entwickelten (entsprechend 32% der zu diesem Zeitpunkt vorliegenden Gesamtkohorte).[116] **(A11)** Während der Großteil (54/77 Fälle) (sub-)akute Reaktionen auf die Medikamenteneinnahme darstellt (Erytheme, Ekzeme, Mucositis, Pruritus), bestätigten wir Herpesvirus-assoziierte Erkrankungen als die zweithäufigsten Hautreaktionen.

Besondere Aufmerksamkeit erregten allerdings auch zwei Fälle von Autoimmunerkrankungen (leukozytoklastische Vaskulitis, Alopecia areata), welche in Zusammenschau mit weiteren Autoimmunphänomenen in der Kohorte (insb. ein Fall einer Glomerulonephritis[117]) zu besonderer Wachsamkeit drängen. Ferner fielen vier Fälle von (Prä-)Kanzerosen der Haut auf, wobei diese teilweise bei Patienten mit Risikoprofil (insb. ungeschützte Sonnenexposition in der Anamnese) auftraten, teils aber auch unabhängig hiervon.

Zusammenfassend zeigen unsere Arbeiten, dass das Sicherheitsprofil von Cladribin grundsätzlich konsistent ist zu den Studiendaten. Bezüglich Totimpfungen konnten wir ermutigende Daten erheben.

Bei Patienten, die vormals eine Therapie mit Fumaraten erhielten, sind jedoch schwerwiegende Lymphopenien möglich, welche dann auch mit Komplikationen vergesellschaftet sein können. Ferner zeigten wir, dass Hautreaktionen häufig sind und dass Patienten hierüber aufgeklärt werden sollten. Inwiefern die Detektion von kutanen Malignomen die Debatte über ein grundsätzlich erhöhtes Risiko von Neoplasien unter Therapie mit Cladribin erneut anstößt, ist aktuell nicht abzusehen; vielleicht sollte Cladribin bei Patienten mit entsprechender Risikokonstellation (UV-Exposition, positive Anamnese für kutane Malignome) zurückhaltend eingesetzt werden und Patienten sollten über die Notwendigkeit eines entsprechenden Sonnenschutzes informiert werden. Das regelmäßige Hautkrebsscreening scheint in jedem Fall indiziert unter Therapie mit Cladribin.

2.3 Ocrelizumab

2.3.1 Zulassungsdaten

Der monoklonale Antikörper Ocrelizumab ist seit Anfang 2018 in Deutschland in der Regelversorgung verfügbar, nachdem eine positive Evaluation in der schubförmigen Multiplen Sklerose in den OPERA-Schwesterstudien (OPERA1&2) gelang.[16] Auch wenn hier nicht weiter darauf eingegangen werden soll, ist erwähnenswert, dass Ocrelizumab aktuell auch die einzige zugelassene Therapieoption bei primär progredienter Multipler Sklerose darstellt, nachdem die entsprechende ORATORIO-Studie ebenfalls positiv ausfiel. [118]

Ocrelizumab bindet an das CD20-Oberflächenantigen verschiedener B-Zell-Subtypen und löst den Zelluntergang aus.[68] Sowohl die entsprechenden Progenitoren als auch die reifen Plasmazellen selbst exprimieren CD20 nicht und werden ausgespart. Wie bereits vormals beschrieben, beginnt die Repletion von B-Zellen nach einem Zeitraum von drei bis sechs Monaten. Dies war Grundlage für das zugelassene Dosisschema von Ocrelizumab mit der Applikation alle sechs Monate.

Prototypisch für die B-Zell-Therapie war der erste zur Therapie beim Menschen zugelassene Antikörper Rituximab, welcher auch an CD20 bindet und bereits 2008 in der HERMES-Studie positiv bei MS-Patienten evaluiert wurde.[119] Eine Zulassung von Rituximab selbst erfolgte nicht, formal wurde auch erst unlängst eine eigenständige Phase-III-Studie hierzu durchgeführt (RIFUND-Studie).[120]

2.3.2 Offene Fragen zur Wirksamkeit in der Regelversorgung

Angesichts des spätestens seit 2008 in verschiedenen Ländern bereits erfolgenden, „*off label*“-Einsatzes von Rituximab[121] ist das Wirksamkeits- und Sicherheitsprofil der B-Zell-Depletion grundsätzlich gut definiert. Es gilt jedoch auch im Rahmen der OPERA-Studien das bereits wiederholt thematisierte Dilemma, dass neben den Plattform-Therapien weitere immunmodulatorische Vortherapien weitgehend abwesend waren.

Ferner besteht seit jeher Unklarheit über die Möglichkeiten und Limitationen des Therapiemonitorings der B-Zell-Depletion mittels Bestimmung der peripheren $CD19^+$ B-Zellen. Während der Ära des *off label*-Einsatzes von Rituximab bei verschiedenen neuroimmunologischen Erkrankungen wurden therapeutische Entscheidungen oft hieran ausgerichtet. Dies war durch zahlreiche Studien gedeckt, in denen ein Wiederanstieg der B-Zellen einer erneuten Krankheitsaktivität vorausging.[122-124] Im Gegensatz zu Rituximab zeigte sich unter Ocrelizumab in den Zulassungsstudien allerdings kein ausgeprägter Wiederanstieg der B-Zellen nach sechs Monaten.[16]

Während der weiteren Evaluation der Ocrelizumab-Kohorte kam aber im Rahmen der COVID-19-Pandemie die berechtigte Sorge vor schweren Krankheitsverläufen nach B-Zell-Therapie und einem möglicherweise suboptimalen Ansprechen auf eine Impfung auf. Spätestens in dieser Situation bestand großes Interesse, die Bedeutung der peripheren B-Zellen als prädiktiven Marker zu evaluieren.

Insgesamt bestanden also folgende Fragen zur Wirksamkeit von Ocrelizumab in der Regelversorgung:

1. Inwiefern beeinflusst die Therapiesequenz die Wirksamkeit von Ocrelizumab in der Regelversorgung?
2. Welche Bedeutung hat die Bestimmung der peripheren B-Zellen in der Therapiesteuerung und kann unter Berücksichtigung dessen gegebenenfalls das Therapieintervall verändert werden?

2.3.3 Eigene Arbeiten zur Wirksamkeit in der Regelversorgung

In dieses Kapitel eingeflossene eigene Arbeiten:

A12 **Pfeuffer S**, Rolfes L, Ingwersen J, Pul R, Kleinschnitz K, Korsen M, Räuber S, Ruck T, Schieferdecker S, Willison AG, Aktas O, Kleinschnitz C, Hartung HP, Kappos L, Meuth SG. Effect of Previous Disease-Modifying Therapy on Treatment Effectiveness for Patients Treated With Ocrelizumab. Neurol Neuroimmunol Neuroinflamm. 2023 Apr 11;10(3):e200104. doi: 10.1212/NXI.0000000000200104.
A13 Rolfes L, Pawlitzki M, **Pfeuffer S**, Nelke C, Lux A, Pul R, Kleinschnitz C, Kleinschnitz K, Rogall R, Pape K, Bittner S, Zipp F, Warnke C, Goereci Y, Schroeter M, Ingwersen J, Aktas O, Klotz L, Ruck T, Wiendl H, Meuth SG. Ocrelizumab Extended Interval Dosing in Multiple Sclerosis in Times of COVID-19. Neurol Neuroimmunol Neuroinflamm. 2021 Jul 14;8(5):e1035. doi: 10.1212/NXI.0000000000001035.

Wir untersuchten die Wirksamkeit von Ocrelizumab unter anderem in einer multizentrischen, prospektiven Studienkohorte von insgesamt 280 Patienten.[125] **(A12)** Grundsätzlich bestätigten wir die Studiendaten dahingehend, dass innerhalb des vormals hochaktiven Patientenkollektivs (im Median ein Schub pro Jahr) die Schubrate nach Therapieeinleitung mit Ocrelizumab auf 0.07 Schübe pro Jahr fiel. Vergleichbare Ergebnisse beobachteten wir auch im Hinblick auf die Behinderungsprogression und das Auftreten neuer T2-hyperintenser MRT-Läsionen.

Nach Analyse der Effektivität in Abhängigkeit von der letzten immunmodulatorischen Vortherapie zeigten wir die Wirksamkeit bei vormals therapienaiven Patienten und auch bei Patienten, die von Plattform-Substanzen, Teriflunomid, DMF oder Natalizumab wechselten. Allerdings fiel auch hier eine relative Einschränkung der Wirksamkeit bei Patienten auf, die als letzte Vortherapie Fingolimod erhielten. Diese Patienten wiesen ein 3,5 bis 5,9fach erhöhtes Risiko fortgesetzter Krankheitsaktivität auf, je nachdem, ob es sich bei Ocrelizumab um die zweite oder dritte Eskalationsbehandlung in der Therapiesequenz handelte (darauf bezogen, dass

Fingolimod als erste bzw. zweite Eskalationstherapie verabreicht wurde). Vergleichbare Ergebnisse sahen wir auch in Hinblick auf die weiteren Endpunkte.

Wir evaluierten entsprechend, ob einerseits der initiale Depletionserfolg nach Vorbehandlung mit Fingolimod eventuell eingeschränkt ist, beispielsweise durch anhaltende Retention von B-Zellen in den lymphatischen Geweben. Andererseits untersuchten wir auch, ob eine vorzeitige Repletion der B-Zellen im peripheren Blut nach Vorbehandlung mit Fingolimod nachzuweisen ist. Beides konnten wir letztlich verneinen.

Die genauen pathophysiologischen Zusammenhänge, die der verminderten Wirksamkeit nach vorherigem Einsatz von Fingolimod zugrunde liegen, sind bis heute nicht abschließend verstanden. Prinzipiell übt Fingolimod neben der Retention von Immunzellen in lymphatischen Geweben aber auch weitere, anhaltende Effekte auf das Immunsystem aus. Insbesondere führt Fingolimod auch zu einem Anstieg regulatorischer B-Zellen im peripheren Blut und exponiert diese womöglich zur Depletion.[126, 127]

Um die Bedeutung der Bestimmung der peripheren B-Zellen und einer Anpassung des Dosisintervalls weiter zu eruieren, untersuchten wir eine multizentrische Kohorte von 318 Patienten aus fünf Schwerpunktzentren in Deutschland. Wir verglichen die Krankheitsverläufe von Patienten unter Standard-Dosierungsintervall (SID) und extendiertem Dosierungsintervall (EID, d.h. Verlängerung um ≥4 Wochen). Die Verlängerung des Dosisintervalls betrug in unserer Kohorte im Mittel acht Wochen.[128] **(A13)** Wir konnten letztlich keinen signifikanten Unterschied bezüglich des Wiederauftretens von Krankheitsaktivität ausmachen, auch weil in beiden Therapiegruppen jeweils etwa 90% der Patienten frei von jeglicher Krankheitsaktivität

blieben. Die Zahl der zirkulierenden B-Zellen verblieb in beiden Gruppen in der überwiegenden Zahl der Patienten supprimiert. Soweit bei geringer Fallzahl angesichts der wenigen Krankheitsschübe beurteilbar, zeigte sich in unserer Kohorte keine Assoziation zwischen einer Repopulation der zirkulierenden B-Zellen und einer erneuten Krankheitsaktivität.

Zusammenfassend konnten wir in unseren Arbeiten zeigen, dass die immunmodulatorische Vortherapie auch bei Patienten unter B-Zell-depletierender Therapie einen Einfluss auf die Wirksamkeit hat. Insbesondere scheint die Vorbehandlung mit einem S1PRM wie Fingolimod die Wirksamkeit von Ocrelizumab zu reduzieren. Hierüber sollten Patienten informiert werden. Ferner konnten wir in unseren Arbeiten zeigen, dass im begründeten Einzelfall, z.B. zur Sicherstellung einer suffizienten Impfantwort, eine Verzögerung der erneuten Infusion von Ocrelizumab für einige Wochen nicht mit einem gesteigerten Risiko wiederkehrender Krankheitsaktivität assoziiert ist. Insgesamt erschien die Bestimmung der $CD19^+$ B-Zellen im peripheren Blut aber nicht nützlich, da sie in keiner der beiden Studien geeignet war, die Patienten mit fortgesetzter/wiederkehrender Krankheitsaktivität zu identifizieren.

3. ZUSAMMENFASSUNG UND AUSBLICK

In der bisherigen Zeit meiner wissenschaftlichen und klinischen Laufbahn habe ich bereits eine beeindruckende Wandlung der therapeutischen Möglichkeiten bei Patienten mit Multipler Sklerose miterlebt, da zeitgleich mit dem Beginn meiner Weiterbildungszeit Alemtuzumab in der klinischen Regelversorgung eingesetzt wurde. In den folgenden Jahren wurden mit Cladribin und Ocrelizumab zahlreiche Immunrekonstitutionstherapeutika neu zugelassen und zusammen mit den weiteren neu entwickelten Substanzen im Bereich der Erhaltungstherapeutika wurde das Ziel einer kompletten Krankheitsfreiheit auch in der Regelversorgung erreichbar und zum therapeutischen Goldstandard erklärt.

Während in dieser Zeit faktisch jedes Jahr eine neue Substanz verfügbar wurde, beträgt die Generationszeit wissenschaftlicher Evidenz mittels randomisierter kontrollierter Studien allein von der Registrierung der Studie bis hin zur Bekanntgabe über das Vorliegen von Ergebnissen durchaus sechs bis sieben Jahre (am Beispiel der OPERA-Studien[16]). In der klinischen Regelversorgung besteht dadurch bei den neu zugelassenen Substanzen eine „*challenge of choice*“[77], da sich aus den Zulassungsstudien regelhaft keine weiteren Therapiealgorithmen ableiten lassen, beziehungsweise eine Evidenz für Therapiesequenzen fehlt.

Im Falle von Alemtuzumab lagen zwischen Zulassung und Publikation der ersten Fallserie, die eine verminderte Wirksamkeit bei mit Fingolimod vorbehandelten Patienten zeigte, vier Jahre.[85]

Der Einfluss einer Therapiesequenz kann jedoch nicht sinnvoll im Rahmen einer Zulassungsstudie evaluiert werden, da hierzu bereits auf der Ebene einer Vortherapie

eine erste Randomisierung erforderlich wäre und dementsprechend der Umfang der notwendigen Stichprobe um den Faktor X^n ansteigen würde (wobei X die eigentlich zu vergleichenden Substanzen und n die Anzahl der verfügbaren Vortherapien seien).

Dementsprechend besteht die Notwendigkeit, möglichst unmittelbar nach Markteintritt einer neuen Substanz, prospektive, multizentrische Kohortenstudien zu initiieren, um die hier erörterten Analysen durchführen zu können. Dass die Betrachtung der immunmodulatorischen Vortherapie dabei gesondert erfolgen muss, ist beispielsweise daran zu ersehen, dass noch 2017 in einer über 4300 Patienten fassenden Kohorte adjustierte Vergleiche zwischen Fingolimod, Natalizumab und Alemtuzumab sowie weiteren Substanzen erfolgten, ohne dass der spezifische Nachweis ungünstiger Therapiesequenzen in dieser darauf nicht ausgerichteten Analyse augenscheinlich wurde. [129]

Prospektive Beobachtungsstudien haben gegenüber kontrollierten Interventionsstudien selbstverständlich Nachteile. Einerseits besteht ein mögliches Selektionsbias, da präferenziell die hochaktiven Patienten langfristig an das tertiäre Zentrum angebunden verbleiben, während sich langfristig stabile Patienten im Verlauf häufiger an „periphere“ Institutionen (in Deutschland im Speziellen auch niedergelassene Kollegen) wenden.

Allein aufgrund der Anforderungen an das Infusionsmanagement ist dieser Effekt in unserer Kohorte bezüglich Alemtuzumab und Ocrelizumab zu vernachlässigen und bei Cladribin stellen die erheblichen Kosten der Therapie mit entsprechenden Schwierigkeiten bezüglich der Abrechnungsbudgets womöglich ein „ausreichend starkes Hindernis“ für eine Auslagerung der Patienten dar.

Grundsätzlich sind natürlich nur nationale longitudinale Kohorten über ein Selektionsbias erhaben. Derartige Kohorten existieren beispielsweise in Dänemark oder Schweden und diese decken weit über 95% der in den Ländern behandelten Patienten ab.[130] In Deutschland existieren zahlreiche Versuche, entsprechende Kohorten zu etablieren, wobei diese jeweils deutlich kleiner sind und darüber hinaus oft konkurrieren.

Die hier vorgestellten Arbeiten und die beobachteten Effekte sind womöglich durch (unbekannte) Confounder bedingt. Der Fokus dieser Studien liegt nun aber nicht primär auf dem pathophysiologischen Erkenntnisgewinn, sondern ist in dieser Hinsicht allenfalls als hypothesengenerierend anzusehen. Eigentliches Ziel war und ist die Evaluation der Versorgungsrealität, sodass die Ergebnisse zwar stets mit Augenmaß interpretiert werden sollten, aber dennoch erhebliche praktische Bedeutung haben.

Die systematische Nachbeobachtung von Patienten im Rahmen longitudinaler Kohorten ist auch zwingende Voraussetzung zur Detektion von neu aufgetretenen MRT-Läsionen, klinischen Schüben oder aber zunehmender Behinderung und dies ist in der Regelversorgung oft allenfalls eingeschränkt zu gewährleisten.

Unsere Arbeiten begegnen dieser Einschränkung vor allem dadurch, dass die Patienten unter den jeweiligen Immuntherapien im Rahmen systematischer longitudinaler Studien verfolgt wurden, die mit aufeinander abgestimmter Datenerhebung an den jeweiligen Standorten durchgeführt wurden. Der Erfolg dieses Aufwandes schlägt sich vor allem in der hohen Retentionsrate in unseren Studien als auch in der hohen Verfügbarkeit von MRT-Daten und anderen Parametern nieder.

Bezüglich der eingangs formulierten Fragen konnte nun also das Wirksamkeitsprofil von Alemtuzumab und Ocrelizumab dahingehend schärfer definiert werden, dass bei

vormals naiven Patienten und solchen, die vorher eine Plattformtherapie, Fumarate oder Teriflunomid erhielten, die Wirkung vergleichbar den Ergebnissen der Zulassungsstudien war. Bei Patienten, die vormals Natalizumab erhielten, zeigte sich ebenfalls ein konsistentes Bild; hier konnten wir auch zeigen, dass Alemtuzumab bei notwendigem Absetzen von Natalizumab der Therapie mit Fingolimod überlegen ist. Auch das Wirkungsprofil von Cladribin war im Wesentlichen konsistent zu den Studiendaten, auch wenn seine Wirksamkeit bei Patienten, die von Natalizumab umstellten nicht ausreichend zu sein schien.

Bei Patienten, die vorher S1PRM erhielten, ist hingegen die Wirkung von beiden monoklonalen Antikörpern reduziert und dieser Effekt scheint auch in langjähriger Nachverfolgung anhaltend. Die Literatur diskutiert aber insbesondere im Hinblick auf die Sequenz „S1PRM→Ocrelizumab“ die Stärke und Persistenz des Effekts weiterhin. Auch ist die Frage, inwiefern dies durch eine verlängerte Auswaschphase moduliert werden kann, noch offen.[131]

Patienten sind meiner Meinung nach dennoch entsprechend aufzuklären und dies sollte schon bei der Einstellung auf einen S1PRM bedacht werden, da von den weiteren Eskalationsmöglichkeiten aktuell nur eine Therapie Natalizumab keinen Anhalt für eine Einschränkung der Wirksamkeit liefert. Inwiefern künftige DMT auch nach S1PRM-Vortherapie uneingeschränkt wirksam sind, bleibt abzuwarten.

Bezüglich der Sicherheit fanden sich in Hinblick auf Ocrelizumab keine durchgreifenden Änderungen gegenüber dem in den Zulassungsstudien dokumentierten Wirkungsprofil. Wir konnten auch zeigen, dass eine Extension des Dosisintervalls zumindest über acht Wochen über das Standardintervall hinaus

weiterhin sicher und wirksam war. Dies wurde zwischenzeitlich von weiteren Arbeitsgruppen bestätigt.[132]

Das Sicherheitsprofil von Alemtuzumab hat sich die Jahre hinweg deutlich komplexer gestaltet. Wir haben einerseits sowohl neuartige Autoimmunphänomene beschrieben als auch insbesondere die ursprünglich angenommene Pathophysiologie einer rein B-Zell-getriebenen Autoimmunität weiterentwickeln können und damit das Verständnis der Lymphopenie-induzierten Autoimmunität vorangetrieben. Klinische Relevanz haben unsere Analysen insbesondere dahingehend, dass die Risikokonstellation bei Baseline viel stärker zu berücksichtigen ist, sofern sie zu erfassen ist (z.B. in Hinblick auf die Schilddrüsen-Autoantikörper). In den letzten Jahren wurde Alemtuzumab einerseits aufgrund der zunehmenden Beschreibungen sekundärer Autoimmunphänomene als auch aufgrund möglicher kardiovaskulärer Komplikationen[133], die auch eine zeitweilige Indikationseinschränkung im Rahmen eines sogenannten „Artikel-20-Verfahrens“ der Europäischen Arzneimittelbehörde bedingten, deutlich seltener eingesetzt.

Das Sicherheitsprofil von Cladribin wurde im Rahmen unserer Arbeiten ebenfalls weiter eingeordnet und inzwischen auch durch externe Arbeiten bestätigt. Die bedeutsamsten Infektionserkrankungen, die nach Cladribin-Exposition gehäuft beobachtet wurden, waren Varizella-Zoster-Virus-assoziierte Erkrankungen.

VZV-Reaktivierungen sind bei MS-Patienten (mit und ohne Immuntherapie) gegenüber der Allgemeinbevölkerung grundsätzlich gehäuft, neigen unter Immunmodulation jedoch häufiger zu Komplikationen und Residuen (Post-Zoster-Neuralgie).[23] Gemäß des Wirkmechanismus von Cladribin ist die Infektionsneigung eng mit der Zahl der Lymphozyten im Blut assoziiert. Dies bestätigte sich in unseren Arbeiten.[134] Die rapide

Zunahme von Lymphopenien und entsprechender Infektionserkrankungen nach Fumarat-Vorbehandlung erscheint nicht unerwartet angesichts der bisherigen Literatur.[135] Wir konnten aber auch beobachten, dass selbst bei Patienten, die bei Beginn der Therapie mit Cladribin normale Lymphozytenwerte aufwiesen, prolongierte Lymphopenien nach Behandlung auftraten. Dies erscheint womöglich durch die langanhaltenden Veränderungen von T-Zell-Subpopulationen nach Fumarat-Behandlung bedingt, welche sich in der Gesamtlymphozytenzahl bei Baseline jedoch nicht abbildeten.[136]

Dies sei an dieser Stelle nochmals erwähnt, da die Prävalenz immunmodulatorisch behandelter Patienten jenseits des 50. Lebensjahres weltweit deutlich zunimmt und Cladribin insbesondere in dieser Patientenkohorte zunehmend als therapeutische Option thematisiert wird (u.a. auch auf der von uns mitentwickelten Erkenntnis, dass Impfantworten unter dieser Therapie erhalten bleiben).[137] Nachdem ältere Patienten in der eigenen Erfahrung besonders selten therapienaiv vor Behandlung mit hochaktiven Substanzen sind, kommt dem Risikomanagement hier besondere Bedeutung zu.

Dies soll auch zum Ausblick überleiten, der angesichts der fortwährenden Entwicklung neuer Substanzen zur Therapie sowie auch des zunehmenden Anteils älterer, womöglich mehrfach vorbehandelter Patienten meiner Meinung nach von zunehmend schwierigeren Therapieentscheidungen geprägt sein wird. Analogien zu den hier vorgestellten Daten werden wohl auch weiterhin nicht aus Phase-III-Studien ableitbar sein.

Auch in den jüngst komplettierten Zulassungsstudien von Evobrutinib waren zwei Drittel der eingeschlossenen Patienten therapienaiv[138] und dieser Trend setzt sich in den weiteren laufenden Phase-III-Studien in der Multiplen Sklerose ungebrochen fort.

Ein Lichtblick ist hier womöglich die HERCULES-Studie, in welcher Tolebrutinib bei Patienten mit inaktiver, sekundär-progredienter Multipler Sklerose in der Phase-III evaluiert wird. Die hier evaluierten Patienten wurden durchgängig mit den derzeit gängigen Substanzen vorbehandelt und weisen mit einem Median von 49 Jahren ein höheres Lebensalter auf[139], sodass Sicherheitssignale aus spezifischen Therapiesequenzen womöglich sichtbar werden. Da es sich bei HERCULES um eine Studie zur sekundär-progredienten MS mit entsprechenden klinischen Endpunkten handelt, werden Rückschlüsse im Hinblick auf die anti-inflammatorische Wirksamkeit spezifischer Therapiesequenzen aber wohl nur schwer zu ziehen sein und stehen grundsätzlich unter dem Vorbehalt, dass diese Studie überhaupt positiv verläuft (als erste Studie bei dezidiert inaktiver, progredienter MS).

Die Bruton-Tyrosin-Kinase (BTK)-Inhibitoren zu denen Tolebrutinib und Evobrutinib gehören, gelten als die wahrscheinlichsten Kandidaten, bei denen eine Zulassung zumindest einzelner Substanzen in den nächsten Jahren denkbar erscheint.[140,141]

Nachdem aufgrund ihres Wirkmechanismus, sowohl auf das angeborene als auch das erworbene Immunsystem, diese Substanzen als mögliche Therapieoptionen „über die gesamte Lebenszeit hinweg“ glorifiziert wurden, hat dieses Bild durch die negativen Studien zu Evobrutinib Risse bekommen. Auch wenn diese Substanzen in den kommenden Jahren zum Einsatz kommen werden, wird dies also wohl in Therapiesequenzen geschehen.

In diesem Kontext ist auch zu berücksichtigen, dass künftige Therapiesequenzen nicht mehr nur die „klassische“ Eskalation von Plattform-Therapien hin zu hochaktiven Substanzen umfassen werden, sondern dass auch Sequenzen mehrerer hochaktiver Substanzen hintereinander wahrscheinlich werden.

Beispielsweise wurde bereits der sequenzielle Einsatz der BTK-Inhibitoren (siehe oben) und der B-Zell-depletierenden CD20-Antikörper (zum Beispiel Ocrelizumab) diskutiert. Derartige Regime werden bereits bei anderen Indikationen (lymphatische Leukämie), bei denen BTK-Hemmer und CD20-Antikörper bereits zugelassen sind, eingesetzt.[142,143]

Auch dieses wird uns – sofern BTK-Hemmer in der MS verfügbar werden – entweder „geplant“ oder „ungeplant“ begegnen.

Die „*challenge of choice*“ gepaart mit der Notwendigkeit, systematischer und gründlicher Nachbeobachtungen in der Regelversorgung werden in jedem Fall nicht weniger bedeutsam werden.

4. EIGENE ARBEITEN

4.1 Impact of previous disease-modifying treatment on effectiveness and safety outcomes, among patients with multiple sclerosis treated with Alemtuzumab

Multiple sclerosis

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Original research

Impact of previous disease-modifying treatment on effectiveness and safety outcomes, among patients with multiple sclerosis treated with alemtuzumab

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ABSTRACT

Objectives Alemtuzumab is effective in patients with active multiple sclerosis but has a complex safety profile, including the development of secondary autoimmunity. Most of patients enrolled in randomised clinical trials with alemtuzumab were either treatment naïve or pretreated with injectable substances. Other previous disease-modifying treatments (DMTs) were not used in the study cohorts, and therefore, associated risks might yet remain unidentified.

Methods We retrospectively evaluated a prospective dual-centre alemtuzumab cohort of 170 patients. We examined the baseline characteristics as well as safety and effectiveness outcomes, including the time to first relapse, the time to 3 months confirmed disability worsening and the time to secondary autoimmunity.

Results The regression analysis showed that, among all previously used DMTs, the pretreatment with fingolimod (n=33 HRs for the time to first relapse (HR 5.420, 95% CI 2.520 to 11.660; p<0.001)) and for the time to worsening of disability (HR 7.676, 95% CI 2.870 to 20.534; p<0.001). Additionally, patients pretreated with fingolimod were more likely to experience spinal relapses (55% vs 10% among previously naïve patients; p<0.001) and had an increased risk of secondary autoimmunity (HR 5.875, 95% CI 2.126 to 16.27; p<0.001).

Conclusion In the real-world setting, we demonstrated suboptimal disease control and increased risk of secondary autoimmunity following alemtuzumab, among patients previously treated with fingolimod. These data can provide guidance for improving MS therapeutic management.

Linked

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INTRODUCTION

Despite the approval of several disease-modifying treatments (DMTs) within the past decade, the therapeutic management of relapsing-remitting multiple sclerosis remains challenging. Most of patients are initially treated with low-risk first-line treatments and are switched to highly active therapies, which are potentially associated with more severe side effects, only if further disease activity occurs.

Alemtuzumab (ALEM) is an anti-CD52 monoclonal antibody,[1] which was shown to be highly efficacious in controlling the disease activity, among both treatment naïve patients (CARE-MS I) and those, who had poor response to first line DMT (CARE-MS II).[2 3] Patients enrolled in the CARE-MS II trial had been previously treated mainly with beta-interferon (IFN) or glatiramer acetate, although a minority had received natalizumab (NTZ), azathioprine or mitoxantrone.[2] Ongoing real-world cohorts, such as the TREAT-MS registry,[4] provide important information regarding the use of ALEM and demonstrated that its effectiveness proportionally reduces with the number of previously administered DMTs.[5]

Despite a variety of new DMTs having entered the clinical routine, real-world data on specific treatment sequences remains sparse and the optimisation of the escalating therapeutic management remains short of general consensus. The effectiveness and safety profile of ALEM, among patients pretreated with NTZ, has been suggested to be consistent to the core study results.[6 7] However, evidence of the ALEM effectiveness, among patients who previously failed to respond to fingolimod (FTY) has been conflicting. While a British case series suggested that the escalation to ALEM might not achieve a good control of the disease activity,[8] more retrospective analyses demonstrated good effectiveness and safety profile of ALEM following FTY pretreatment.[9 10] In addition, data on the effect of ALEM among patients previously treated with teriflunomide, dimethyl fumarate (DMF) or ocrelizumab are currently missing.

Real-world data are also needed to assess the potential impact of DMT sequencing on the immune system in order to assist the decision making process in clinical practice. This is particularly relevant to the ALEM, as its use has been recently restricted by medical authorities to a subset of pretreated and highly active patients, because of its complex safety profile, including infusion-associated reactions (IAR), cerebrovascular complications and potential development of secondary autoimmunity, even years after the last administration.[2 3] In this context, we analysed a large real-world prospective cohort of patients with MS in order to assess the potential impact of pretreatment on the efficacy and safety of ALEM infusion.

METHODS

Patients

Between February 2014 and April 2018, adult patients with active MS, according to 2010

Table 1 Distribution of baseline data in the PROGRAM^{MS} cohort

		Last previous DMT				
	Whole cohort	NTZa	NTZs	FTY	Basic	Naïve
Patients, no	170	29	21	33	52	35
Age at baseline ALEM infusion, years, median (IQR)	34 (26–41)	36 (29–43)	34 (31–46)	35 (27–42)	32 (25–36)	27 (22–36)
Male patients, no (%)	57 (34)	9 (31)	2 (10)	6 (18)	23 (44)	17 (49)
Baseline-ARR, median (IQR)	1 (1–2)	2 (1–2)	0 (0–0)	1 (1–2)	1 (1–2)	1 (1–2)
Baseline-EDSS, median (IQR)	2.5 (1.5–3.5)	3 (2–4)	3.5 (2.5–4)	3 (2–4)	2 (1–3)	2 (1–2.5)
Disease duration since onset, years, median (IQR)	6 (2–10)	9 (7–12)	10 (7–15)	7 (4–11)	5 (3–8)	1 (0–2)
Treatment duration of last previous DMT (non-naïve pat.), months, median (IQR)	21 (8–37)	27.5 (16.75–46.75)	21 (7–33)	14 (7.5–25)	14 (7–34)	–
Previous DMT						–
0 (no (%))	35 (21)	0 (0)	0 (0)	0 (0)	0 (0)	
1 (no (%))	42 (25)	5 (17)	4 (19)	4 (12)	29 (56)	
2 (no (%))	39 (23)	8 (28)	9 (43)	13 (39)	9 (17)	
3 (no (%))	31 (18)	8 (28)	4 (19)	9 (27)	10 (19)	
≥4 (no (%))	23 (14)	8 (28)	4 (19)	7 (22)	4 (8)	
Washout duration of last previous DMT, (non-naïve pat.), days, median (IQR)	38 (7–51)	41 (37–49)	56 (46–65)	43 (35–67)	0 (0–14)	–
Follow-up duration, months, median (IQR)	44 (35–52)	39 (29–49)	51 (43–65)	48 (37.5–54.5)	42 (32–50)	44 (36–53)

Basic treatment group includes patients previously treated with either beta-interferon formulations or dimethyl fumarate.
ALEM, alemtuzumab; ARR, annualised relapse rate; DMT, disease-modifying treatment; EDSS, Expanded Disability Status Scale; FTY, fingolimod; NTZa, natalizumab (active subgroup); NTZs, natalizumab (stable subgroup).

revised McDonald criteria,[11] who were considered eligible for treatment with ALEM, based on the most recent prescription criteria, were enrolled in our prospective PROGRAM^{MS} cohort (NCT04082260). ALEM infusions were administered at two tertiary MS centres (Muenster & Essen, Germany). All patients received ALEM according to the manufacturer's guidelines. Exclusion criteria were: any progressive form of MS, inability to undergo MRI examination, presence of autoimmune disorders other than MS, systemic disease that interfere either with disability due to MS or the safety profile of ALEM; a more detailed description has been previously published.[12]

Outcome measurements

Epidemiological data were validated on screening and baseline infusion including disease and treatment history as well as determination of smoker-status since this was previously identified as possible risk factor for development of secondary autoimmunity.[13] IARs were documented and graded according to the common terminology criteria for adverse events (CTCAE). Patients were evaluated every 3 months with standardised neurological examinations by two trained neurologists per site; the level of disability was scored by using the Expanded Disability Status Scale (EDSS). The occurrences of relapses, including their exact date of onset, the performed treatment and symptoms characterising the affected functional system, were recorded. Furthermore, localisation of symptomatic inflammation in the central nervous system (CNS) was determined by clinical evaluation and MRI data from semiannual scans. In the case of multifocal relapses, the localisation driving the relapse-related disability increase was counted.

In this study, we included only patients with a documented follow-up of at least 1 year following the second ALEM infusion course and with complete data on previous DMT, including treatment duration, date and reason for treatment cessation. The washout duration was defined as time from last drug intake to the first ALEM infusion.

Statistical analysis

Baseline parameters in our cohort were assessed using descriptive statistics. Patients receiving basic treatments (beta-IFN, DMF, glatiramer acetate, teriflunomide) were merged into one group (referred to as 'basic' group) since baseline characteristics (online supplemental table S1) and outcomes (online supplemental figure S1) were similar. For analysis of efficacy and safety outcomes, we used the Kaplan-Meier method and the Cox proportional hazard model. We defined 'time to first clinical relapse', 'time to first confirmed worsening of disability' and 'time to first manifestation of secondary autoimmune disease (SAD)' (each measured in months since baseline infusion) as meaningful outcome parameters for the regression analysis. Our regression models included the following covariates with an enter method: sex, age (above vs below median, since data were not normally distributed in naïve patients), annualised relapse rate at baseline, baseline-EDSS, disease duration since onset and last previous DMT. Multivariate HRs are stated throughout the manuscript. Worsening of disability was considered clinically relevant if two independent clinical assessments 3 months apart indicated an increase of the EDSS as follows: +1.5 points (baseline=0.0), +1.0 point (baseline=1.0–4.0), +0.5 points (baseline ≥4.5). To determine the progression to secondary progressive MS, Lorscheider criteria[14] were used. Further analyses were carried out using Fisher's exact test or the χ^2 test for categorical variables and Wilcoxon's paired rank-sum test for continuous variables. A $p<0.05$ was considered significant. All analyses were considered exploratory. The analysis was carried out using SPSS Statistics 27.

RESULTS

Patients

We identified 170 patients who were treatment naïve or previously failed to respond to NTZ, FTY, IFN or DMF, and were treated with at least two courses of ALEM. In total, data from 2425/2498 (97.1%) scheduled visits were available. All patients had received their previous DMT for more than 6 months. Majority of patients (n=108) were switched to ALEM because

of the occurrence of disease activity, although 21 NTZ-treated patients were switched because of the increased risk for developing progressive multifocal leukoencephalopathy (PML), while having experienced stable disease.

Baseline clinical and demographic features were similar, among treatments groups. However, there were higher proportions of male patients in the 'naïve' and 'basic' groups. In addition, treatment naïve patients were younger and had shorter disease duration at ALEM commencement (table 1).

Relapses and disability worsening

In the total population, 78 patients (45.9%) experienced at least one relapse within the observation period; this occurred in three patients (1.8%) within the first 3 months and in 34 patients (20.0%) within 1 year following the ALEM infusion. We evaluated whether treatment effectives of ALEM depended on the number of previously administered DMT. Patients who received ALEM as third-line treatment, had a significantly increased hazard for relapses (HR 2.651, 95% CI 1.279 to 5.497; p=0.009), compared with those treated withALEM, as first or second line therapy (online supplemental table S2A). In the same group, a similar trend was observed for increased risk of disability worsening (HR 2.527, 95% CI 0.961 to 6.649; p=0.060; online supplemental table S2B). No relevant differences between patients having received ALEM as first-line or second-line treatment were noted.

Next, we investigated the response to ALEM based on the last previously used DMT. Among all treatments, the exposure to FTY was found to have the most significant association with an increased hazard of experiencing a clinical relapse, compared with treatment naïve and basic groups (figure 1A). The multivariate model confirmed that the previous use of FTY exerted the strongest predictive effect for an increased risk of relapses following ALEM infusion (HR 5.420, 95% CI 2.520 to 11.660; p<0.001) (table 2).

Additionally, the model identified the relapse rate at baseline (HR 1.460, 95% CI 1.098 to 1.940; p=0.009) and the previous exposure to NTZ, as significant predictors of the occurrence of clinical relapses.

Among previously NTZ-treated patients, we evaluated separately those who switched to ALEM because of increased PML risk while having been clinically stable('stable' patients) and those who did because of ongoing disease activity ('active' patients). Compared with naïve patients, we observed higher hazards for relapses as well among 'active' patients (HR 3.888, 95% CI 1.375 to 10.990; p=0.010) as also among 'stable' patients (HR 2.732, 95% CI 1.138 to 6.560; p=0.025) (table 2).

Similar results were found when assessing the hazard for developing 3 months confirmed worsening of disability, which was significantly higher among patients previously treated with FTY (HR 7.676, 95% CI 2.870 to 20.53; p<0.001), compared with naïve patients. However, in the multivariate regression model, no further covariates, including the previous exposure to NTZ, were shown to affect the disability outcome (figure 1B and table 3).

We also evaluated the number of total relapses during the first 2 years following ALEM induction (and thereby prior to any additional courses that were eventually administered). We found a strong reduction of the annualised relapse rate, compared with baseline, among treatment naïve patients (0.33 vs 1.15; p=0.004) and among those who had received basic treatment (0.16 vs 1.47; p<0.001). Similar trend was observed among NTZ-pretreated patients with previously stable disease

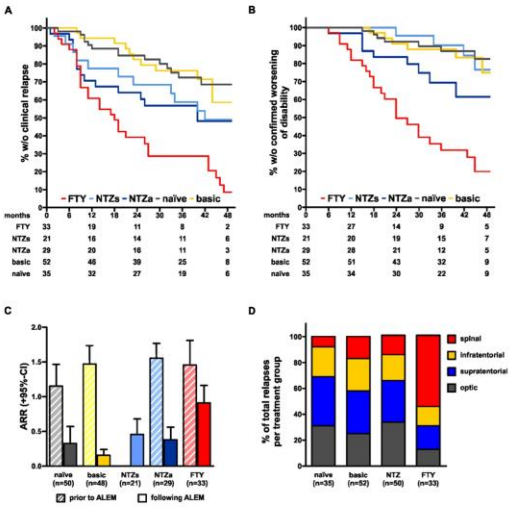

Figure 1 Analysis of efficacy outcomes in the PROGRAM^{MS} cohort. (A) Kaplan-Meier plot depicting time to first clinical relapse of patients stratified to last previous disease-modifying treatment. Numbers below the x-axis indicate patients at risk at the respective time point. (B) Kaplan-Meier plot depicting time to first 3 months confirmed worsening of disability. (C) Analysis of annualised relapse rates 1 year prior to ALEM induction (left, striped bars) and in year 1 and 2 following induction (right bars) divided by last previous disease-modifying treatment. Data are shown as mean+95% CI. Numbers above bars indicate significance levels determined using the Wilcoxon-paired rank-sum test. (D) Depiction of relative relapse distribution in different treatment groups. ALEM, alemtuzumab; FTY, fingolimod; NTZa, natalizumab (previously active); NTZs, natalizumab (previously stable).

(0 vs 0.45; p<0.001), and among previously active NTZ-treated patients (0.38 vs 1.55; *p<0.001*). In the FTY pretreatment group, following ALEM there was smaller yet significant reduction of the relapse rate within first 2 years (0.90 vs 1.46; p=0.010; figure 1C).

Table 2 Regression model for analysing time to first clinical relapse

Time to first relapse	HR	95% CI	P value
Sex (male (57) vs female (113; ref.))	0.707	0.415 to 1.206	0.203
Age (<34 years (84; ref.) vs ≥34 years (86))	1.201	0.729 to 1.981	0.472
Annualised relapse rate at baseline	1.460	1.098 to 1.940	**0.009**
Baseline-EDSS	1.004	0.805 to 1.252	0.973
Disease duration since onset (yrs)	0.945	0.891 to 1.003	0.062
Last previous DMT (naïve=ref. (35)) basic (52)	0.930	0.410 to 2.110	0.983
NTZs (21)	2.732	1.138 to 6.560	**0.025**
NTZa (29)	3.888	1.375 to 10.990	**0.010**
FTY (33)	5.420	2.520 to 11.660	**<0.001**

Results from our Cox proportional hazard model using an enter method to integrate all the covariates in the final analysis. For analysis of age as a covariate, we split our group according to the median. Reference categories are indicated for categorical covariates. Numbers in brackets in the first column indicate sample numbers for the respective covariate.
Bold values indicate p-values below 0.05
DMT, disease-modifying treatment; EDSS, Expanded Disability Status Scale; FTY, fingolimod; NTZa, natalizumab (previously active); NTZs, natalizumab (active subgroup).

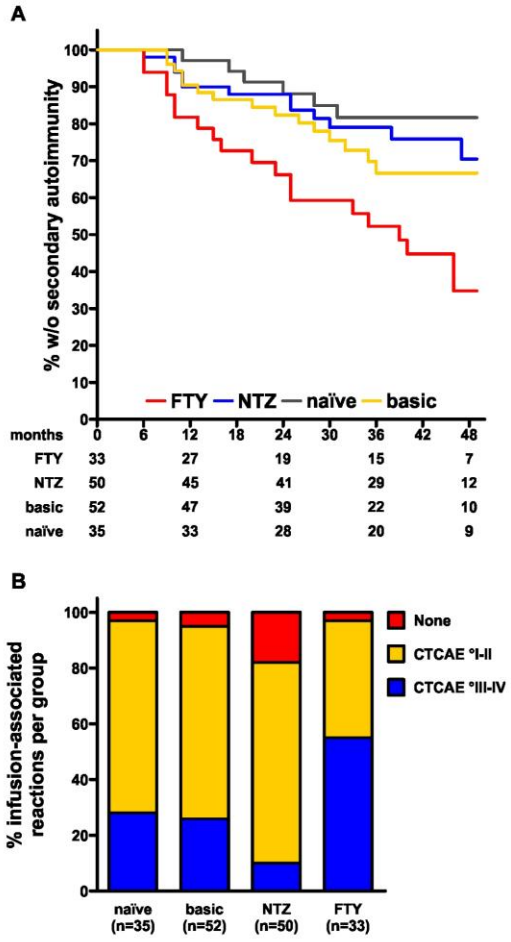

Figure 2 Analysis of safety outcomes in the PROGRAM[MS] cohort. (A) Kaplan-Meier plot depicting time to onset of (first) secondary autoimmune disorder. (B) Analysis of infusion-associated reactions stratified by severity grade in patients who received their first course of ALEM. ALEM, alemtuzumab; CTCAE, common terminology criteria for adverse events; FTY, fingolimod; NTZ, natalizumab.

Table 5 Regression model for analysing time to first secondary autoimmune disorder

Time to first development of secondary autoimmunity	HR	95% CI	P value
Sex (male (57) vs female (113; ref.))	1.514	0.813 to 2.820	0.191
Age (<34 years (84; ref.) vs ≥34 years (86))	0.671	0.356 to 1.266	0.218
Annualised relapse rate at baseline	0.926	0.665-.1290	0.650
Baseline-EDSS	1.004	0.770 to 1.319	0.974
Disease duration since onset (yrs)	0.997	0.931 to 1.066	0.921
Last previous DMT (naïve=ref. (35)) basic (52)	2.043	0.770 to 5.418	0.151
NTZ (50)	1.802	0.602 to 5.393	0.293
FTY (33)	5.875	2.126 to 16.237	**<0.001**

Results from our Cox proportional hazard model using an enter method to integrate all the covariates in the final analysis. For analysis of age as a covariate, we split our group according to the median. Reference categories are indicated for categorical covariates. Numbers in brackets in the first column indicate sample numbers for the respective covariate.
Bold values indicate p-values below 0.05
DMT, disease-modifying treatment; EDSS, Expanded Disability Status Scale; FTY, fingolimod; NTZ, natalizumab.

from the TREAT-MS study, showing a declining efficacy of ALEM proportionally to the number of previously used DMTs and therefore a better response following administration early in the disease course.[5]

We focused on the impact on the treatment response of the last previous DMT administered before ALEM. The analyses demonstrated that patients switching to ALEM from NTZ, compared with those previously on basic therapy or treatment naïve, were more likely to experience relapses. This was observed in both subgroups of previously NTZ-treated patients with or without previous ongoing disease activity. However, in both NTZ-treated subgroups, following the commencement of ALEM there was no significantly increased risk of disability worsening, compared with naïve patients unlike was previously observed in patients switching from NTZ to FTY.[6 19] Overall, our data support the use of ALEM as a valuable treatment option for patients stopping NTZ.

Previous evidence regarding the effectiveness of ALEM in previously FTY-exposed patients remained conflicting. Whereas previous reports raised concerns regarding decreased effectiveness or even aggravation of disease courses within this special treatment sequence,[8] other studies concluded that ALEM is an effective option in patients stopping FTY.[9 10] Here, we showed that patients previously treated with FTY had worse response to ALEM, compared with other treatment groups, as they experienced a less pronounced reduction of annualised relapses rates compared with baseline as well as higher hazards for disability worsening and for the development of secondary autoimmunity. Yet, compared with their respective baseline, annualised relapse rates following ALEM induction were significantly lower in previous FTY-treated patients. We hence consider these patients as 'suboptimal responders' rather than as 'non-responders'.

Previous reports have suggested ongoing lymphopenia as a possible risk factor for suboptimal treatment response. However, we observed normal lymphocyte counts (above 1200 cells/mm^3) in all but three patients following FTY. Nonetheless, we assume that total blood lymphocyte counts might not depict differences in lymphocyte subsets and their respective tissue distribution. Lymphocytes are differentially affected by FTY and relevant populations might be retained in lymphoid tissues and thereby might be relatively spared from depletion.[20] We can only speculate whether such phenomenon is the reason for the relative reduction of IAR in FTY pretreated patients, as such reactions are suggested to directly correlate with immune cell destruction.[21 22]

However, from the current dataset, we can neither confirm nor ultimately rule out that the extension of the wash-out period before commencing ALEM can positively affect outcome parameters (median washout in our cohort: 1.5 months vs 3.5 months in previous cohorts).

It is known that persistent T cell clones can become the source of homeostatic proliferation following ALEM treatment and previous data proved this as a pivotal step for manifestation of

Table 3 Regression model for analysing time to confirmed worsening of disability

Time to first confirmed worsening of disability	HR	95% CI	P value
Sex (male (57) vs female (113; ref.))	0.768	0.400 to 1.473	0.427
Age (<34 years (84; ref.) vs ≥34 years (86))	1.312	0.703 to 2.447	0.394
Annualised relapse rate at baseline	1.239	0.879 to 1.748	0.221
Baseline-EDSS	0.972	0.750 to 0.1260	0.829
Disease duration since onset (yrs)	0.964	0.905 to 1.027	0.258
Last previous DMT (naïve=ref. (35)) basic (52)	0.855	0.279 to 2.615	0.783
NTZs (21)	1.533	0.349 to 6.723	0.571
NTZa (29)	2.92	0.868 to 8.349	0.086
FTY (33)	7.676	2.870 to 20.534	**<0.001**

Results from our Cox proportional hazard model using an enter method to integrate all covariates in the final analysis. For analysis of age as a covariate, we split our group according to the median. Reference categories are indicated for categorical covariates. Numbers in brackets in the first column indicate sample numbers for the respective covariate.
DMT, disease-modifying treatment; EDSS, Expanded Disability Status Scale; FTY, fingolimod; NTZa, natalizumab (previously active); NTZs, natalizumab (stable subgroup).

The clinical and MRI-based analysis of relapse anatomic localisation showed similar distribution of symptoms, among patients who had received basic treatment, NTZ or who were treatment naïve, with most lesions located within the cerebral hemispheres or optic nerves. In contrast, among patients pretreated with FTY there was a high preponderance of spinal relapses (55%) following treatment with ALEM (figure 1D), although previous medical reports showed no trend in favour of spinal cord symptoms while receiving FTY.

We also evaluated the transition to secondary progressive MS and found a single patient who met the Lorscheider criteria at 46 months after the first administration of ALEM (previously treated with FTY).

Additional treatment courses

Because of ongoing disease activity, 4 patients in the basic treatment group, 6 patients pretreated with NTZ, 6 treatment naïve patients and 11 patients pretreated with FTY received a further course of ALEM after 32 median months from the baseline infusion (range: 24–58 months). In addition, two patients (one pretreated with NTZ and one pretreated with FTY) received a fourth course of ALEM at 45 and 55 months, respectively. Following retreatment, all patients in the basic and treatment naïve groups remained clinically stable, while three patients in the NTZ group experienced one further relapse within the observation period. In the FTY group, we observed seven further relapses, with one patient having experienced two relapses (and subsequently having received a fourth course).

SAD and IAR

Among 52 patients (30.6%), we observed SAD which are listed in table 4. Five patients developed two different autoimmune disorders (two patients: thrombocytopenia + Graves' disease; two patients: vitiligo + Graves' disease; one patient: idiopathic Castleman's disease + Graves' disease). Most of secondary autoimmunity presented with thyroid dysfunctions, although three patients experienced immune thrombocytopenia and a in a single case there was immune neutropenia. Furthermore, we observed four cases of vitiligo (previously published in ref. 15), one case of idiopathic multicentric Castleman's disease (previously published in ref. 16) and two cases of autoimmune hepatitis.

Table 4 Overview on observed secondary autoimmune disorders in the PROGRAM^{MS} cohort

Secondary autoimmune disorder	Cases	Onset from baseline infusion (months)
Graves' disease	28	6–47 (median: 20)
Autoimmune thyroiditis	12	6–39 (median: 17.5)
Autoimmune thrombocytopenia	5	9, 11, 26, 27, 35
Vitiligo	4	17, 24, 45, 51
Autoimmune hepatitis	2	12, 19
Autoimmune neutropenia	1	25
Idiopathic Castleman's disease	1	40

Given the high abundance, we did not indicate the time of onset from baseline in patients with thyroid autoimmunity but showed median and range. In other diseases, the numbers indicate the months of onset from baseline in the respective patients.

Similar to effectiveness analyses, we observed an increased hazard for development of secondary autoimmunity among patients treated with ALEM as third-line agent, compared with the first-line and second line groups (HR 2.850; 95% CI 1.060 to 7.424; p=0.038; online supplemental table S2C). In the multivariate model pretreatment with FTY was the only variable significantly influencing the risk of developing SAD, (HR 5.875; 95% CI 2.126 to 16.237; p<0.001), compared with naïve patients (figure 2A, for full regression model, see table 5). Notably, in the FTY pretreated group, the wash-out duration did not impact on the time to first manifestation of SAD (HR: 1.054 per additional day washout (95% CI 0.978 to 1.136; p=0.0172)). We also evaluated whether a history of smoking was associated with the development of SAD but could not find any significant effect; a history of smoking was recorded among 14 (27%) patients with SAD and among 25 patients (21%) without SAD (p=0.264).

Overall, 121 patients (71.1%) experienced IAR during their first course of ALEM, and 105 patients (61.2%) at the administration of the second course. In the majority of patients symptoms were mild, including fever, rash and tachycardia, each resolving without specific treatment (CTCAE°I–II). However, we also observed severe adverse events (CTCAE°III–IV) following the first course of ALEM, including temporary liver injury (one patient), symptomatic bradycardia (three patients), pneumonitis (three patients) and acalculous cholecystitis (six patients). We also reported laboratory changes indicative of gall bladder inflammation in the absence of symptoms in three of six patients during the second course. After stratification according to the last previous DMT, we found that patients who had received FTY were less likely to develop IAR during their first course of ALEM (figure 2B), whereas patients who had received NTZ were prone to develop such symptoms. Notably, the vast majority of severe IAR was observed in patients switching from NTZ (p<0.001).

DISCUSSION

In this real-world study, we observed, among patients with MS treated with ALEM, different treatment responses, based on the previous use of DMT. Patients who had previously received either basic treatment or who were treatment naïve experienced outcomes for clinical relapses and disability progression rates comparable to those reported in randomised clinical trials.[2 3 17 18] Furthermore, we confirmed previous data

secondary autoimmunity.[23] Although it has not been shown yet, we assume that similar mechanisms might underlie re-emerging disease activity, as distinct changes in the T cell receptor repertoire were visible prior to relapses in event-driven analyses.[24]

These hypotheses are further supported by the qualitative changes of relapse following FTY, which in large proportion localised in the spinal cord and indeed were persistent also in patients with longer washout durations. It has been shown that specific lymphocyte subsets have preferences for different parts of the CNS.[25] Furthermore, the spinal cord involvement is an important driver of disability progression and is likely to underlie the observed increased risk of disability accumulation in our FTY pretreated patients.[26]

Besides differences in lymphocyte distribution and their accessibility for depletion, lots of other effects mediated by FTY have been described. These especially involve qualitative changes in the immune network, such as transcriptomic changes of CD4+ T cells,[27] the modulation of T helper cell phenotype balances as well as the increase in regulatory T cell abundance[28] and the increase and functional changes in regulatory B cells.[29 30] We speculate that these effects interfered with ALEM-induced depletion and immune reconstitution in an unfavourable manner, resulting in increased risk for disability progression and for the development of secondary autoimmunity.

These long-lasting changes in the immune repertoire following induction with ALEM after FTY can also explain the absent association between washout duration and time to manifestation of SAD in our cohort. We assume that—unlike lymphocyte sequestration—the qualitative changes in the immune network following FTY treatment and subsequent impact of ALEM re-shape the immune system in an irreversible manner and that this cannot be overcome by re-exposition to ALEM.

Interestingly, core study data already indicated that the risk of developing SAD is mostly defined by the first course of ALEM with only minor changes in risk exerted by further courses.[31]

We did not observe specific patterns of autoimmunity in our treatment groups but we assume that other risk factors could define the organ direction of autoimmunity. We have previously shown that a genetic predisposition via human leucocyte-antigen haplotypes is visible in vitiligo patients and a high abundance of thyroid antibodies at baseline in patients with secondary thyroiditis.[15 32] Additionally, the identification of a family history of autoimmunity or a history of smoking as risk factors for development of secondary autoimmunity corroborate the concept of dormant autoimmunity being unravelled by ALEM.[13]

We are aware that the absence of randomisation and a potential sample bias at our tertiary centres represent our study's limitations. However, we should not expect randomised clinical trials with designs capable of evaluating hypotheses such as ours; such limitations have been repeatedly noted.[33] Consequently, real-world longitudinal studies like PROGRAM^{MS} remain invaluable for determining a definite safety and efficacy profile.

Although the efficacy of FTY has been proven in various clinical trials, such as FREEDOMS, TRANSFORMS or most recently PARADIGMS,[34–36] our data indicate FTY pre-treatment as a risk factor for suboptimal therapeutic response to ALEM and for developing secondary autoimmunity. Additionally, our data provide an interesting insight into the complex interaction between immune cell distribution and qualitative immune cell function for treatment success in patients with MS and how immunomodulatory treatment persistently modifies this interaction.

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Contributors SP: study concept and design, acquisition of data, analysis of data, writing of the manuscript. TR: analysis of data, critical revision of the manuscript for intellectual content. RP: acquisition of data, analysis of data, critical revision of the manuscript for intellectual content. LR: analysis of data, critical revision of the manuscript for intellectual content. CK: acquisition of data, critical revision of the manuscript for intellectual content. MP: acquisition of data, critical revision of the manuscript for intellectual content. BW: critical revision of the manuscript for intellectual content. LK: critical revision of the manuscript for intellectual content. CK: critical revision of the manuscript for intellectual content. AS: critical revision of the manuscript for intellectual content. HW: study concept and design, critical revision of the manuscript for intellectual content. SGM: study concept and design, critical revision of the manuscript for intellectual content.

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4.2 Alemtuzumab-induced immune phenotype and repertoire changes: implications for secondary autoimmunity

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ORIGINAL ARTICLE

Alemtuzumab-induced immune phenotype and repertoire changes: implications for secondary autoimmunity

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Alemtuzumab is a monoclonal antibody that causes rapid depletion of CD52-expressing immune cells. It has proven to be highly efficacious in active relapsing–remitting multiple sclerosis; however, the high risk of secondary autoimmune disorders has greatly complicated its use. Thus, deeper insight into the pathophysiology of secondary autoimmunity and potential biomarkers is urgently needed. The most critical time points in the decision-making process for alemtuzumab therapy are before or at Month 12, where the ability to identify secondary autoimmunity risk would be instrumental. Therefore, we investigated components of blood and CSF of up to 106 multiple sclerosis patients before and after alemtuzumab treatment focusing on those critical time points.

Consistent with previous reports, deep flow cytometric immune-cell profiling ($n = 30$) demonstrated major effects on adaptive rather than innate immunity, which favoured regulatory immune cell subsets within the repopulation. The longitudinally studied CSF compartment ($n = 18$) mainly mirrored the immunological effects observed in the periphery. Alemtuzumab-induced changes including increased numbers of naïve $CD4^+$ T cells and B cells as well as a clonal renewal of $CD4^+$ T- and B-cell repertoires were partly reminiscent of haematopoietic stem cell transplantation; in contrast, thymopoiesis was reduced and clonal renewal of T-cell repertoires after alemtuzumab was incomplete. Stratification for secondary autoimmunity did not show clear immununological cellular or proteomic traits or signatures associated with secondary autoimmunity. However, a restricted T-cell repertoire with hyperexpanded T-cell clones at baseline, which persisted and demonstrated further expansion at Month 12 by homeostatic proliferation, identified patients developing secondary autoimmune disorders ($n = 7$ without secondary autoimmunity versus $n = 5$ with secondary autoimmunity). Those processes were followed by an expansion of memory B-cell clones irrespective of persistence, which we detected shortly after the diagnosis of secondary autoimmune disease.

In conclusion, our data demonstrate that (i) peripheral immunological alterations following alemtuzumab are mirrored by longitudinal changes in the CSF; (ii) incomplete T-cell repertoire renewal and reduced thymopoiesis contribute to a proautoimmune state after alemtuzumab; (iii) proteomics and surface immunological phenotyping do not identify patients at risk for secondary autoimmune disorders; (iv) homeostatic proliferation with disparate dynamics of clonal T- and B-cell expansions are associated with secondary autoimmunity; and (v) hyperexpanded T-cell clones at baseline and Month 12 may be used as a biomarker for the risk of alemtuzumab-induced autoimmunity.

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Abbreviations: AHSCT = autologous haematopoietic stem cell transplantation; ALEM = alemtuzumab; BCR = B-cell receptor; PBMC = peripheral blood mononuclear cell; RRMS = relapsing–remitting multiple sclerosis; SAID = secondary autoimmune disorder; Simoa = single molecule arrays; TCR = T-cell receptor; T_{reg} = regulatory T cell

Introduction

Alemtuzumab (ALEM) is a humanized monoclonal antibody directed against CD52 that has been approved for the treatment of highly active relapsing–remitting multiple sclerosis (RRMS). ALEM leads to a rapid depletion of CD52-expressing immune cells in peripheral blood with profound quantitative and qualitative effects on immunological networks, principally leading to long-lasting T-cell lymphopenia.[1–4] In contrast to maintenance therapies (e.g. natalizumab and fingolimod), ALEM can induce long-term treatment-free remission and is therefore considered to be an immune reconstitution therapy.[5–8] However, high therapeutic efficacy and treatment freedom is accompanied by potential serious adverse events, of which secondary autoimmune disorders (SAIDs) are the most significant for long-term outcomes.[9–12] SAID was reported in up to 48% of ALEM-treated RRMS patients, with thyroid autoimmunity as the most frequent condition.[11] The onset of SAID is delayed and ranges from 6 to 61 months, peaking in Years 2 and 3 and declining thereafter.[11–13] Frequent ALEM-related SAIDs include glomerulonephritis and immune thrombocytopenia, which in addition to thyroid autoimmunity are all considered to be B-cell-driven and autoantibody-mediated pathologies.[14] As ALEM causes fast, partially overshooting B-cell repopulation with simultaneous T-cell paucity, the most established hypothesis of this lymphopenia-associated autoimmune phenomenon assumes proliferation of autoreactive B cells under insufficient T-cell control.[15,16] However, this hypothesis does not sufficiently explain onset of SAID being delayed by several years and the appearance of clearly T-cell-mediated autoimmunity, for example sarcoidosis and vitiligo.[17–20]

SAID development has also been reported in the context of autologous haematopoietic stem cell transplantation (AHSCT). AHSCT is considered as the prototypic immune reconstitution therapy. Consistent with effects reported for ALEM, naïve B- and T-cell repopulation, a reduction of Th17 cells and a significant surge of regulatory T- and NK-cell subsets have been reported for AHSCT.[5,21–24] Therefore, mechanisms underlying SAID development might show overlaps for both treatment modalities.[5] However, the reported frequencies of SAID after AHSCT in multiple sclerosis patients are much lower than for ALEM and range around 2.8–6.4% of patients.[25–27] Interestingly, ALEM-containing conditioning regimens for AHSCT in patients with multiple sclerosis have been associated with a high incidence (14%) of late immune thrombocytopenia compared to non-ALEM regimens (0–2.8%).[26] Further to this, ALEM used in conditioning regimens treating autoimmune diseases generally has been associated with the occurrence of SAID in a significantly higher number of patients (16%) compared to regimens that did not use lymphocyte-depleting antibodies (0%).[28] For both ALEM and AHSCT, the proliferation of self-antigen-responsive T cells in the context of lymphopenia, so-called homeostatic proliferation, has been implicated as an important factor for SAID development.[27,29,30] For ALEM, early T-cell repopulation is mainly driven by homeostatic proliferation, whereas multiple sclerosis patients with SAID demonstrated reduced thymopoiesis and exhibited clonal restriction of the T-cell repertoire.[29] However, these alterations were demonstrated early following the initiation of ALEM, so how this relates to SAID occurrence peaking in Years 2–3 remains to be elucidated.

In a previous report, IL-21 was shown to promote homeostatic proliferation and IL-21 serum levels correlated with the incidence of SAID in ALEM-treated RRMS patients.[1] Thus, IL-21 measurement was proposed as a biomarker for SAID risk. However, the findings could not be validated in larger prospective cohorts with commercially available IL-21 enzyme-linked immunosorbent assay kits.[31] Baseline thyroid autoantibodies were associated with increased thyroid SAID after ALEM, but lymphocyte repopulation dynamics did not predict SAID occurrence.[32,33] Interestingly, previous treatment and treatment sequence impacts the efficacy and safety profile of ALEM.[34]

Thus, a comprehensive mechanistic hypothesis as well as potential biomarkers/signatures for ALEM-related SAID are currently lacking and are urgently needed in the clinical setting. We here performed in-depth multidimensional immune phenotyping and repertoire analysis predominantly in blood but also in CSF, comparing both compartments [including flow-cytometry, T- and B-cell receptor (TCR/BCR) sequencing, single molecule arrays (Simoa), and proteomics]. This parallel assessment of the TCR and BCR provides a novel hypothesis for the development of SAID, whereas no significant cellular or humoral immune signature was associated with the development of SAID.

Materials and methods

Cohort

This patient collective was established as part of the prospective PROGRAM^{MS} cohort ($n = 106$ patients; for further details see Supplementary material). Ethical approval was granted by the local authority (Institutional Review Board of the Medical Council Westphalia-Lippe, 2014-398-f-S). CSF analysis was conducted in a subgroup of patients who were enrolled in the ALAIN01 study, in which extensive immunoprofiling of ALEM-treated patients was performed. The study protocol has been published previously.[35] The study was approved by local authorities (2014-545-f-A) and has been officially registered (NCT02419378). Besides those 15 patients, CSF analysis was conducted in 3 additional patients after informed consent. For extensive analyses different subcohorts of the PROGRAM^{MS} cohort were analysed, which—aside from the ALAIN01 cohort—were randomly assigned (Table 1 and Fig. 1A).

Biomaterial

For this study, biological parameters from peripheral blood and CSF were investigated. While EDTA–blood and CSF cells were analysed within 1 h of withdrawal, serum, CSF supernatant and peripheral blood mononuclear cells (PBMCs) were cryopreserved following standardized processes as described previously.[36]

Flow cytometry

Flow-cytometric analyses of EDTA blood and CSF cells were performed as a part of routine clinical prectice and for therapy surveillance, as described previously.[36,37] In addition, PBMCs from a subcohort of ALEM patients were analysed according to a flow-cytometric functional immune phenotyping matrix as described before.[37] For this purpose, cryopreserved PBMCs were thawed and stained with distinct sets of fluorochrome-conjugated antibodies (Supplementary material). For intracellular staining, cells were treated with fixation/permeabilization solution (eBiosciences) for 20 min, subsequently washed with permeabilization buffer (eBiosciences) and finally incubated with antibodies directed against intracellular target molecules of interest. To investigate the capacity to produce cytokines, PBMCs were rested overnight in X-VIVO™ 15 Serum-free Hematopoietic Cell Medium (Lonza) and subsequently stimulated with Leucocyte Activation Cocktail, with BD GolgiPlug™ [with phorbol myristate acetate (PMA), Ionomycin and Brefeldin A; BD Pharmigen™] for 4 h before extracellular staining for lineage markers and intracellular staining for cytokines. Flow-cytometric data were analysed using Kaluza Analysis Software Version 2.1 (Beckman Coulter). Immune cell subsets were defined according to a prespecified gating hierarchy (Supplementary material). Gating strategy and representative stainings can be found in Supplementary Figs 1 and 2)

Simoa

CSF supernatant was processed as described above. To ensure high comparability, all samples were analysed on the same day. IL-10, IL-12p70, IFNγ, TNFα, IL-6, IL-17 and GM-CSF were measured in several rounds by Simoa HD-1 (Quanterix) using the Simoa HD-1 Analyzer™ (Quanterix) using human kits for the respective cytokines or the Simoa® NF-light™ Advantage Kit (Quanterix) for serum

Table 1 Baseline characteristics of investigated cohorts

Parameter	Cohort A $n = 106$	Subcohort B $n = 30$	Subcohort C $n = 18$	Subcohort D $n = 12$	P subcohort B versus excluded	P subcohort C versus excluded	P subcohort D versus excluded
Age at baseline, median (IQR)	34 (29–43)	36 (28–46)	34 (28–37)	33 (28–46)	0.277[a]	0.503[a]	0.897[a]
Male sex, *n* (%)	37 (36)	14 (44)	4 (24)	5 (42)	0.200[b]	0.180[b]	0.454[b]
Duration since multiple sclerosis diagnosis, years, median (IQR)	6 (2–10)	5 (2–10)	6 (4–10)	5 (2–10)	0.937[a]	0.656[a]	0.546[a]
Duration since multiple sclerosis onset, years, median (IQR)	7 (3–12)	7 (3–10)	6 (5–11)	7 (3–9)	0.717[a]	0.564[a]	0.731[a]
Number of previous DMT, median (IQR)	2 (1–3)	2 (1–3)	3 (2–4)	2 (1–3)	0.133[a]	0.080[a]	0.514[a]
EDSS at baseline, median (IQR)	2.5 (1.5–4.0)	3 (2.0–3.5)	4 (2.0–4.5)	3 (2.0–4.0)	0.655[a]	0.069[a]	0.103[a]
Number of relapses within last two years before ALEM, median (IQR)	2 (1–3)	2 (1–3)	2 (1–4)	2 (1–3)	0.702[a]	0.431[a]	0.359[a]
Last previous DMT, *n* (%)					0.212[b]	0.304[b]	0.773[b]
None	15 (14)	9 (28)	1 (6)	1 (8)			
Basic	33 (31)	9 (28)	6 (35)	4 (33)			
Escalation	58 (55)	14 (44)	10 (59)	7 (59)			

DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale.
[a,b]P-values were calculated using the [a]Mann–Whitney rank sum test and [b]Fisher's exact test, respectively.

neurofilament light chain from the same batch according to manufacturer's instructions. Samples were measured in duplicate.

TCR/BCR repertoire sequencing

RNA from Magnetic Activated Cell Sorting-sorted $CD4^+$, $CD8^+$ and $CD19^+$ cells from frozen PBMCs was purified using the RNeasy® Plus Micro Kit (Qiagen) and transcribed into cDNA. $CD4^+$ TCR variable beta chain and $CD19^+$ BCR immunoglobulin heavy chain (IgH) high-throughput sequencing was performed using the immunoSEQ® Assay (Adaptive Biotechnologies), as previously described.[38] The analyses were performed using the R package divo: Tools for Analysis of Diversity and Similarity in Biological Systems to measure the Morisita–Horn index (for quantification of sample overlap).[39] The Efron–Thisted estimator was calculated according to Efron and Thisted[40]; Simpson clonality was calculated as previously described by Pruessmann *et al.*[41] The healthy control and TERI datasets shown in Fig. 3 were processed and analysed from raw data previously published by Klotz *et al.*[38] The AHSCT dataset shown in Fig. 3 was processed and analysed from raw data previously published by Muraro *et al.*[42] All used datasets were sex- and age-matched. For SAID-related TCR beta chain and BCR IgH repertoire sequencing, cells [$CD4^+$ T cells ($CD3^+CD4^+$), $CD8^+$ T cells ($CD3^+CD8^+$) and memory B cells ($CD19^+CD27^+IgD^{+/-}$)] were sorted using a MoFlo XDP High-Speed Cell Sorter (Beckman Coulter). For further sample processing a custom protocol was used, which is comparable to the immunoSEQ® Assay (Adaptive Biotechnologies) and is described in detail in the Supplementary material. Approximately 25 million sequence reads were generated per Illumina Miseq run. Raw sequence reads were processed using the MiXCR software pipeline (https://milaboratories.com/software).[43] MiXCR pre-processed data were post-analysed using VDJtools to evaluate clonal expansions, repertoire diversity, clonotype overlap and clonotype tracking (new and persisting clones; https://vdjtools-doc.readthedocs.io/en/master/install.html, https://immunarch.com/index.html).[44,45]

Proteomics

Cryopreserved serum and CSF samples were sent to Olink for proteomic analysis by the Explore 1536 panel (Olink proteomics) by proximity extension assay as described in a recently published article.[46]

Statistics

Given the heterogeneity of clinical and experimental data, non-parametric tests of association were used throughout this study unless otherwise specified. Comparisons between patient groups were performed using the Mann–Whitney U test (unpaired) or signed-rank test (paired) for continuous variables and Fisher's exact test for categorical variables. Comparisons of multiple groups were made using the Kruskal–Wallis test followed by Dunn's test. Longitudinal analyses of leucocyte compositions in respective patients were analysed using the Friedman test, including Dunn's test. An exploratory analysis of potential markers associated with SAID and treatment response was performed using the volcano plot, which visualizes the *P*-values derived from the testing of a respective parameter and the corresponding $\log_2$ fold change of the medians. Correlation analyses were performed in GraphPad Prism version 9.1 (GraphPad Software, San Diego, California, USA; www.graphpad.com) by linear non-parametric Spearman correlation. For all calculations, a *P*-value below 0.05 was considered statistically significant. Due to the exploratory nature of the dataset, *P*-values were not corrected for type I error. Thus, the findings of this study should be considered as hypothesis-generating and must be independently confirmed.

Data availability

Individual data-points for Figs 1 and 2 can be found in Supplementary Table 2. Further data will be shared upon personal request to the corresponding author.

Results

Alemtuzumab leads to profound immune repertoire changes in the peripheral blood and CSF

Immune depletion and repopulation patterns following ALEM have been reported previously.[2,29,47,48] We first evaluated whether our cohort provides findings compatible with the literature ensuring their transferability. We performed broad immunophenotyping with the complete cohort of 106 ALEM-treated patients (cohort A) and more detailed analyses with subcohorts (cohorts B–D) of those 106 patients (Fig. 1A). The baseline parameters were similar for the different cohorts and consistent with the phase 3 trials (Table 1).[6,7] In cohort A, ALEM treatment led to the predominant depletion of B and T lymphocytes and to a lesser extent innate immune cells (NK, NK-T, monocytes and granulocytes; Supplementary Fig. 3A–J). Absolute and relative lymphocyte counts did not recover to baseline levels during the 24 months of observation (Supplementary Fig. 3A). B cells repopulated quickly, whereas $CD4^+$ and $CD8^+$ T cells remained reduced (Supplementary Fig. 3E–G and J). None of the patients showed the previously described depletion failure with ALEM.[49]

Figure 1 Continued
Bold lines indicate immune compartments with profound changes. Statistically significant changes are coloured and *P*-values are indicated. Colour filling shows the $\log_2$ fold change after 12 months of treatment versus baseline ($n = 30$ for baseline and $n = 24$ for Month 12). (**C**) Serum cytokines measured by Simoa ($n = 9$ for baseline and $n = 15$ for Month 12). (**D**) Change in absolute cell numbers of indicated immune cell subsets in the CSF compartment ($n = 18$ for baseline and Month 12). (**E**) CSF cytokines measured by Simoa ($n = 17$ for baseline and $n = 18$ for Month 12). (**F**) Analysis of BCR and TCR sequencing of $CD4^+$, $CD8^+$ T cells and B cells ($n = 12$ for baseline and Month 12). Simpson clonality measures how evenly receptor sequences are distributed in the repertoire, where 0 represents an even and 1 a monoclonal sample; repertoire richness measures how many clones are present in the repertoire; expansion of persisting clones indicates whether pretreatment clones occupy higher volumes of the repertoire ($n = 12$ for baseline and Month 12). Statistical analysis was performed using the Mann–Whitney test (unpaired comparisons). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns = not significant. ADCC = antibody-dependent cellular cytotoxicity; Breg = regulatory B cell; CM = central memory; CTLA-4 = cytotoxic T-lymphocyte-associated Protein 4; DNAM-1 = DNX-Accessory Molecule-1; EM = effector memory; FACS = flow cytometry; NK = natural killer cell; NKT = natural killer T cell; mDC = myeloid dendritic cells; ILC = innate lymphoid cell; tTreg = thymus-derived Treg; pTreg = peripherally induced Treg; RTE = recent thymic emigrants; seq = sequencing; TEMRA = T effector memory expressing CD45RA.

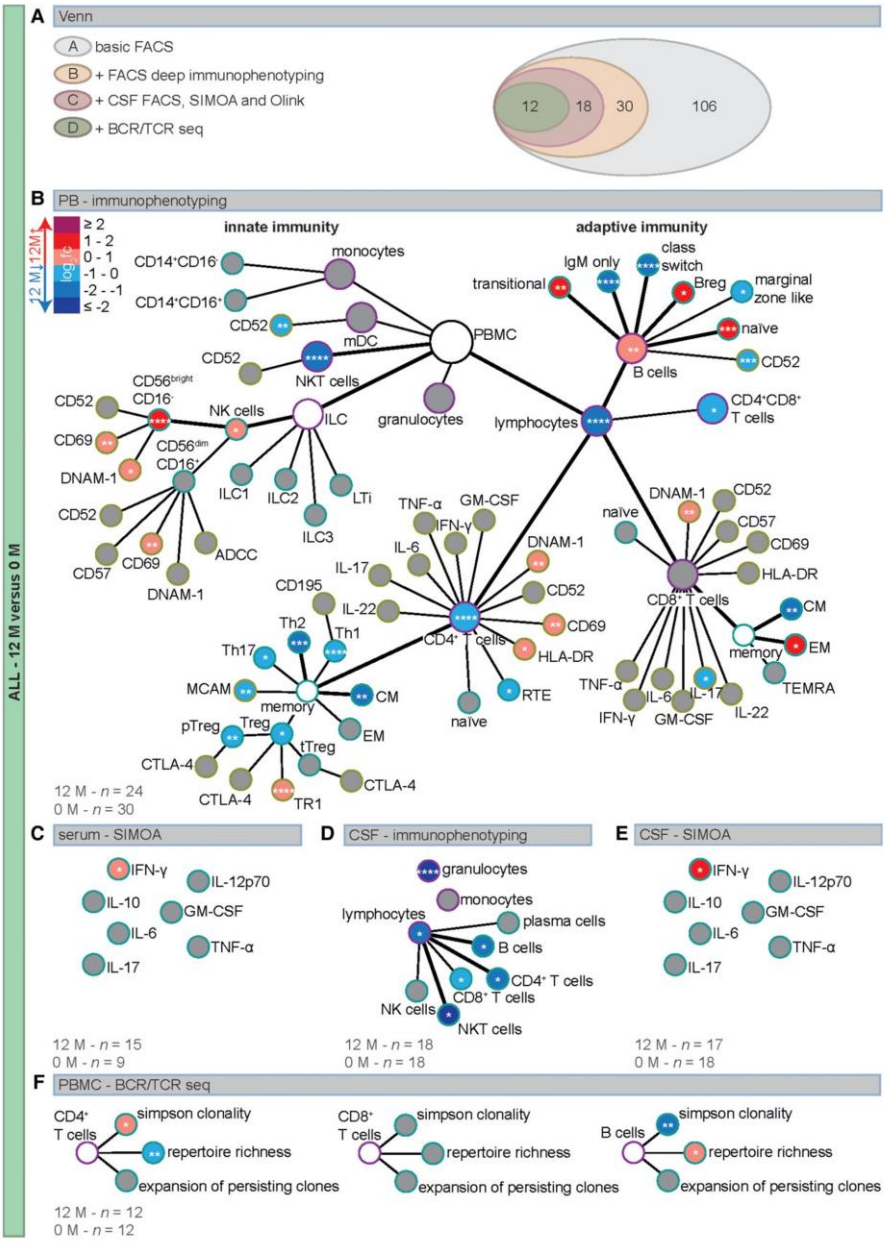

Figure 1 ALEM induces profound changes in the immune repertoire. (**A**) Venn diagram displaying the study cohort and subcohorts with patient numbers and corresponding analyses. For baseline characteristics of the different cohorts see Table 1. (**B**) Hierarchical illustration of the changes to absolute cell numbers of different immune cell subsets in ALEM-treated RRMS patients. All data-points compare values at baseline and after 12 months of treatment; individual values can be found in Supplementary Table 2. Lines display relationships between populations and subpopulations. (Continued)

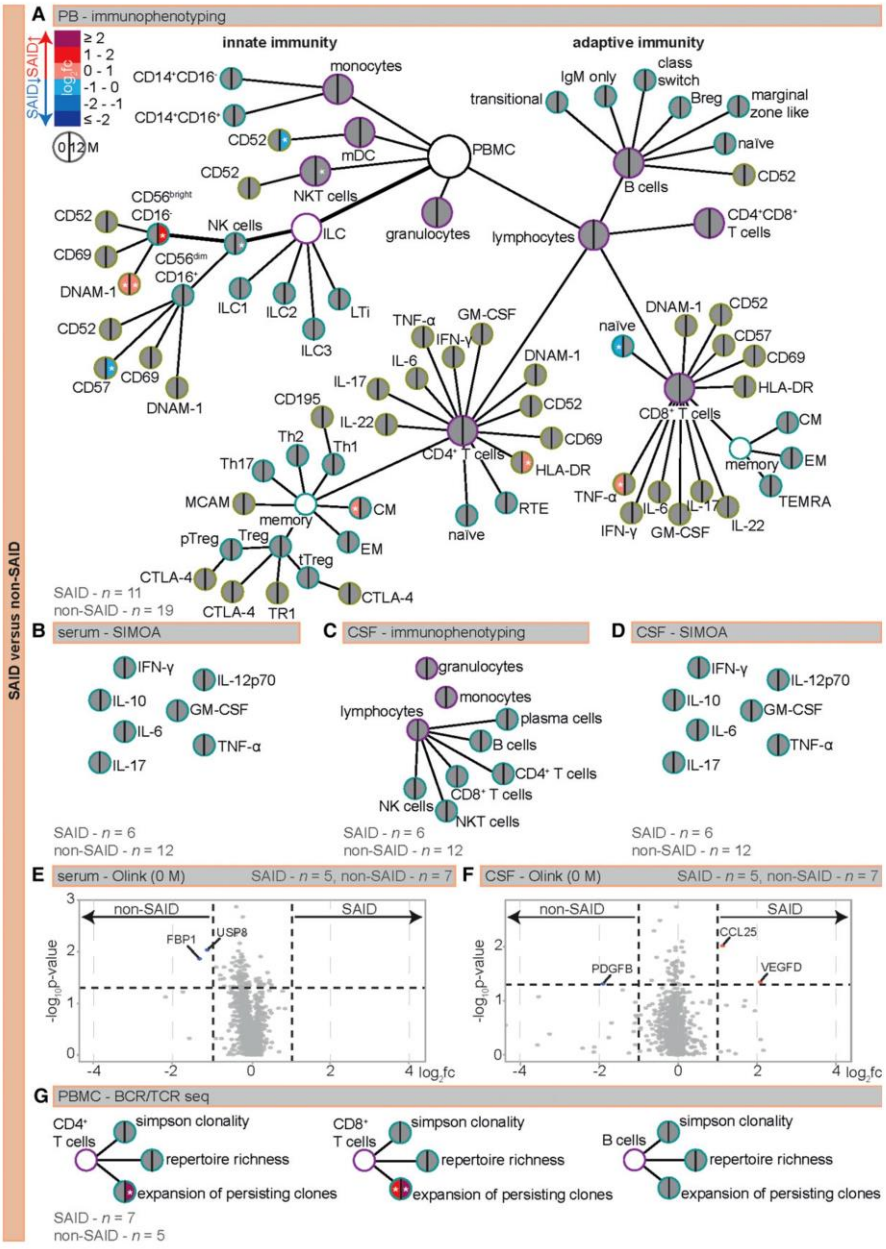

Figure 2 SAID is associated with distinct immune alterations in the TCR repertoire. (**A**) Hierarchical illustration of the changes to absolute cell numbers of different immune cell subsets in ALEM-treated RRMS patients with or without SAID. Statistically significant differences are coloured and indicated by their *P*-values. All data-points are presented as split circles with the left half indicating the comparison of SAID versus non-SAID patients at baseline and the right half indicating the comparison of SAID versus non-SAID patients after 12 months of treatment; individual values can be found in

(Continued)

More in-depth immunophenotyping in subcohort B demonstrated reduced central memory $CD4^+$ T-cell numbers, inflammatory T helper subsets (T_h1, T_h2, T_h17) and recent thymic emigrants as surrogate markers for thymopoiesis (Fig. 1B) 12 months after ALEM. In the $CD8^+$ T-cell compartment, central memory cells were decreased, whereas effector memory cells were more abundant (Fig. 1B). The production of anti- or pro-inflammatory cytokines was comparable to baseline (cell-type-specific: Fig. 1B, except for less IL-17 in $CD8^+$ T cells; and in serum: Fig. 1C, except for higher IFN-γ levels). In the B-cell compartment, we detected enhanced numbers of immature B-cell subsets (naïve and transitional B cells), whereas memory B-cell subsets (class-switched, IgM only and marginal zone-like memory B cells) were reduced at Month 12 (Fig. 1B). In the regulatory cell subsets, we obsereved a reduction in regulatory T cells ($FoxP3^+$ T_{reg}), which was mainly attributable to peripherally induced T_{reg} (Fig. 1B). In contrast, T regulatory type 1 (T_R1) cell numbers were absolutely and relatively increased. However, relative proportions of both T_{reg} and T_R1 cells were enhanced 12 months after ALEM (Supplementary Fig. 4A). Of note, within the T_{reg} compartment the relative increase was pronounced for $Helios^+$ T_{reg}, which show enhanced immunosuppressive characteristics (Supplementary Fig. 4A).[50,51] T_{reg} (Supplementary Fig. 4) and $CD56^{bright}$ NK cells (Fig. 1B) expressed higher levels of markers that indicate facilitated immunosuppressive function (T_{reg}: Helios, PD-1, GITR[50,52,53]: Supplementary Fig. 4A and B; $CD56^{bright}$ NK cells: DNAM-1: Fig. 1B).[54]

In CSF (subcohort C), ALEM treatment led to significantly reduced absolute cell numbers and a reduced IgG ratio. However, the remaining basic CSF parameters such as total protein and oligoclonal bands demonstrated no relevant changes over the 12 months (cohort A, Supplementary Table 1). Flow-cytometric immunphenotyping showed that ALEM significantly reduced granulocyte, $CD4^+$ T, $CD8^+$ T, B cell and NK-T cell counts (cohort C, Fig. 1D). Cytokine measurements using Simoa showed no significant changes in anti- (IL-10) or pro-inflammatory cytokines (TNF-α, IL-12p70, IL-6, IL-17 and GM-CSF, except for increased IFN-γ levels) in the CSF (subcohort C, Fig. 1E).

Next, we investigated alterations and dynamic changes in the peripheral TCR and BCR repertoires (subcohort D). The $CD4^+$ T-cell compartment showed higher repertoire clonality (Simpson clonality) and reduced richness (Efron–Thisted estimator). Clonality metrics describe the extent to which one or a few clones dominate the sample repertoire, whereas richness measures focus on how many clones are present in the sample repertoire. In marked contrast, B-cell repopulation was characterized by an increased richness and lower clonality after 12 months. The $CD8^+$ TCR repertoire was heterogeneous for the investigated patients (subcohort D, Fig. 1F).

In summary, changes after depletion and during immune repopulation are measurable on numerous immunological levels, both cellular and humoral, which is consistent with the previously reported immunological effects of ALEM.[15,33,47,55] The CSF compartment had not yet been investigated, but broadly mirrored the findings in peripheral blood. Alterations were seen on many adaptive but also innate immune components and support the notion that immune repopulation rebalances towards a more regulatory and anti-inflammatory phenotype. The longitudinal TCR and BCR repertoire changes argue for durable and profound immunological effects. Supplementary Table 2 contains the numeric data that are illustrated in Fig. 1.

Differences in the TCR repertoire characterize patients developing secondary autoimmunity after alemtuzumab

ALEM has proven high efficacy that is sustained for several years without any further treatment.[12] However, uncertainty regarding ALEM therapy arises from the risk of SAID. Hence, biomarkers identifying patients at risk for the development of SAID and deeper insights into the pathophysiology of ALEM-associated SAID are of great clinical interest. The most critical time points in the decision-making process for ALEM therapy are at baseline (whether to start therapy) or at Month 12 (whether to continue therapy), where information on SAID risk would be instrumental. Data, however, suggest that only the initial lymphocyte depletion is decisive for the development of SAID.[56] We therefore investigated the immunophenotypic differences at those time points for ALEM-treated RRMS patients who did or did not develop SAID within the 48 months of follow-up after therapy initiation.

Consistent with previous observations,[33,47] peripheral immune cell subset phenotyping and serological analyses showed only marginal differences between patients with or without SAID. Further, the observed alterations did not affect any clear immunological trait or show any conclusive pattern to generate meaningful hypotheses based on peripheral blood findings (subcohort B, Fig. 2A and B). Additionally, in the CSF, we found no differences in cellular composition (subcohort C, Fig. 2C) or in cytokine signatures (subcohort C, Fig. 2D). To add more depth to the solute-targeted analyses, we performed proteomic analyses by the Olink Explore 1536 panel at baseline in serum and CSF. However, neither serum nor CSF proteins demonstrated relevant differential regulation with only very few proteins marginally passing the selected cut-offs (subcohort C, Fig. 2E and F).

The most prominent alteration between SAID and non-SAID patients among all investigated parameters was the expansion of persisting $CD4^+$ and $CD8^+$ T-cell clones (subcohort D, Fig. 2G). The expansion of persisting clones indicates whether pretreatment

Figure 2 Continued
Supplementary Table 2. Colour filling shows the $\log_2$ fold change of the corresponding comparison ($n = 11$ for SAID and $n = 19$ for non-SAID patients). (**B**) Serum cytokines measured by Simoa ($n = 6$ for SAID and $n = 12$ for non-SAID patients). (**C**) Immunophenotyping of the CSF compartment ($n = 6$ for SAID and $n = 12$ for non-SAID patients). (**D**) CSF cytokines measured by Simoa ($n = 6$ for SAID and $n = 12$ for non-SAID patients). (**E** and **F**) Olink analysis comparing serum and CSF of SAID and non-SAID patients as demonstrated by volcano plot. Volcano plots were constructed by calculating the $\log_2$ fold change of the median and the $-\log_{10}$ *P*-value ($n = 6$ for SAID and $n = 12$ for non-SAID patients). (**G**) Analysis of BCR/TCR sequencing of $CD4^+$, $CD8^+$ and B cells ($n = 7$ for SAID and $n = 5$ for non-SAID patients). Statistical analysis was performed using the Mann–Whitney test (unpaired comparisons). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns = not significant. ADCC = antibody-dependent cellular cytotoxicity; Breg = regulatory B cell; CM = central memory; CTLA-4 = cytotoxic T-lymphocyte-associated Protein 4; DNAM-1 = DNX-Accessory Molecule-1; EM = effector memory; FACS = flow cytometry; NK = natural killer cell; NKT = natural killer T cell; mDC = myeloid dendritic cells; ILC = innate lymphoid cell; tTreg = thymus-derived Treg; pTreg = peripherally induced Treg; RTE = recent thymic emigrants; seq = sequencing; TEMRA = T effector memory expressing CD45RA.

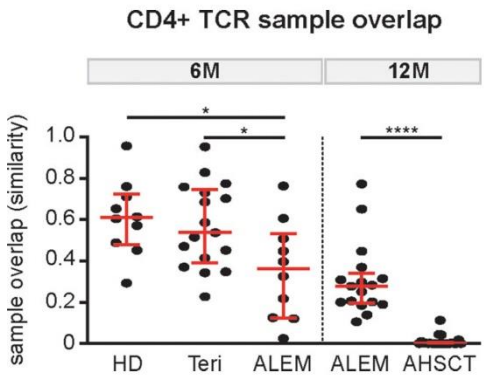

Figure 3 TCR sample overlap in ALEM-treated patients compared to healthy donors, AHSCT and teriflunomide. Dot plot displaying the median sample overlap (similarity of repertoires) of TCR sequencing of $CD4^+$ T cells comparing either baseline and 6-month time points in cohorts of healthy donors, teriflunomide- and ALEM-treated multiple sclerosis patients (*left*) or baseline and 12-month time points in ALEM- and AHSCT-treated multiple sclerosis patients (*right*). Median and IQR are indicated. Statistical analysis was performed by Mann–Whitney test (unpaired comparisons). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns = not significant. HD = healthy donor; Teri = teriflunomide.

clones occupy higher volumes of the repertoire. Supplementary Table 2 contains the numeric data that are illustrated in Fig. 2.

Alemtuzumab leads to incomplete renewal of the $CD4^+$ TCR repertoire

As an expansion of T-cell clones was observed in ALEM-treated SAID patients, ALEM might have unique effects on the T-cell repertoire that contributes to the high risk of SAID development. Immune repertoire exchange due to ALEM was compared to teriflunomide, a platform therapy with no reported cases of SAID,[57] and AHSCT treatment by determining the sample overlap, which describes repertoire similarity according to the Morisita–Horn index.[39] An index of 0 demonstrates that in a given patient the $CD4^+$ TCR repertoires at baseline and the indicated time point do not overlap, whereas an index of 1 represents a perfect overlap of the samples. However, even in healthy donors the sample overlap 6 months from baseline analysis is 0.61 (IQR 0.24) due to ongoing thymopoiesis (Fig. 3, left). In comparison to healthy donors, teriflunomide-treated (TERI) multiple sclerosis patients displayed no relevant differences in the $CD4^+$ TCR repertoire sample overlap (0.54, IQR 0.35) between baseline and 6 months (Fig. 3, left), thus indicating no relevant exchange in the TCR repertoire exceeding physiological parameters. In contrast, ALEM led to a significant but incomplete TCR repertoire exchange (0.36, IQR 0.41) compared to healthy donors and TERI 6 months after first infusion (Fig. 3, left). Twelve months after AHSCT we observed an almost complete TCR repertoire exchange (<0.01, IQR < 0.01) in the $CD4^+$ TCR repertoire, whereas the repertoire remained incompletely exchanged after ALEM (0.28, IQR 0.15; Fig. 3, right). Thus, TERI demonstrated no specific effect on the TCR repertoire, AHSCT led to an almost complete renewal, whereas ALEM induced an incomplete $CD4^+$ TCR repertoire exchange. This unique effects of ALEM on the immune repertoire might be related to the high risk of SAID.

TCR and BCR repertoire changes with specific temporal dynamics are associated with secondary autoimmunity

As we observed specific $CD4^+$ T-cell immune repertoire changes at Month 12 after ALEM treatment, we then investiagted whether the repertoire changes that distinguish SAID patients are restricted to the first year and the $CD4^+$ T-cell compartment. We compared data from five ALEM-treated multiple sclerosis patients with SAID to seven patients without SAID. Clinical details on the investigated cohort are presented in Supplementary Table 3. At 4–6 months, persisting clones occupied significantly larger proportions of both the $CD4^+$ (subcohort D, Fig. 4A, left) and $CD8^+$ TCR (Fig. 4A, right) repertoires in SAID patients. In general, persisting clones (green) made up larger proportions of $CD8^+$ T-cell repertoires than of $CD4^+$ T-cell repertoires (Fig. 4A). In the B-cell compartment, we found that persisting IgG^+ memory B-cell clones occupied only small fractions (1.67% ± 0.42%) of the post-ALEM repertoires in both SAID and non-SAID patients (Supplementary Fig. 5A).

Investigating the dynamics of the cumulative volumes of the 'Top 100' persisting T-cell clones over time, we detected maximum expansion of persisting clones, whereas individual clones were reduced, in both $CD4^+$ (Fig. 4B, middle) and $CD8^+$ TCR (Fig. 4C, middle) repertoires in SAID patients at months 4–6, which then declined over the observation period (Supplementary Fig. 5B and C, left and middle). In non-SAID patients, we found no significant expansion of T-cell clones (Fig. 4B and C, left). Expansion of persistent T-cell clones in SAID patients was significantly different from non-SAID patients at months 4–6 for both $CD4^+$ and $CD8^+$ T cells and at months 18–24 for $CD4^+$ T cells (Fig. 4B and C, right). Regarding B cells, we did not observe significant expansions of persisting memory B-cell clones over the 18–24 months following ALEM treatment for SAID and non-SAID patients (Fig. 4D). However, as the occurrence of SAID peaks around Years 2–3,[11–13] we analysed further time points. To correct for the different timing of SAID occurrence, PBMCs were analysed 1–3 months after SAID diagnosis (Fig. 4E, SAID) and 9–12 months prior to diagnosis (Fig. 4E, before SAID). Comparing repertoire volumes occupied by the 'Top 100' memory B-cell clones, irrespective of their persistence over time, we discovered remarkable expansions shortly after the manifestation of SAID (Fig. 4E). The repertoire dynamics of 'Top 100' memory B-cell clones in the individual SAID patients were visualized at baseline, before and at the first repertoire analysis following manifestation of SAID in Supplementary Fig. 5B and C, right panel. In all five SAID patients we found that hyperexpanded and large B-cell clones occupied significantly increased volumes of the B-cell repertoire after SAID development. (Supplementary Fig. 5D). Hyperexpanded and large clones were defined as clones occupying more than 1% and up to 100% or 0.1% up to <1% of the repertoire, respectively. Thus, SAID patients demonstrate persistence and expansion of T-cell clones as early as after the first ALEM course, peaking around 4–6 months, as well as a consecutive expansion of memory B-cell clones shortly after SAID development.

In SAID patients hyperexpanded T-cell clones are overrepresented even at baseline

As we observed that the 'Top 100' persisting $CD4^+$ and $CD8^+$ T-cell clones of SAID patients showed a strongly pronounced expansion

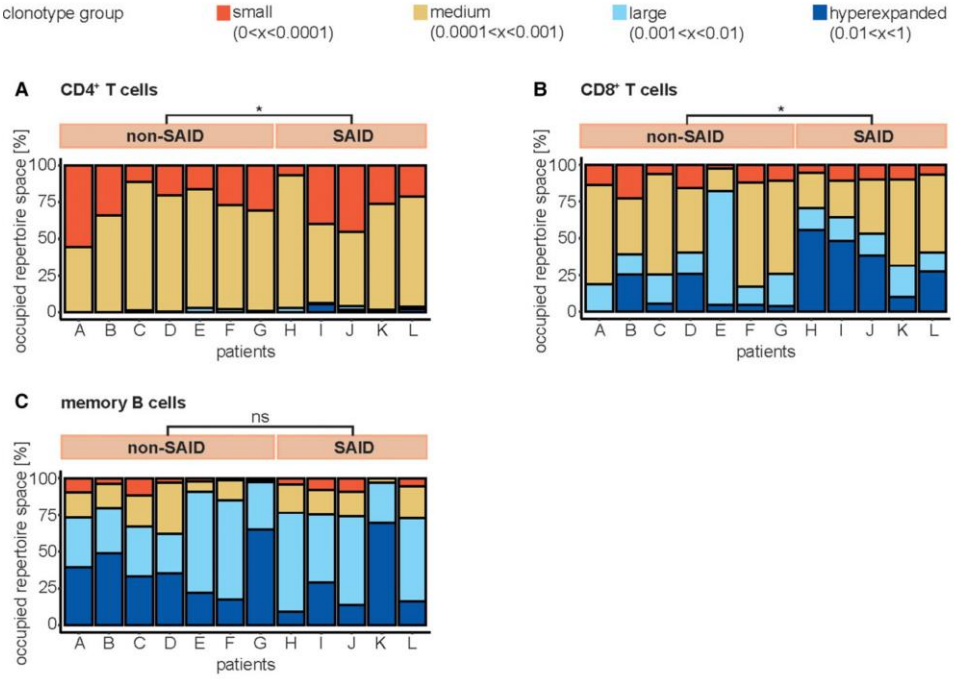

Figure 5 Hyperexpanded T-cell clones can be found in SAID patients already at baseline. Proportions of peripheral (**A**) $CD4^+$ T cell, (**B**) $CD8^+$ T cell and (**C**) $CD19^+$ memory B cell immune repertoire occupied by different clonotype groups in non-SAID ($n = 7$, Patients A–G) and SAID patients ($n = 5$, Patients H–L) at baseline. Bar graphs display proportions of different clonotypes: hyperexpanded T-cell clones (1% to 100% of the repertoire, dark blue) as well as T-cell clones with large (0.1% to <1%, light blue), medium (0.01% to <0.1%, yellow) and small expansion (<0.01%, red). Statistical analysis was performed by Mann–Whitney test (unpaired comparisons). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, ns = not significant.

after the first ALEM treatment course, we investigated whether hyperexpanded clones were already present before ALEM treatment. Remarkably even at baseline, hyperexpanded clones (Fig. 5, dark blue bars) occupied significantly higher proportions of both the $CD4^+$ (Fig. 5A) and $CD8^+$ (Fig. 5B) T-cell repertoire volumes in SAID patients compared to non-SAID patients. At baseline, hyperexpanded clones occupied between 10% and 55% of the $CD8^+$ and between 0% and 5% of the $CD4^+$ T-cell repertoires in SAID patients, whereas their abundance in non-SAID patients was significantly smaller in $CD8^+$ (0%–25%) and not detected within $CD4^+$ TCR repertoires. Differences of hyperexpanded clone abundance at baseline were not significant in the memory B-cell populations (Fig. 5C). Thus, hyperexpanded T-cell clones at baseline might indicate patients at risk for SAID development under ALEM-treatment.

Discussion

ALEM is a highly efficacious therapy for RRMS. However, the high risk of infusion-related adverse events and SAID significantly affects its risk–benefit ratio. We therefore sought a deeper understanding of the pathophysiologic mechanisms underlying SAID and potential biomarkers using a unique cohort of well-defined patients with longitudinal follow-up for ≥48 months, adjacent cellular and non-cellular biomaterials as well as longitudinal CSF analysis.

Consistent with previous reports, ALEM treatment led to pronounced adaptive rather than innate immune repertoire changes, which were in favour of regulatory components of the immune system. The CSF compartment mainly mirrored the immunological effects of ALEM in the periphery. Increased numbers of naïve $CD4^+$ T cells and B cells as well as a clonal renewal of $CD4^+$ T- and B-cell repertoires including diversification of the B-cell repertoire resembled features of AHSCT. However, in comparison to AHSCT the clonal renewal by ALEM appeared somewhat incomplete.

Comparing the differentiation of the immune architecture following depletion between SAID and non-SAID, the expansion of persisting T-cell clones at Month 12 was the most prominent alteration, whereas further in-depth cellular and non-cellular phenotyping parameters provided no clear immunological traits or signatures characterizing this difference. In SAID patients, however, hyperexpanded T-cell clones were present even at baseline; their expansion peaked around 4–6 months after ALEM treatment and was followed by a subsequent expansion of memory B-cell clones, which we detected shortly after SAID development.

In 2019, considerable side effects of ALEM including SAID led to a European Medicines Agency review process with subsequent restriction of ALEM use to, among others, RRMS patients without

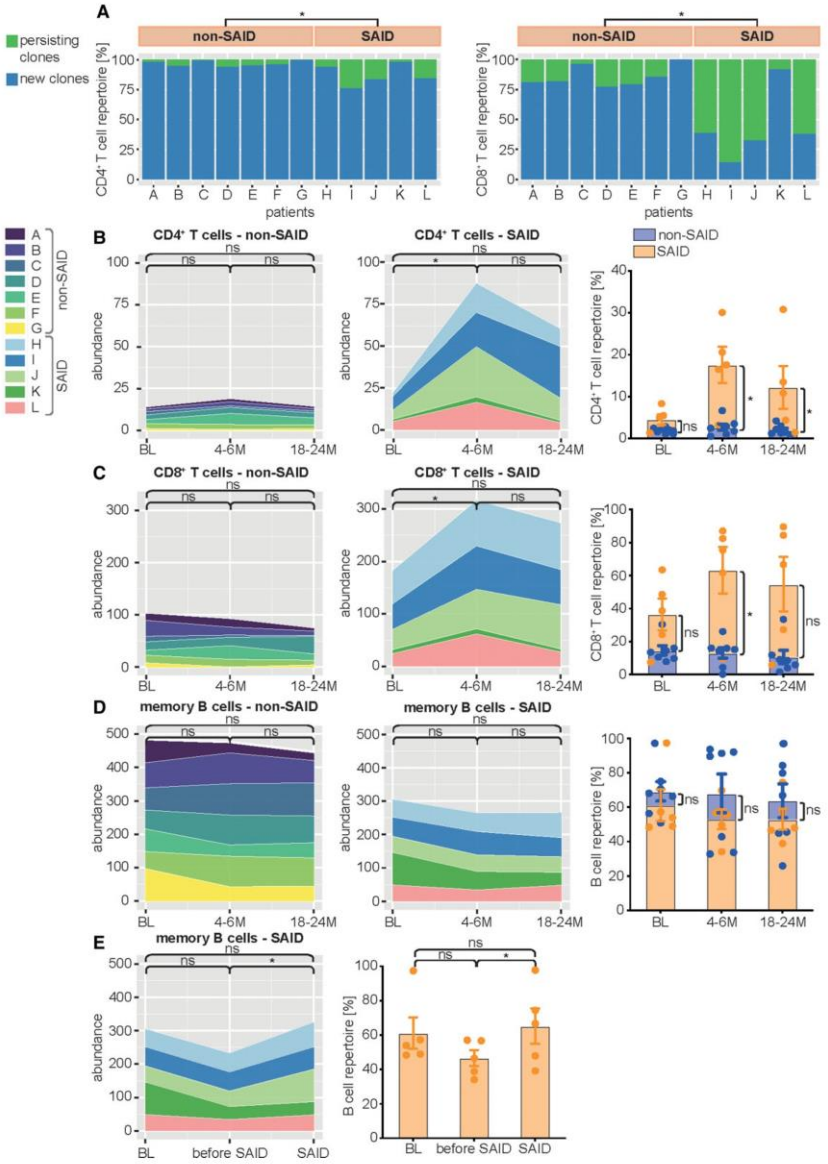

Figure 4 TCR and BCR repertoire changes in multiple sclerosis patients with or without manifestation of SAID after ALEM treatment. (**A**) Proportions of immune repertoire volumes occupied by persisting (green bars) versus new (blue bars) $CD4^+$ (*left*) and $CD8^+$ (*right*) T-cell clones in peripheral blood from non-SAID ($n = 7$, Patients A–G) and SAID patients ($n = 5$, Patients H–L) at 4–6 months after the first ALEM treatment course. Cumulative volumes (abundance) of the 'Top 100' persisting (**B**) $CD4^+$ T cell, (**C**) $CD8^+$ T cell and (**D**) 'Top 100' (irrespective of persistence) $CD19^+$ memory B-cell clones. Longitudinal values (baseline, 4–6 months, 18–24 months) for individual non-SAID (*left*, $n = 7$, Patients A–G), SAID (*middle*, $n = 5$, Patients H–L) and comparison of both (*right*) is depicted. (**E**) Cumulative volumes of 'Top 100' memory B-cell clones (irrespective of persistence) 1–3 months after SAID diagnosis (SAID) and 9–12 months before (pre-SAID). *Left*: Longitudinal values for individual patients; *right*: comparison of pre-SAID and SAID for the different time points ($n = 5$, Patients H–L). Bar graphs depict the median and IQR unless otherwise indicated. Statistical analysis was performed using the Mann–Whitney test (unpaired comparisons) and Wilcoxon signed rank test (paired comparisons). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns = not significant.

any pre-existing autoimmune disorder other than multiple sclerosis.[58,59] Some national guidelines on multiple sclerosis therapy are consequently recommending ALEM use only if natalizumab or CD20-depleting agents are contraindicated.[60,61] Given the high efficacy and the advantages of potential treatment freedom, those regulatory processes support the urge for a deeper understanding of ALEM-related SAID, as ALEM is still a valuable treatment option for some patients.

Currently, B- and T-cell-centric hypotheses have been proposed to explain ALEM-related SAID. Considering the B-cell population, a surge of immature B-cell subsets in the absence of T-cell-mediated regulation is believed to give rise to autoreactive B cells and subsequently to antibody-driven SAID.[15,16,62] High IL-21 levels might influence B-cell function and IL-21 has been shown to be a driver for antibody-mediated autoimmunity.[63] Consistent with limited B-cell control by T_{reg}, we found that absolute T_{reg} numbers are significantly reduced at Month 12 after ALEM. The relative increase of T_{reg} as well as enhanced numbers of other regulatory immune cell subsets (T_R1, B cells, NK cells) might still not be sufficient to control emerging autoimmunity. However, we and others did not find differences in T_{reg} numbers and proportions that would discriminate between SAID and non-SAID patients.[33] Further, the B-cell-centric hypothesis does not explain the occurrence of significant—although less so compared to ALEM—frequencies of SAID after AHSCT, where early B-cell hyperpopulation has not been reported.[64] Further, it is not an explanation for the clearly T-cell-mediated SAID after ALEM, for example sarcoidosis or vitiligo.[17–20]

In the context of lymphopenia, some mechanisms that maintain host tolerance are temporarily suspended.[65] T-cell-centric hypotheses suggest that T-cell repopulation during ALEM-induced lymphopenia is dominated by homeostatic proliferation in response to self-antigens, which limits repertoire skewing and predisposes to autoimmune responses. In accordance, previous studies reported IL-21 facilitated homeostatic proliferation of T cells with reduced thymopoiesis and a restricted TCR repertoire as drivers of SAID.[1,29] At Month 12 after ALEM, we consistently observed reduced absolute recent thymic emigrant numbers as a surrogate for diminished thymic function. The work by Jones *et al.* further confirmed this by observing low Sj/β TCR excision circle ratios, which were significantly reduced in patients developing SAID. In our study, RTE numbers were not able to differentiate SAID and non-SAID patients. This might be explained by the fact that TCR excision circle ratios specifically reflect intrathymic T-cell proliferation, whereas recent thymic emigrant numbers are potentially compromised by peripheral cell division.[29] Consistent with our data, the same study reported a restriction of the TCR repertoire in multiple sclerosis patients with ALEM-related SAID in the first 12 months after treatment. In contrast to our study, those observations were restricted to the TCR repertoire and the time before SAID development.

To the best of our knowledge and although provisional in nature due to small sample size, our sequencing data provide for the first time deeper insight into the longitudinal changes of both the TCR and BCR repertoire in the context of SAID and support the synthesis of both hypotheses. Consistent with enhanced homeostatic proliferation as a driver for SAID, we observed a significantly increased persistence and expansion of $CD8^+$ more than of $CD4^+$ T-cell clones, which peaked around 4–6 months after ALEM and slowly declined over the observation period of 18–24 months. This implies that thymopoiesis does contribute to T-cell repopulation, at least in the long term.[66] Interestingly, core study data indicated that the SAID risk is mostly defined by the first ALEM treatment course, thus supporting the relevance of those early immunological processes.[56] The B-cell compartment, however, demonstrated no relevant expansion of persisting clones. Irrespective of clonal persistence, we found a clonal expansion of memory B-cell clones shortly after SAID diagnosis compared to pre-SAID time points. Whether those dynamics are just coincidence or are causally linked processes leading to autoimmunity remains to be elucidated. However, it is tempting to speculate that expanded T-cell clones might provide T-cell help for autoreactive B cells that bind the same auto-antigens resulting in B-cell proliferation and maturation, auto-antibody production and consequently SAID.[67] As the regeneration of $CD4^+$ T cells requires an average of 35 months,[9,68] the number of T-cell clones might not be sufficient to induce clonal B-cell expansion before years 2 or 3, thus potentially explaining the late occurrence of B-cell-mediated autoimmunity. This hypothesis is supported by our observation of delayed clonal memory B-cell expansion shortly after SAID diagnosis. As B-cell repopulation kinetics were not different in patients with or without SAID,[33,47] the observed B-cell hyperpopulation may be indicative of an autoimmunity permissive environment rather than representing the underlying pathophysiology.

For T-cell-mediated SAID, the pathophysiologic pathway might be different with expansion of autoreactive $CD4^+$ or $CD8^+$ T cells that directly induce autoimmune responses. Vitiligo is induced by antigen-specific and clonally expanded $CD8^+$ T cells.[69] In ALEM-related vitiligo, we previously found a predominance of single CD8 T-cell clones supporting a directly T-cell-induced pathogenesis after ALEM treatment.[20] In this case, the proposed strategy of CD20 depletion after ALEM to mitigate SAID should be less efficient.[70]

As the ALEM treatment procedure is standardized, patient-specific prerequisites are the most likely factor to be decisive for SAID risk and whether SAID pathogenesis is B- or T-cell-mediated. Consistent with this, we found hyperexpanded T-cell clones even at baseline in patients that went on to develop SAID. As previously suggested, impaired or dysfunctional thymopoiesis may contribute to this proautoimmune state and in combination with exaggerated homeostatic proliferation may facilitate SAID development under ALEM.[29] The individual genetic risk profile might be the basis for these factors and thus for the increased susceptibility to SAID in parallel to that of multiple sclerosis. In support of this, linked genetic risk factors and genetic associations have been described for multiple sclerosis and other autoimmune disorders such as Graves' disease, Goodpasture syndrome, immune thrombocytopenia and vitiligo.[10,71–74] As SAID is not a feature among patients receiving ALEM for cancer,[75] the high SAID rates in multiple sclerosis patients might reflect this proautoimmune background. Further, anti-thyroid autoantibodies detected before treatment predisposed patients to ALEM-related thyroid autoimmunity.[32]

The SAID frequencies after ALEM are about 10 times higher than after AHSCT. We here observed that in comparison to AHSCT the clonal renewal through ALEM was rather incomplete. In the context of extensive lymphopenia, this incomplete renewal might lead to increased probability of persisting autoreactive T-cell clones and their expansion by homeostatic proliferation. Further, we and others observed reduced thymopoiesis after ALEM treatment,[29] whereas AHSCT has been reported to enhance thymic function.[66] Thus, the ALEM-characteristic changes to the TCR repertoire might be associated with higher SAID risk. Consistent with this, cyclophosphamide leads to contrary effects on the TCR repertoire with reduction of high-frequency T-cell clones, high TCR diversity and sample overlap[76] and is associated

with lower SAID risk when included in AHSCT conditioning regimens than ALEM.[26]

In addition, previous treatments might increase the probability of autoreactive T cells that escape ALEM-mediated depletion. Consistent with this, fingolimod pretreatment was associated with a higher risk of SAID.[34] Fingolimod blocks lymphocyte egress from lymphoid tissues, which are supposedly less susceptible to ALEM,[77] and might thus prevent the depletion by ALEM.[78]

We are aware that the exploratory nature of our analyses, the small sample size, particularly also the subcohorts for extensive analyses and a potential sample bias in our tertiary centre represent our study's limitations. Sample size might have particularly affected multiparameter analyses such as Olink, although this supports the relevance of cellular analyses. The immunological changes in our ALEM cohort were largely comparable to previous reports,[2,29,47,48] thus we expect that our findings are transferable to ALEM treatment in general. Particularly for the TCR/BCR sequencing data, a validation in larger cohorts is required based on this hypothesis-generating project. Differences in single parameters might be related to variations in patient cohorts, marker selection and detection methods.

Our findings imply that hyperexpanded T-cell clones, already present at baseline, may be predictive for the development of SAID with ALEM treatment. Following exploration of these findings in a larger cohort, the analysis of hyperexpanded T-cell clones may be used as a biomarker to exclude patients prone to SAID from ALEM therapy at baseline. However, sequencing technologies are expensive and cut-offs for normal values do not exist. Thus, a large-scale application of our findings in clinical practice might rather be reserved for the future.

In conclusion, our findings support ALEM-specific immune repertoire changes (restriction of TCR repertoire, reduced thymopoiesis, homeostatic proliferation, disparate dynamics of clonal T- and B-cell expansion) that provide a conceptual basis for ALEM-related SAID development in predisposed patients, combining current B- and T-cell-centric hypotheses. The deeper understanding of the immunological changes by ALEM may be instrumental in guiding its optimal use as a durable therapeutic strategy.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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4.3 Alemtuzumab therapy changes immunoglobulin levels in peripheral blood and CSF

ARTICLE OPEN ACCESS CLASS OF EVIDENCE

Alemtuzumab therapy changes immunoglobulin levels in peripheral blood and CSF

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Abstract

Objective
The use of alemtuzumab, a humanized monoclonal anti-CD52 antibody has changed the therapy of highly active relapsing-remitting MS (RRMS). Alemtuzumab infusion depletes most lymphocytes in peripheral blood, whereas differential recovery of immune cells, probably those with a less CNS-autoreactive phenotype, is supposed to underlie its long-lasting effects. To determine whether alemtuzumab significantly reduces immunoglobulin levels in blood and CSF of treated patients, we analyzed blood and CSF samples of 38 patients with MS treated with alemtuzumab regarding changes in immunoglobulin levels.

Methods
Blood and CSF samples of patients were collected at the beginning of alemtuzumab treatment and at 12, 24, and 36 months after the first administration of the drug. Specimens were analyzed regarding immunoglobulin concentrations in blood and CSF.

Results
We observed significant and dose-dependent reductions of immunoglobulin levels (IgG, IgM, and IgA) in serum and CSF 12 and 24 months following 2 courses of alemtuzumab. Patients with persistent or returning disease activity who were treated with a third course of alemtuzumab exhibited even further decrease in IgG levels compared with matched controls treated twice. Here, alemtuzumab-treated patients with IgG levels below the lower limits of normal were more susceptible to pneumonia, sinusitis, and otitis, whereas upper respiratory tract and urinary tract infections were not associated therewith.

Conclusions
Our results suggest to monitor IgG levels for safety reasons in patients treated with alemtuzumab—in particular when additional treatment courses are required—and to consider preventive action in critical cases.

Classification of evidence
This study provides Class IV evidence that for patients with RRMS alemtuzumab reduces immunoglobulin levels.

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Glossary

DMT = disease-modifying therapy; **OCB** = oligoclonal band; **RA** = rheumatoid arthritis; **RRMS** = relapsing-remitting MS.

Alemtuzumab was approved for therapy of active relapsing-remitting MS (RRMS) in Europe in November 2014.[1–3] The humanized monoclonal antibody selectively binds CD52, a human glycosylphosphatidylinositol-anchored protein. CD52 is highly expressed on the surface of T and B lymphocytes and, at lower levels, on cells of the innate immune system, namely monocytes and macrophages.[4–6] Consequently, alemtuzumab administration leads to rapid depletion of circulatory CD52-positive cells through antibody-dependent cell-mediated cytolysis and complement-dependent cytolysis.[7] Subsequent to depletion, immune cells derived from hematopoietic stem cells slowly repopulate. The repopulation dynamics are distinct for different immune cell types with B lymphocytes recovering faster than T cells. B lymphocyte numbers return to the baseline level after approximately 3 months; after 12 months, they can even exceed baseline levels.[8,9]

The trade-off for clinical efficiency of alemtuzumab is the occurrence of frequent and sometimes serious adverse events.[10] The most interesting and not yet fully understood adverse events are secondary autoimmune phenomena.[11]

Several case reports also described either viral or complex bacterial or fungal infections, highlighting consequences of T-cell depletion.[12] Common and usually noncomplicated infections, such as pneumonia or bacterial upper respiratory tract infections fostered by hypogammaglobulinemia, have received much less attention to date. Because of new reports of immune-mediated and partly fatal cardiovascular adverse events, the European Medicines Agency (EMA) restricted the use in April 2019.

Autoantibody production leading to consecutive autoimmune phenomena after alemtuzumab treatment has already been reported; however, physiologic immunoglobulin production following alemtuzumab administration has not yet been analyzed in greater depth.

Methods

Study design and setting

Between January 2015 and December 2016, 38 patients with diagnosed active RRMS were treated with at least 2 cycles of alemtuzumab at the Neurology Clinic of the University Hospital Münster. Serum (n = 38) and CSF (n = 24) samples were collected at treatment start and at 12, 24, and 36 months after the first alemtuzumab administration. None of the patients had received previous immunosuppressive or B cell–depleting therapy, had any history of chronic (autoimmune or infective) disease other than RRMS, or experienced a clinical relapse within the 4 weeks before sample collection. Patients were interviewed and clinically examined in regular 3-monthly follow-up visits (table 1).

This study provides Class IV evidence that long-term treatment with alemtuzumab can reduce immunoglobulin levels in patients with RRMS.

Participants

Demographic data collected included age, sex, duration of MS before alemtuzumab therapy, baseline EDSS score, number of previous disease-modifying therapies (DMTs), and number of MS relapses within the 2 years before alemtuzumab therapy. Diagnostic data included analysis of CSF cell count, CSF protein levels, CSF lactate, serum/CSF albumin ratio, CSF and serum analysis of immunoglobulin concentrations (IgG, IgA, and IgM) including serum/CSF immunoglobulin ratios as an indicator for intrathecal synthesis of IgG, IgA, and IgM, and oligoclonal bands (OCB) as an indicator for intrathecal IgG synthesis. During alemtuzumab treatment cycles, patients were examined for opportunistic infections, and data on disease activity and MS relapses were gathered.

Standard protocol approvals, registrations, and patients consents

Patients gave written informed consent for data publication, and the study was approved by the institutional review boards at both centers (University of Muenster, 2014-398-f-S; Hannover Medical School, 3142-2016).

Table 1 Baseline characteristics of the study cohort

Patients, no.	38
Blood samples available, no. (%)	38 (100)
CSF samples available, no. (%)	24 (63.2)
Age, yr, median (range)	33 (18–58)
Male sex, no. (%)	18 (47.3)
MS duration, yr, median (range)	
Since manifestation	4 (0–27)
Since diagnosis	4 (0–17)
Previous DMTs, no., median (range)	2 (0–6)
Baseline EDSS score, median (range)	2.5 (0–6)
Relapses within past 2 years, median (range)	3 (0–9)
OCBs positive at baseline (%)	22 (79)

Abbreviations: DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; OCB = oligoclonal band.

CSF and serum analysis

CSF and serum were analyzed using routine methods.[13] Immediately after CSF sampling via lumbar puncture, CSF cell count, total protein, and lactate were analyzed. CSF cells were counted manually with a Fuchs-Rosenthal counting chamber. For further analyses, the residual CSF was centrifuged (145*g* for 15 minutes), and the supernatant was frozen at −80°C. The corresponding serum was also frozen at −80°C. CSF total protein was determined by a Bradford dye-binding procedure. Albumin, IgG, IgA, and IgM were examined in serum and CSF in the same latex-enhanced assay by kinetic nephelometry (Siemens BN ProSpec, Münster; Beckman Coulter IMMAGE, Hannover) according to the guidelines by the manufacturer. The function of the blood-CSF barrier was estimated as CSF-serum albumin quotient (QAlbumin). The age-adjusted upper reference limit of QAlbumin (QAlb) was calculated using the formula QAlb = 4 + (age in years/15). Intrathecal synthesis of immunoglobulins (IgG, IgA, and IgM) was calculated based on the method of Reiber-Felgenhauer, referring IgG, IgA, and IgM quotients to the albumin quotient.[14] CSF and serum OCBs were determined by isoelectric focusing (Pharmacia Biotech) in polyacrylamide gels (EDC) with consecutive silver staining (GE Healthcare) according to the manufactures' recommendations. Five patterns of OCBs were distinguished following the recommendations of the first European consensus on CSF analysis in MS, whereas the type 2 and 3 pattern is an indicator for intrathecal IgG synthesis.[15]

To analyze immunoglobulin changes after 3 courses of alemtuzumab, we thawed serum samples obtained immediately before additional courses. Those samples were processed differently to samples from the previous time point and were eventually not comparable to previous values. We therefore decided to also analyze matched samples from patients with MS with ongoing natalizumab therapy and treatment-naive patients with MS, which underwent a similar thawing process to exclude process-related effects on serum immunoglobulin concentrations. To compare between groups, patients were matched for age, sex, disease duration since manifestation, and storage duration of samples. In accordance with the inclusion criteria for alemtuzumab-treated patients, none of the controls had received previous immunosuppressive or B cell–depleting therapy, and samples were obtained minimally 4 weeks after the last clinical relapse.

Statistical analysis

To compare between the groups at treatment start and at 24 months, the Mann-Whitney rank-sum test was used. To compare samples from 36 months after treatment start with respective controls, the Kruskal-Wallis test for multiple-group comparison including the Dunn post-test was used. Continuous variables (e.g., quantitative fraction of intrathecal IgG synthesis over time) were analyzed using the Wilcoxon paired test. To test categorical variables (proportion of patients with positive OCBs at different time points), The McNemar test with Edwards correction was applied. Statistical analysis was performed using SPSS 25 (IBM, CA). p Values below 0.05 were considered significant.

Data availability

All authors have full access to all data sets and take full responsibility for the integrity of the data and accuracy of the data analysis. Data will be shared on request from any qualified investigator.

Results

Patient characteristics

In total, we analyzed blood samples of 38 patients treated with alemtuzumab. For 24 patients, additional CSF samples were available. The patient characteristics at baseline are summarized in table 1.

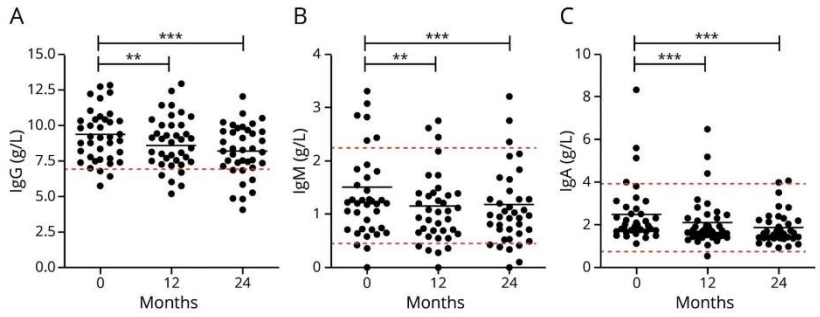
Serum IgG, IgM, and IgA concentrations of alemtuzumab-treated patients are reduced after 12 and 24 months

We measured serum immunoglobulin before the first administration of alemtuzumab and at 12 and 24 months. All patients received at least 2 cycles of alemtuzumab therapy according to SmPC. Eight patients required a third course 24 months after the first infusion due to ongoing disease activity. We measured immunoglobulin concentrations before start of each therapy course, respectively. We found a statistically significant decrease of immunoglobulin concentrations compared with baseline values for each subgroup (IgG, IgM, and IgA; figure 1, A–C). In all examined immunoglobulin subgroups, we found a stronger reduction after 24 months than after 12 months. All median values and p values are listed in an additional table (table e-1, links.lww.com/NXI/A172). Within the IgA group, 3 outliers with noticeably increased values could be identified before and 12 months after the first administration of alemtuzumab. At 24 months, no outliers remained. Notably, the majority of patients showed IgG levels within the normal range. At 24 months after therapy start, only 6 patients presented with IgG levels under 7 g/L (normal value >7 g/L).

Intrathecal IgG synthesis decreases during alemtuzumab therapy

For the majority of cases, we observed a significant decrease of the quantitative fraction of intrathecal IgG synthesis at 12 and 24 months after the first alemtuzumab administration. Except for 1 case, the decrease was progressive over time. We identified 3 patients with rising intrathecal IgG synthesis between therapy initiation and 12 months. For these 3 patients, synthesis significantly decreased thereafter. Furthermore, median CSF IgG, IgM, and IgA concentrations significantly decreased over time. All median values and p values are listed in an additional table (table e-2, links.lww.com/NXI/A172). At baseline, 79% of all patients exhibited positive OCBs in the CSF. At 12 and 24 months after therapy start, their percentage amounted to 75% ($p = 1.000$) and 71% ($p = 0.0662$), respectively. In fact, for 2 patients, OCBs were no longer detectable at 24 months (figure e-1, links.lww.com/NXI/A172).

Figure 1 Measurement of serum immunoglobulin (IgG, IgA, and IgM) levels in alemtuzumab-treated patients



Serum IgG (A), IgM (B), and IgA (C) levels (in g/L) were analyzed before the first alemtuzumab course and at 12 and 24 months. Stars represent levels of significance (**$p < 0.01$; ***$p < 0.001$).

Patients exhibit reduced serum IgG levels after the third course of alemtuzumab

Eight patients required a third course of alemtuzumab due to sustained disease activity (new relapses: 4/8; EDSS progression: 1/8, cMRI with new/enlarging T2 lesions: 7/8). We measured serum immunoglobulin levels 12 months after the third course of alemtuzumab (36 months after initiation of therapy). Unlike previous measurements, those samples underwent freezing and thawing before analysis, and our quality control indicated slight differences in 4 samples. We performed a quality control measurement of 4 samples that were both freshly analyzed stored and found slight differences in IgG levels (5%). We therefore decided not to perform comparison between 36-month samples and other time points but compared our findings with similarly processed samples from patients having not received a third course. In addition, we included samples from naive patients with MS as further control.

Compared with patients treated twice and treatment-naive patients with MS (table e-3, links.lww.com/NXI/A172), patients with a third alemtuzumab course showed further, significant reduction of serum IgG levels, ranging from 5.0 to 7.5 g/L ($p = 0.006$ compared with patients with 2 courses). The majority of controls presented with serum IgG levels of about 10.0 g/L at 36 months. For the other immunoglobulin subgroups (IgM and IgA), no differences were detectable. Because specimens were thawed and serum immunoglobulin levels were measured afterward, values are comparable among each other but not with regard to reference values.

Patients with reduced immunoglobulin levels were more likely to have otitis, sinusitis, and pneumonia

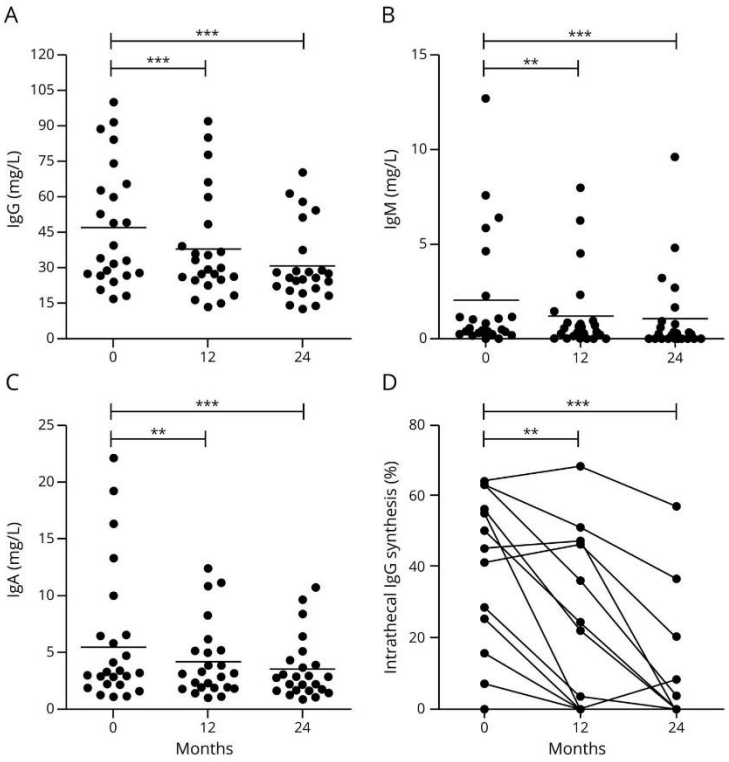
In adults, the lower limit of normal for serum IgG levels is about 7 g/L. For lower values, IgG deficiency is diagnosed. For the 38

Table 2 Frequency of different infections during alemtuzumab treatment

	Total events with qualified IgG measurement	Serum IgG >7 g/L in closest proximity to event	Serum IgG <7 g/L in closest proximity to event	Time from last course of ALEM, range (months)
Upper respiratory tract infection	57	50	7	3–35
Otitis media	9	3	**6**	13–32
Sinusitis	10	2	**8**	11–26
Pneumonia	3	0	**3**	14–26
Skin infection	2	2	0	5–12
Urinary tract infection	48	42	6	2–30

Abbreviation: ALEM = alemtuzumab.
Patients with serum IgG levels >7 g/L were compared with patients with serum IgG levels <7 g/L. Numbers in columns 1-3 represent observed events. Bold numbers indicate infections associated with hypogammaglobulinemia.

Figure 2 Measurement of CSF immunoglobulin (IgG, IgA, and IgM) levels in alemtuzumab-treated patients



CSF IgG (A), IgM (B), and IgA (C) concentrations (in mg/L) were analyzed before the first alemtuzumab course and at 12 and 24 months. In addition, quantitative fraction of intrathecal IgG synthesis over time is shown (D). Stars represent levels of significance (**p < 0.01; ***p < 0.001). OCB = oligoclonal band.

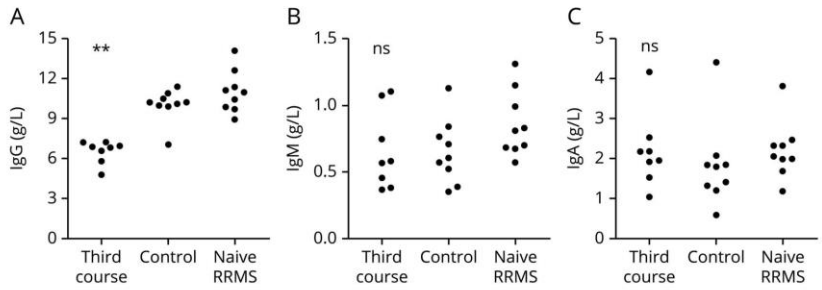
alemtuzumab-treated patients, we documented any infections as events in a 3-year follow-up. In 36/38 patients (95%) of patients, we observed infections as adverse events. Mainly, patients had upper respiratory tract or urinary tract infections (44% and 37% of all events, respectively). Several cases of otitis and sinusitis were also observed. Three individuals had pneumonia, and 2 had soft tissue infections. We analyzed IgG levels when patients presented to the neurologic department for their regular 3-monthly visit after alemtuzumab treatment. Some patients required repeated measurement. We evaluated all events for which serum IgG levels were available within a 3-month period before reported infections. We checked whether preexisting IgG deficiency was associated with an increased susceptibility toward different types of infections. In case of multiple measurements within the specified time, we included the measurement closest to onset of infection.

Within the group of patients with upper respiratory tract infections and urinary tract infections, IgG levels were above 7 g/L in the majority of cases. Patients having otitis, pneumonia, or sinusitis frequently exhibited reduced serum IgG levels during their previous examination.

Discussion

Administering alemtuzumab leads to rapid depletion of circulating CD52–positive immune cells. However, immune cells in lymphoid organs are less affected.[16] T cells recover within 11–12 months, whereas the B-lymphocyte count typically normalizes within 6 months posttreatment. However, Jones and colleagues observed early T-cell recovery after alemtuzumab treatment, largely driven by homeostatic expansion of cells that escaped depletion. Furthermore, they demonstrated that homeostatic proliferation increased the risk of secondary autoimmunity.[17] Between 30% and 41% of alemtuzumab-treated patients develop thyroid autoimmune disease. Less common, but potentially more critical, are immune thrombocytopenia and glomerulonephritis.[11] A possible mechanism behind secondary autoimmunity is the faster repopulation of B cells in the absence of effective T-cell regulation in individuals with genetic susceptibility for autoimmunity.[18] However, T cell–mediated secondary autoimmune phenomena have also been described[19] and the mechanisms of reeducation of immune regulatory networks after CD52 depletion remain largely elusive.[20]

Figure 3 Serum immunoglobulin levels of patients with third alemtuzumab course compared with controls and treatment-naive patients with MS



Samples were collected 36 months after initiation of therapy; serum IgG (A), IgM (B), and IgA (C) levels (in g/L) were measured. Controls had received 2 courses of alemtuzumab treatment and were matched to third-course patients. Samples taken from third-course patients and treatment-naive patients with MS were analyzed after thawing. Stars represent levels of significance (**$p < 0.01$ vs control).

Despite the faster recovery of B cells, our results demonstrate significantly decreased immunoglobulin levels in alemtuzumab-treated patients 12 and 24 months after administering the first dose (figure 1). The levels even further decrease after a third therapeutic cycle. In patients without a third course, IgG but not IgM and IgA concentrations increased 36 months after therapy initiation (figure 3). The ongoing reduction of immunoglobulin levels despite normalized B-cell counts indicates a decrease of immunoglobulin concentrations in serum independent from B-lymphocyte repopulation. Recently, it has been shown that B-cell distribution shifts toward a naive phenotype following alemtuzumab treatment. $CD19^+CD24hiCD38hi$ and $CD19^+PD-L1hi$ cells, which are deficient in peripheral blood of patients with RRMS, increase after alemtuzumab administration.[21] These cells are known for supporting an anti-inflammatory environment by limiting Th1 and Th17 differentiation and maintaining regulatory T cells.[22] This may serve as an explanation for continuously low immunoglobulin concentrations. Wray and colleagues found that patients in phase 2 (CAMMS223) and phase 3 (CARE-MS I and CARE-MS II) studies exhibited similar absolute counts of $CD4^+$ T-cell, $CD8^+$ T-cell, and $CD19^+$ B-cell subsets whether they developed an infection or not. This finding was common to all time points examined.[23] The authors hypothesized that unchanged concentrations of immunoglobulins to common viruses, as shown by Clark and colleagues,[24] result from CD52-negative antibody-secreting long-lived plasma cells.[25] While we did not measure specific antibodies, the total immunoglobulin concentration decreased in our patient cohort.

The ongoing reduction of IgG levels in peripheral blood seems to be of clinical relevance. Among our 38 patients, those with IgG values below 7 g/L showed a higher susceptibility to develop infections such as pneumonia, sinusitis, and otitis (table 2), whereas the more common infections such as upper respiratory tract and urinary tract infections were not related to reduced serum IgG levels. It has to be noted that we did not differentiate between mild hypogammaglobulinemia (defined as IgG <7 g/L) and severe hypogammaglobulinemia (defined as IgG <4 g/L). In a study of 389 patients with secondary hypogammaglobulinemia, Blot and colleagues detected no significant difference between patients with mild and severe hypogammaglobulinemia regarding their infectious risk,[26] which indicates that hypogammaglobulinemia generally increases the risk of infection. However, Furst stated that very low levels of IgG are indeed associated with a heightened risk of infections, but he also demonstrated that less severe hypogammaglobulinemia (>5 g/L) appears to be tolerated in most subjects.[27]

Within our cohort, pneumonia, sinusitis, and otitis were observed much later following alemtuzumab compared with urinary tract infections and upper respiratory tract infections, which occurred early after infusions. This could indicate differences in underlying pathophysiology. Hypogammaglobulinemia is also known as a relevant risk factor for infections with cytomegalovirus (CMV). Recently, few case reports have described CMV infections in alemtuzumab patients.[12,28] Although an increased risk of infections is clearly acknowledged in phases around infusions, a clear signal for an increased risk of infections has not been reported to date. Other depleting antibodies used to treat autoimmune diseases, such as the anti-CD20 antibody rituximab, also lead to a significant decrease of blood immunoglobulin levels during therapy.[29] However, several studies on patients with rheumatoid arthritis (RA) treated with rituximab could demonstrate that patients with below normal immunoglobulin levels did not have more serious infections than patients with normal immunoglobulin levels.[29–32]

Our results appear to be in contrast to the findings of McCarthy et al.[33] who performed a pilot study regarding immunologic memory to common viruses and responses to vaccinations in 24 patients with prior alemtuzumab treatment. Their patients exhibited a normal humoral response to diphtheria, tetanus, and poliomyelitis vaccine, haemophilus influenzae type b and meningococcal group C conjugate vaccine, and pneumococcal

polysaccharide vaccine with normal IgG titers. One possible explanation might be that in contrast to the McCarthy cohort, most of our patients had received multiple immunomodulatory therapies before alemtuzumab administration. Being a real-world cohort with patients including complex history of MS treatment despite exclusion of patients with a history of mitoxantrone, azathioprine or anti-CD20 therapy might also explain why the observation of developing hypogammaglobulinemia has not been made in the phase 3 trials and their respective extension studies.[23] Age-dependent effects in our cohort are rather unlikely as the median even undercuts the median age of patients from CARE-MS II. However, real-world cohorts have shown different frequencies or even entities of adverse events following immunomodulatory treatment with progressive multifocal leukoencephalopathy following natalizumab treatment being rapidly present in one's mind.[34]

If immunoglobulin levels would be monitored during and following alemtuzumab therapy, especially in patients with multiple previous DMTs, patients with markedly reduced IgG levels could be identified, and possibly, a prophylactical antibiotic treatment or immunoglobulin substitution therapy could be initiated to prevent serious infections in those patients. Besides McCarthy et al., another group assessed antigenic responses following influenza, polyvalent pneumococcus vaccine (PPV23) and combined diphtheria/tetanus/poliovirus vaccines in patients with RA who had been treated with alemtuzumab 20 years ago. Similar levels of seroprotection following poliovirus (P1-P3), tetanus, and diphtheria vaccination were observed for alemtuzumab-treated patients and controls.[35] As the nature of the observed infections in our cohort (pneumonia, otitis, and sinusitis) indicates that encapsulated bacteria such as *Streptococcus pneumoniae* might be responsible, PPV23 vaccination before alemtuzumab induction seems reasonable.

An interesting aspect of our study is the parallel measurement of CSF immunoglobulins, in addition to the periphery. Because of the decrease of serum IgG concentrations, reduction of IgG levels measured in CSF is to be expected. Of interest, the percentage of intrathecal IgG synthesis itself is lowered in nearly all patients (figure 2). This indicates that reduction of CSF IgG is not merely a consequence of decreased serum IgG concentrations and could suggest that alemtuzumab inhibits the autoimmune process within the CNS. This is also reflected by the disappearance of OCBs in 2 patients (figure 2).

Within our cohort, 8 patients required a third treatment cycle because of ongoing/returning clinical or paraclinical disease activity. They exhibited significantly reduced serum IgG concentrations compared with matched controls who received only 2 treatment cycles and also with treatment-naive patients with MS (figure 3). Therefore, the decrease in serum IgG concentrations is not merely a consequence of thawing the specimens. The fact that patients with 3 treatment courses presented with lower IgG concentrations is not surprising, as they received a higher overall dose of alemtuzumab and their last treatment took place only 12 months ago. Of interest, other immunoglobulin subgroups were not affected. In addition, it can be observed that serum IgG levels of alemtuzumab patients without a third treatment course obviously normalized over time, as they do not differ from IgG concentrations of untreated patients with MS (figure 3).

Our study has several limitations. First, this work is based on a retrospective analysis of clinical data. Because of the nature of this investigation, no causal relations can be interpreted. Moreover, the number of cases especially of patients with a third alemtuzumab course is limited, and their samples have been thawed before the analysis of immunoglobulin levels. As a result, the findings need to be verified in larger samples. In addition, real-world cohorts are needed to determine the exact temporal expansion of hypogammaglobulinemia following alemtuzumab treatment.

Conclusion

We could demonstrate reduced concentrations for all immunoglobulin subgroups at 12, 24, and 36 months after initiation of alemtuzumab therapy. Patients requiring a third treatment cycle due to ongoing disease activity had the most severe drop in IgG concentrations. Reduced IgG concentrations were associated with an increase in pneumonia, otitis, and sinusitis. We therefore suggest that serum IgG levels should be monitored at least in those patients receiving more than 2 treatment courses of alemtuzumab. In addition, we recommend a pretreatment pneumococci vaccination. The decreased intrathecal IgG production in the CSF suggests that alemtuzumab effectively suppresses the autoimmune process within the CNS.

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Tobias Ruck, MD	University of Münster, Münster, Germany	Author	Critical revision of the manuscript for intellectual content
Catharina C. Gross, MD, Prof	University of Münster, Münster, Germany	Author	Supervision of acquisition, analysis, and interpretation of CSF routine parameters and critical revision of the manuscript for intellectual content
Thomas Skripuletz, MD, Prof	Hannover Medical School, Hannover, Germany	Author	Critical revision of the manuscript for intellectual content
Luisa Klotz, MD, Prof	University of Münster, Münster, Germany	Author	Critical revision of the manuscript for intellectual content
Heinz Wiendl, MD, Prof	University of Münster, Münster, Germany	Author	Critical revision of the manuscript for intellectual content
Martin Stangel, MD, Prof	Hannover Medical School, Hannover, Germany	Author	Study concept and design and critical revision of the manuscript for intellectual content
Sven G Meuth, MD, Prof	University of Münster, Münster, Germany	Author	Study concept and design and critical revision of the manuscript for intellectual content

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4.4 Efficacy and safety of alemtuzumab versus fingolimod in RRMS after natalizumab cessation

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ORIGINAL COMMUNICATION

Efficacy and safety of alemtuzumab versus fingolimod in RRMS after natalizumab cessation

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Abstract

Background Natalizumab (NTZ) was the first approved monoclonal antibody for the treatment of relapsing-remitting multiple sclerosis (RRMS). Despite proven and sustained efficacy, its use is limited by the risk of progressive multifocal leukoencephalopathy (PML). Moreover, some patients show ongoing disease activity under NTZ, requiring a switch to another disease-modifying treatment (DMT). However, evidence regarding the optimal DMT for treatment of active RRMS after NTZ-cessation is still scarce.

Objective To evaluate efficacy and safety outcomes of ALEM vs FTY treatment after cessation of NTZ.

Methods We retrospectively identified patients at 12 German neurology centers and analyzed risks for disease activity, adverse events, disability progression, and treatment discontinuation.

Results 195 patients were identified and 144 underwent final analysis (FTY: 101; ALEM: 42). The hazard ratio for clinical relapses was 2.24 favoring ALEM (95% CI 1.12–4.50; $p=0.015$). The hazard ratio for adverse events was 7.78 (95% CI 1.04–57.95; $p=0.006$) and 2.41 for MRI progression (95% CI 1.26–4.60; $p=0.004$). The odds ratio for disability progression after 12 months was 4.84 (95% CI 1.74–13.47, $p=0.003$). Differences remained after adjusting for possible confounders (e.g., age, sex, baseline disability, NTZ treatment duration, washout time).

Conclusion Our findings indicated particular advantages of ALEM compared to FTY in patients stopping NTZ.

Keywords Alemtuzumab · Natalizumab · Fingolimod · Remitting-relapsing multiple sclerosis · Progressive multifocal leukoencephalopathy · Immunomodulatory therapy

Introduction

Natalizumab (NTZ) is a highly efficacious treatment option in relapsing-remitting multiple sclerosis (RRMS). It has proven to reduce relapse rates and to slow disability progression [1, 2]. Unfortunately, its therapeutic potential is limited by a significant risk of progressive multifocal leukoencephalopathy (PML), a JC virus (JCV)-mediated and potentially life-threatening viral infection of the brain [3]. Specific subgroups of patients (treatment for more than 2 years, positive JCV antibodies and previous immunosuppressive therapy) can bear a risk of up to 1:31 for development of PML [4]. Consequently, a significant proportion of patients need to switch treatment to maintain control of disease activity while minimizing PML risk [5]. Furthermore, a small but relevant patient cohort shows continued disease activity despite NTZ infusion. In phase 3 clinical trials, up to 26% of NTZ patients developed more than one new or enlarging T2-hyperintense lesion and up to 8% of patients experienced more than one relapse within 2 years [1].

Since NTZ-cessation is associated with rekindling disease activity, considerations regarding the optimal choice of the following disease-modifying treatment (DMT) are

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important. Depending on when a DMT is subsequently initiated, rebound activity peaks around month 4–7 after NTZ-cessation and can even exceed pre-treatment inflammatory activity [6, 7]. Frequently, patients at increased PML risk are switched to fingolimod (FTY), which has shown superior efficacy compared to beta-interferon and glatiramer acetate in previous studies [8].

Nonetheless, FTY is not capable of stabilizing the disease course in all patients switched from NTZ. Even a shortening of the washout period to 8 weeks prior to FTY initiation still led to paraclinical disease activity 24 weeks after the last NTZ infusion in about half of the patients in a randomized clinical trial [9]. In a larger cohort, around 20% of patients that were switched to FTY experienced clinical relapses within the first year. In this trial, rituximab (RTX) treatment was examined as the other drug of choice after NTZ-cessation. RTX showed favorable outcomes in this situation [10]. Unfortunately, it is restricted to off-label use in RRMS and long-term data on the upcoming anti-CD20 antibodies, e.g., ocrelizumab, in this special situation will take several years of real-world experience.

Another powerful therapeutic option for active RRMS is alemtuzumab (ALEM) [11, 12]. The monoclonal anti-CD52 antibody has proven superior efficacy compared to interferons in trials of active RRMS. So far, there are no reports on a significant risk of PML in alemtuzumab-treated MS patients. Nonetheless, the long-lasting immunological effects of alemtuzumab have to be put in context with carry-over PML. However, the most relevant risk under alemtuzumab is the induction of secondary autoimmune disorders. These mostly affect the thyroid gland and to a much lesser extent platelets and kidneys [13, 14].

Given the specific risks of the mentioned treatments for active RRMS, the individual risk-benefit-profile should be subject to careful considerations. However, currently there are no larger scale studies providing evidence for the decision making process. We, therefore, conducted a multicenter retrospective analysis on efficacy and safety in RRMS patients switched from NTZ to either ALEM or FTY providing relevant information for the development of treatment algorithms in patients with active RRMS.

Methods

We conducted a retrospective analysis at 12 German neurology departments. Patients that were withdrawn from NTZ between 01/2014 and 12/2016 were identified from local databases. Inclusion criteria were NTZ treatment of at least 12 months and subsequent switch to either ALEM or FTY. Exclusion criteria were a washout period of more than 6 months and pregnancy. In-depth medical chart reviews were performed locally using standardized case report forms. Differences between treatment groups (ALEM or FTY) in the distribution of baseline parameters were assessed using Mann–Whitney *U* test for continuous and Fisher's exact test for categorical variables. Baseline was here defined as time point of the respective subsequent DMT initiation. The time to MRI progression, serious adverse events, relapses and drug discontinuation was analyzed using the Cox proportional hazards model and the Kaplan–Meier method. We performed analysis using a complete-case strategy. For the outcome parameter disability progression after 12 months, logistic regression was used for calculation of odds ratios. Multivariable analysis was performed using step-wise variable selection with the drug (FTY vs. ALEM) in the first block and further covariates in a second block (inclusion criterion: score test p value ≤ 0.05, exclusion criterion: likelihood ratio test p value > 0.1). Potential covariates included disease duration from debut and diagnosis, NTZ treatment duration, washout time, sex, baseline expanded baseline disability status (EDSS) and presence of gadolinium-enhancing MRI lesions (GELs) at baseline. Hazard ratios and odds ratios are given with 95% confidence interval (95%CI) and p value of likelihood ratio test. MRI scans for GELs at baseline were included if performed at least 6 weeks before or after initiation of ALEM or FTY treatment. MRI data obtained while on FTY or ALEM or not later than 4 weeks after censoring or drug discontinuation were included for follow-up. Paraclinical disease activity was assessed via detection of new or enlarging T2-hyperintense lesions (T2Ls). Treatment cessation of FTY was defined as day of last intake, whereas ALEM treatment discontinuation was defined as beginning of an alternative DMT within the first year or when the decision against administration of the second treatment course was made. Progression of disability after 12 months was considered clinically relevant if two independent assessments of EDSS at least 6 weeks apart displayed the following: (I) EDSS + 1.5 (baseline = 0.0), (II) EDSS + 1.0 (baseline = 1.0–4.0), (III) EDSS + 0.5 (baseline ≥ 4.5). The confirmatory null hypothesis was H_0: The on drug survival does not differ between the treatment arms (FTY vs ALEM). H_0 was tested by two-sided log-rank test on a significance level of 5%. All remaining analyses were regarded as exploratory with p values being displayed for descriptive reasons to study meaningful effects. Analysis was carried out using SPSS Statistics 24 (IBM, Watson, USA). Ethical approval for conduction was given by local ethical review board (University of Muenster, 2017-297-f-S).

Results

In total, we identified 217 patients stopping NTZ, 195 of which were switched to either FTY (124 patients) or ALEM (71 patients, see Fig. 1); 22 patients remained without

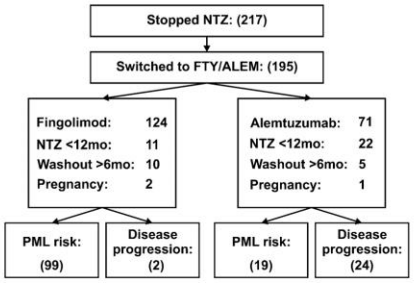

Fig. 1 Flow chart depicting patient identification and inclusion at the study centers. Patients switched from NTZ to different treatment and had follow-up data for 12 months were identified from local databases and underwent in-depth medical chart review

disease-modifying treatment or were switched to other substances (e.g., mitoxantron). After exclusion of patients with only short-term NTZ treatment, prolonged washout or pregnancy, we could include 101 FTY patients and 43 ALEM patients in the final analyses. Table 1 shows baseline criteria for both treatment groups. Data on baseline criteria were missing in only 3 FTY patients (MS duration since disease debut: 2 patients; number of previous DMTs: 1 patient).

Neutralizing antibodies against NTZ were not reported. DMTs were administered according to guidelines with daily intake of 0.5 mg FTY and infusion of 12 mg per day of ALEM for 5 consecutive days. A single case received ALEM for less than 5 days due to serious infusion-related adverse events.

Remarkably, the allocation to the two DMTs was highly dependent on whether a patient was withdrawn from NTZ solely due to PML risk or ongoing disease activity (2.0% of FTY patients and 55.8% of ALEM patients were switched due to disease progression; $p < 0.001$). This is mirrored by a larger proportion of patients with GELs at baseline (3.1% for FTY vs. 21.4% for ALEM; $p < 0.001$). Nonetheless, median washout time was relevantly longer in patients receiving ALEM (63 days for FTY vs. 91 days for ALEM). Remarkably, there was no difference in ALEM patients in terms of washout when considering the reason for stopping NTZ [PML risk: 91 days (66–108); disease progression: 90 days (68.25–118.3); $p = 0.659$]. Within washout period, two relapses occurred prior to ALEM induction, both affecting patients withdrawn from NTZ due to disease progression.

In total, 54 of our patients experienced 83 clinical relapses within the first year, 45 in the FTY group (73 relapses) and 9 in the ALEM group (10 relapses). One-year relapse free survival was 55.4% for FTY patients and 76.7% for ALEM treated patients ($p = 0.024$). Figure 2a shows the decrease of patients without clinical relapse over time. Univariable hazard ratio for time to relapse was 2.24 (95% CI 1.12–4.50; $p = 0.015$) favoring ALEM and was confirmed by multivariable analysis with none of the further covariates described above being selected.

Cranial MRI examination detected ongoing disease activity by either new T2Ls in a total of 51 FTY patients (50.5%) and 11 ALEM patients (25.6%; Fig. 2b). One patient with clinical relapse, but stable cranial MRI was diagnosed with isolated spinal disease activity showing new paraparesis. Cox regression resulted in a crude hazard

Table 1 Baseline characteristics of the ALEM and FTY group

	Fingolimod	Alemtuzumab	*p*
Patients, no.	101	42	–
Age, year, median (IQR)	40 (33–45.75)	34 (27–34)	0.185*
Male sex, no. (%)	27 (26.7)	10 (23.8)	0.835#
MS duration, year, median (IQR)			
Since debut	11 (7–18)	9 (6–13)	0.236*
Since diagnosis	9 (5–14)	6 (3–10)	0.272*
Previous DMTs, no., median (IQR)	2 (1–3)	2 (1–3)	0.484*
Baseline EDSS, median (IQR)	2.5 (2–4)	3.0 (2–4)	0.600
Patients with GELs at baseline, No. (%)	3 (3.1)	9 (21.4)	**< 0.001**#
Natalizumab infusions, no. median (IQR)	34 (20–48)	25 (21–52)	0.517*
Natalizumab duration, months, median (IQR)	34 (20.5–50)	26 (22–54)	0.554*
Washout period, days, median (IQR)	63 (55.5–91)	91 (68–116)	**< 0.001***

Bold—a p value < 0.05 was considered significant in either test

Missing data in the FTY group: MS duration, 2 patients; previous DMT, 1 patient. No data were missing in the ALEM group

EDSS expanded disability status scale, *IQR* interquartile range

*Two-sided p value of Mann–Whitney U test

#Two-sided p value of Fisher's exact test

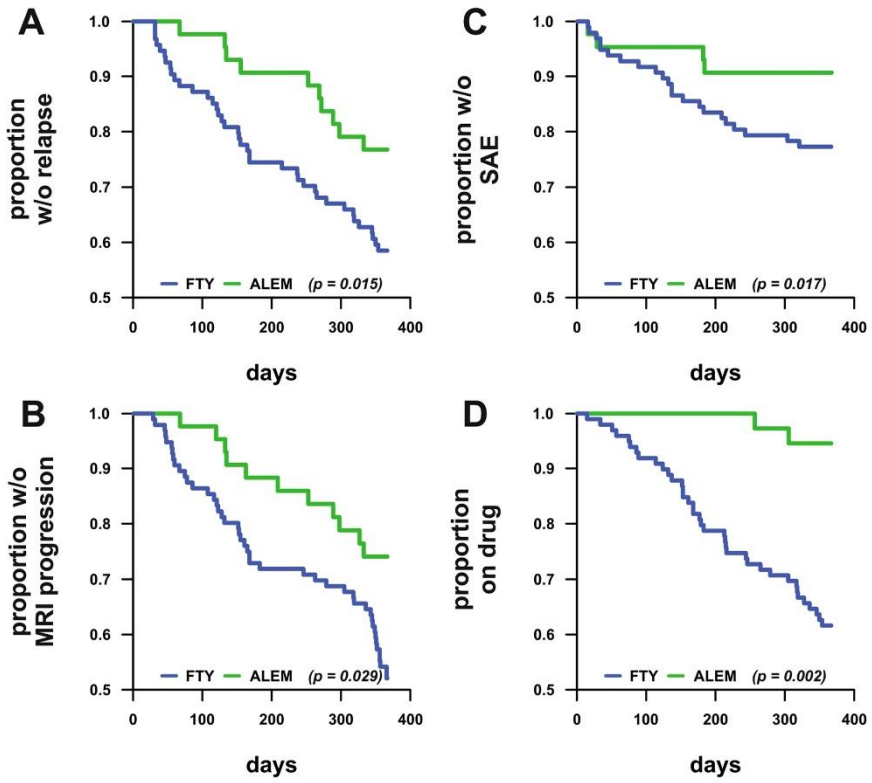

Fig. 2 Kaplan–Meier plots indicating outcomes of both treatment groups within their first year after having been switched from NTZ. **a** Time to first confirmed clinical relapse. **b** Time to first documented serious adverse event. **c** Time to first detection of new T2-hyperintense Lesions or GELs. **d** Time to drug discontinuation. Significance levels were derived from univariate analysis

ratio of 2.17 favoring ALEM (95% CI 1.13–4.20; $p=0.029$), which changed to 2.41 (95% CI 1.26–4.60; $p=0.004$) after adjustment.

The safety profile of both drugs was congruent to previous data and revealed no unexpected (severe) adverse events. First-dosing events were detectable in 5 patients in the FTY group (5%) and in 19 patients in the ALEM group (44.2%; $p>0.001$). Bradycardia was the documented adverse reaction in all 5 FTY-treated patients and 3 of those required temporary surveillance as inpatients (but not exceeding 24 h). In the ALEM treatment group, patients suffered from rash (12), pyrexia (9), tachycardia (6) and bradycardia (1). The latter was judged as a grade 4 adverse event regarding common terminology criteria (CTCAE v4.0) and required intensive care-treatment due to hypotension. Twenty-three adverse events were reported in the FTY group. Of these, persistent grade °IV lymphopenia was present in 8 patients (and led to discontinuation of FTY treatment in all cases), elevated liver enzymes in 5, pneumonia in 2, herpes zoster in 2 and others in 5 patients. Of note, one case of basalioma was diagnosed. In the ALEM-group, 2 case of autoimmune thyroiditis both requiring thyreostatic treatment were diagnosed. Additionally, two patients suffered from temporary elevated liver enzymes of unknown origin. Neither of the patients developed symptoms or required specific treatment. No cases of persistent grade °IV lymphopenia were discovered in the alemtuzumab group and no patient required antiinfective prophylaxis beyond 4-week post-infusion. Apart from first-dosing adverse events, 1-year adverse event free survival was 77.3% and 90.7% in the FTY and ALEM groups, respectively (Fig. 2c). This resulted in an adjusted hazard ratio of 7.78 (95% CI 1.04–57.95; $p=0.006$) favoring ALEM.

Treatment was discontinued in 40 patients in the FTY group and 2 patients in the ALEM group. Accordingly, 1-year drug survival differed significantly between treatment groups with 61.6% and 95.3% in FTY and ALEM groups, respectively ($p = 0.002$) (Fig. 2d). Univariable testing resulted in a crude hazard ratio of 9.09 favoring ALEM (95% CI 2.18–37.84; $p = 0.002$) and multivariable testing gave similar results without inclusion of further covariates. Adverse events, mostly lymphopenia or elevated liver enzymes, were the reason for discontinuation in 11 patients in the FTY group (10.9%) and 1 patient in the ALEM group (3.2%). Twenty-eight patients stopped FTY due to disease progression (27.7%) and one patient required ALEM cessation because of paradoxical disease activity (3.2%). Notably, both patients stopping alemtuzumab were initially switched because of PML risk and not ongoing disease activity. The patient discontinuing ALEM because of her first-dosing adverse event (bradycardia) was subjected to daclizumab treatment after 8 months; the ALEM-patient displaying recurring disease activity with more than 20 new T2Ls after 10 months was treated with RTX. Data on consecutive DMTs in patients stopping FTY are available in 35 patients (87.5%): 17 patients were treated with alemtuzumab (42.5%), 11 were re-exposed to natalizumab (27.5%) and 7 received B cell-depleting therapy with RTX (17.5%).

Finally, 37 patients demonstrated disability progression in the FTY group (37.4%) during the first year after cessation of natalizumab, whereas 5 patients did so in the ALEM group (11.6%). Univariable odds ratio for disability progression was 4.84 favoring ALEM (95% CI 1.74–13.47; $p = 0.003$) and was confirmed by multivariable analysis with none of the further covariates described above being selected.

Discussion

In this study, we compared safety and efficacy outcomes of ALEM and FTY treatment for patients switched from NTZ. Contrasting previous studies, we also included patients that not only switched due to increased PML risk, but also due to ongoing disease activity under NTZ. Only the CARE-MS II trial on ALEM in active RRMS included 15 patients pretreated with NTZ. However, no detailed subgroup analysis was provided [12]. Malucchi and colleagues published a small cohort of patients switched from NTZ to ALEM comprising 16 patients. However, only two patients out of these were followed for at least 1 year and this was somehow limiting the validity of that study [15].

Currently, no randomized, prospective head-to-head studies comparing FTY, NTZ or ALEM exist and their conduction in the future remains unlikely. A large retrospective analysis of the MSBase cohort displayed beneficial outcomes of ALEM compared to FTY during treatment years 1 to 3. Unfortunately, this study included only small numbers of NTZ-pretreated patients in its FTY and ALEM study arms and is, therefore, unsuitable for deriving of recommendations regarding treatment sequences [16].

Of note, risk of disease reactivation largely depends on duration of washout period [9]. But remarkably, washout period was not selected as a confounder in our multivariate analyses despite having shown relevant differences at baseline. Furthermore, only two patients experienced relapses during washout period and prior to ALEM treatment. In terms of NTZ switch to FTY, previous studies have shown a risk ranging from 25 to 30% of reoccurring disease activity in previously stable patients depending on previous disease activity and washout period [10].

In accordance with these observations, we observed that alemtuzumab patients had a lower risk for clinical relapses. Additionally, ALEM was also superior in reducing the risk for MRI disease activity and preventing disability progression. Moreover, a significantly larger proportion of patients remained on ALEM treatment compared to FTY after year one. Finally, around 40% of patients that stopped FTY treatment ultimately switched to ALEM, but experienced sustained disability progression in the meantime. Additionally, some case reports suggest an impaired efficacy of ALEM in previously FTY-treated patients, eventually putting these patients at risk for further accumulation of disability [17].

We also collected safety data, but incomplete reporting, especially in mild adverse events including non-complicated infections, might be a relevant bias to the analyses. Consequently, we only reported data on severe adverse events and events that led to drug withdrawal which were more likely to be covered in the medical charts in total.

As expected from the literature, first-dosing adverse reactions were more common in the ALEM group as cytokine-release syndrome is frequently observed in this antibody-based treatment [18]. Surprisingly, we detected a frequency lower than measured in the phase three clinical trials [12, 19]. It remains unclear whether this is due to common application of intravenous corticosteroids (5 instead of 3 days during first ALEM treatment), other symptomatic treatments (including antihistaminic drugs, non-steroidal anti-inflammatory drugs and proton pump inhibitors), or because of underreporting. A single case was of special interest showing symptomatic bradycardia with hypotension during infusion leading to treatment discontinuation. However, the most important long-term risk of ALEM treatment, the development of secondary autoimmunity affecting thyroid and other organs, is insufficiently represented in our study due to peaking incidence in year three and four after treatment induction [13]. Therefore, these limitations should be carefully considered in interpretation of safety data.

A valid concern of physicians switching patients from NTZ to other DMT is the risk of unidentified PML ("carry-over PML") [20]. Common precautions include repetitive MRI examination and even lumbar puncture for assessment of JCV-DNA in the cerebrospinal fluids. Taken together with long-term T cell suppression with ALEM, this might have prolonged the washout period. Although no cases of carry-over PML have been observed after switch from NTZ to RTX despite a shorter washout period of about 45 days in the Swedish cohort, we acknowledge the wish for an additional level of confidence by an increased washout period [10]. Of course, the existence of PML cases after FTY treatment also warrants a thorough exclusion of PML in patients aiming for FTY after NTZ [21].

As always in retrospective analyses, our study is challenged by potential confounders at baseline that we could not account for. Especially, data on relapse rate prior to NTZ treatment in our cohort were mostly unobtainable despite our best intentions. However, baseline and outcome criteria of our FTY group are in line with other publications [10]. Furthermore, we were not able to analyze the influences of different treatment centers due to limited patient numbers. One of the biggest challenges in this real-world cohort remained the uneven distribution of the respective reasons for NTZ-cessation to the subsequent treatment groups. However, the prevalent admission of active patients to ALEM compared to FTY in our opinion results in a bias against ALEM and was somehow overcome by the still positive findings made here.

In summary, our study reports the first comparative real-world cohort of NTZ-stopping patients treated with ALEM. Despite limitations in prediction of long-term adverse events, we found favorable outcomes for ALEM at least during the first year compared to FTY even though our study was likely to even be biased against ALEM because of the larger proportion of patients with ongoing disease activity subjected to ALEM. However, the specific long-term risks of ALEM treatment in patients stopping NTZ have to be evaluated by longer follow-up of patient cohorts such as the presented one. Of course, our study cannot replace a prospective, randomized head-to-head trial and results have to be interpreted with caution.

We nonetheless conclude that ALEM is a reasonable treatment alternative in patients discontinuing NTZ, even in patients with ongoing disease activity. Our data warrant further (long-term) evaluation of ALEM in these patients, maybe even comparison to B cell-depleting therapy with the recently approved antibody ocrelizumab for further development of optimal treatment sequelae. Thorough balancing of risk factors and the potential benefit of either therapy has to be performed. Patients should be well informed prior to NTZ-cessation and availability of different treatment strategies and should determine their priorities in terms of drug safety and freedom from disease activity.

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Author contributions SP: study concept and design, acquisition of data, analysis and interpretation of data, writing of the manuscript. RS: analysis and interpretation of data, writing of the manuscript. FAS: acquisition of data, analysis and interpretation of data. RP: acquisition of data, critical revision of manuscript for intellectual content. CK: acquisition of data, critical revision of manuscript for intellectual content. MW: acquisition of data. DL: acquisition of data, critical revision of manuscript for intellectual content. RL: acquisition of data, critical revision of manuscript for intellectual content. SD: acquisition of data, critical revision of manuscript for intellectual content. VS: acquisition of data, critical revision of manuscript for intellectual content. SW: acquisition of data, critical revision of manuscript for intellectual content. MP: acquisition of data, critical revision of manuscript for intellectual content. CA: acquisition of data, critical revision of manuscript for intellectual content. ML: acquisition of data, critical revision of manuscript for intellectual content. CE: acquisition of data, critical revision of manuscript for intellectual content. BT: acquisition of data, critical revision of manuscript for intellectual content. VL: acquisition of data, critical revision of manuscript for intellectual content. BW: acquisition of data, critical revision of manuscript for intellectual content. JH: acquisition of data, critical revision of manuscript for intellectual content. LK: acquisition of data, critical revision of manuscript for intellectual content. HW: critical revision of manuscript for intellectual content. TR: study concept and design, acquisition of data, analysis and interpretation of data, critical revision of manuscript for intellectual content. SGM: study concept and design, analysis and interpretation of data, critical revision of manuscript for intellectual content.

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Compliance with ethical standards

Conflicts of interest Steffen Pfeuffer: received travel reimbursements from Sanofi-Genzyme and Merck and honoraria for lecturing from Sanofi Genzyme, Biogen and Mylan. Rene Schmidt: declares no conflicts of interest. Frederike Anne Straeten: declares no conflicts of interest. Refik Pul: received travel reimbursements from Merck, research support by Novartis, speaker honoraria from Merck Serono, Biogen, Novartis, Sanofi-Genzyme, Mylan and Roche. Christoph Kleinschnitz: received travel reimbursements, honoraria for lecturing and research support from Ablynx, Amgen, Bayer Vital, Bristol-Mayers Squibb, Biotronik, Boehringer Ingelheim, Biogen, CSL Behring, Daiichi-Sankyo, Desitin, Eisai, Ever Pharma, Sanofi Genzyme, Merck Serono, Mylan, Medday, Novartis, Pfizer, Roche, Siemens, Stago and Teva. Marinus Wieshuber: declares no conflict of interest. De-Hyung Lee: received compensation for activities with Biogen, Merck, Novartis, Roche, and Sanofi-Genzyme. Ralf A. Linker: received compensation for activities with Biogen, Merck, Novartis, Roche, and Sanofi-Genzyme. Sebastian Doerck: reports no conflicts of interest. Vera Straeten: received travel reimbursements from Novartis, Merck and Biogen and honoraria for lecturing from Sanofi Genzyme, Biogen and Novartis. Susanne Windhagen: declares no conflict of interest. Marc Pawlitzki: received honoraria for lecturing and travel reimbursements from Biogen, Sanofi Genzyme, Merck Serono, Roche and Novartis. Christoph Aufenberg: received travel reimbursements from Bayer. Michael Lang: received travel grants, honoraria for lecturing, financial research sup-

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Ethical standards The local institutional review board (IRB) has approved the conduction of this trial. Further details are listed in the "Methods" chapter.

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4.5 Pretreatment anti-thyroid autoantibodies indicate increased risk for thyroid autoimmunity secondary to alemtuzumab: A prospective cohort study

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Pretreatment anti-thyroid autoantibodies indicate increased risk for thyroid autoimmunity secondary to alemtuzumab: A prospective cohort study

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ABSTRACT

Background: Alemtuzumab is approved for the treatment of active relapsing-remitting multiple sclerosis (RRMS). Alemtuzumab-related secondary autoimmune disorders (sAID) are common, with thyroid sAID being the most frequent, and fundamentally affect the risk-benefit ratio. Therefore, biomarkers indicating the development of sAID are urgently needed to instruct clinical decisions.
Methods: We evaluated whether the anti-thyroid autoantibodies (ThyAb) anti-thyroglobulin (anti-TG) and anti-thyroid-peroxidase (anti-TPO) detected at baseline by standard testing are able to indicate increased risk for thyroid sAID following alemtuzumab treatment in a multicentre prospective cohort of 106 alemtuzumab-treated RRMS patients. We here present an interim-analysis with a median follow-up of 36 months.
Findings: Baseline characteristics demonstrated no significant differences between patients with or without thyroid sAID. 29/106 (27·4%) patients developed thyroid sAID between 5 and 51 months following alemtuzumab treatment initiation. 14/29 patients (48·3%) were positive for ThyAb at baseline and developed thyroid sAID. Hazard ratio for time to thyroid autoimmunity was 12.15 (95% CI 4.73–31.2) indicating a highly increased risk for ThyAb positive patients. Baseline ThyAb were associated with shorter time to sAID, but not with a specific disease entity of thyroid sAID. Hazard ratios for age, sex, previous treatment, disease duration, disability and smoking status demonstrated no significant association with thyroid autoimmunity.
Interpretation: Standard ThyAb-testing for anti-TPO and anti-TG antibodies at baseline was able to indicate increased risk for clinically manifest thyroid sAID and should therefore be used in clinical decisions concerning alemtuzumab treatment initiation.
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1. Introduction

Alemtuzumab is used for the treatment of active relapsing-remitting multiple sclerosis (RRMS) and depletes B- and T-lymphocytes followed by a prolonged repopulation phase with profound quantitative and qualitative changes within immunological networks. Secondary autoimmune disorders (sAID) following alemtuzumab treatment are common and fundamentally affect the risk-benefit ratio and clinical decisions [1]. Hence, there is an urgent need for biomarkers instructing risk-estimation for sAID. Thyroid sAID are the most common and affects approximately 38% to 41% of alemtuzumab-treated patients peaking in year 3 after therapy initiation [2–4].

The most frequent alemtuzumab-associated sAID are mainly B-cell- and autoantibody-driven [1]. Therefore, it is currently believed that the disproportionately high B-cell over T-cell recovery leads to unregulated expansion of autoreactive B cells and consequently to sAID. However, alopecia, haemophagocytic syndrome, sarcoidosis and vitiligo after alemtuzumab infusion, which are all T cell-mediated, have been reported and hint to a more complex pathogenesis [5–8]. Previously, serum IL-21 concentrations have been shown to correlate with the incidence of sAID after alemtuzumab and were proposed as potential biomarker [9]. However, the findings have not been substantiated in

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Research in context

Evidence before this study

Secondary autoimmune disorders (sAID) are the most relevant risk related to alemtuzumab treatment in RRMS patients and thyroid sAID are by far the most common. However, biomarkers indicating increased risk for sAID in clinical routine are currently missing. We searched PUBMED, SCOPUS and COCHRANE databases from inception until May 21st 2019. In addition, ECTRIMS and ACTRIMS conference abstracts as well as reference lists of published articles were screened for further articles to be included. We combined search terms for multiple sclerosis, alemtuzumab, secondary autoimmune disorders, thyroid autoantibodies and thyroid autoimmunity. There were no language restrictions.
IL-21 serum levels were proposed as a biomarker for the risk of developing autoimmunity post alemtuzumab, however currently available detection kits show no predictive utility. A retrospective case-control-study demonstrated that anti-thyroid autoantibodies (ThyAb) precede the development of clinical overt thyroid autoimmune disorders in putative healthy controls. Two retrospective analyses of the CAMMS223 and a Welsh cohort support the indicative value of ThyAb for alemtuzumab-related thyroid sAID. However, data from prospective studies are missing.

Added value of this study

To our knowledge, this is the first study providing evidence for ThyAb detected by standard-testing as valuable tool for risk-estimation prior to alemtuzumab therapy from a prospective, multicentre observational study cohort. We found that anti-thyroglobulin (anti-TG) and anti–thyroid-peroxidase (anti-TPO) autoantibodies yielded good to strong performance for risk-estimation. ThyAb positivity was associated with a highly increased risk for and a shorter time to thyroid sAID.

Implications of all the available evidence

Thyroid sAID following alemtuzumab is often delayed by several years and the ability to reliably estimate the risk of individual patients would facilitate risk-benefit considerations in clinical decisions and allow for targeted monitoring as well as possibly early intervention in alemtuzumab-treated patients. In female patients with active family planning this might be of special relevance since thyroid disorders display a particular risk. Since the ThyAb testing applied in this study is readily available at most clinics in the world, this study can immediately instruct and improve clinical practice. However, positive ThyAb at baseline should not preclude alemtuzumab treatment per se, but instruct the informed consent building process before treatment initiation.

larger prospective cohorts and currently available IL-21 ELISA kits were not able to predict sAID after alemtuzumab [10]. A recent small retrospective study using custom-made high-sensitivity anti-TSHR (anti-thyroidea-stimulating-hormone-receptor) testing demonstrated that baseline measurement of anti-TSHR and anti–thyroid-peroxidase (anti-TPO) autoantibodies indicated increased risk for thyroid sAID following alemtuzumab treatment [11]. However, data from prospective studies evaluating the prognostic value of anti-thyroid autoantibodies (ThyAb) for alemtuzumab-related thyroid sAID have been missing so far.

We therefore evaluated whether baseline ThyAb including anti-thyroglobulin (anti-TG) and anti-TPO by standard tests are able to instruct risk-assessment for thyroid sAID in a prospective cohort of 106 alemtuzumab treated patients.

2. Materials and methods

2.1. Study design and participants

All patients were recruited at the Departments of Neurology at the University Hospital Münster (59 patients), University Hospital Essen (28 patients) and the Clinics Osnabrück (19 patients) in Germany. Patients were recruited starting from 1st April 2014 to 1st October 2018. All patients who received alemtuzumab were approached to participate in the study and 106 of 128 (83%) patients agreed to participate. Alemtuzmab patients received pre-treatments including azathioprine, beta interferons, glatiramer acetate, teriflunomide, fingolimod, natalizumab, mitoxantron, and siponimod (within a clinical trial). All patients were included in our prospective PROGRAM^{MS} ("Signatures of immune reprogramming in anti-CD52 therapy of MS: markers for risk stratification and treatment response") cohort study. Here, detailed clinical and MRI data are collected in combination with blood samples following established SOPs developed within the KKNMS (German competence network for multiple sclerosis). Patients are evaluated at baseline (before first course of alemtuzumab) and every 3 months thereafter including standardized neurological examination, assessments (e.g. EDSS, MSFC) and additional laboratory testing for a study duration of five years. In the current study anti-TG and anti-TPO are measured at baseline and thyroid sAID is monitored prospectively by clinical and laboratory assessments.

Alemtuzumab treatment and monitoring (creatinine, blood count and urine testing monthly and TSH every 3 months) was conducted according to summary of product characteristics (SmPC).

In case of clinical or laboratory signs indicating thyroid sAID patients were referred to a specialized endocrinologist. Diagnosis of Graves' disease was defined by anti-TSHR positivity and hyperthyroidism/hypothyroidism; autoimmune thyroiditis was defined by anti-TPO positivity and hypothyroidism (including cases with transient hyperthyroidism).

None of our patients showed (clinical or laboratory) signs of thyroid dysfunction before alemtuzumab initiation, and all were negative for anti-TSHR.

This study was performed according to the Declaration of Helsinki and approved by the local ethics committee of the University of Münster (Ethik-Kommission der Ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität Münster, 2014-398-f-S). All patients gave written informed consent. The manuscript conforms to the STROBE reporting standards for cohort studies.

2.2. Detection of anti-thyroid autoantibodies

ThyAb were detected by ECLIA (ElectroChemiLumineszenzImmunAssay) on an automated Cobas® e801 analyzer (Roche) used in clinical routine in a blinded manner and in an independent certified laboratory. Analysis was performed using specific kits and following the manufacturer's instructions for anti-TG (Elecsys Anti-Tg, Roche; cut off >15 IU/ml), anti-TPO (Elecsys Anti-TPO, Roche; cut off 15 IU/ml) and anti-TSHR (Elecsys Anti-TSHR, Roche; cut off >1·1 IU/ml) testing.

2.3. Statistical analysis

Statistical analysis was performed using R 3.6.1. To determine development of thyroid sAID over time, we performed Cox proportional hazards model with the R packages survival and survminer [12]. First, we used a univariate Cox regression to evaluate the hazards over time for each thyroid antibody separately and combined. Next, we used a multivariate cox regression to adjust for possible confounders including sex, age, disease duration, number of previous DMT, type of last DMT,

baseline disability (EDSS) and smoking history. Statistical significance of each variable was evaluated by the Wald statistic value and the predictive value by Harrell's c statistic (concordance index). The global *p*-value was determined by the likelihood ratio test. Statistical significance between survival curves was tested by pairwise log rank test adjusted for multiple testing by Benjamini-Hochberg's false discovery rate correction. A *p*-value below 0·05 was considered statistically significant.

3. Results

We here present data of a safety-concern triggered interim analysis with a median follow-up of 36 months. Baseline characteristics demonstrated no significant differences between patients with or without thyroid sAID (Table 1). 46/75 (61·3%) of patients without sAID and 21/29 (72·4%) with thyroid sAID were female. Patients in the no sAID versus the thyroid sAID group had a median age of 35 versus 33 years at alemtuzumab initiation, a median disease duration of 5 versus 6 years and both had a median of 2 previous disease-modifying therapies (DMTs). No DMT was found at significantly higher frequencies in those groups. The proportions of patients with higher disability (EDSS step ≥3) and history of smoking were comparable.

31/106 (29·2%) patients developed sAID between 5 and 51 months following treatment initiation. While 29 patients presented with thyroid sAID, two patients developed non-thyroid sAID (vitiligo and immune-thrombocytopenia, respectively) and were therefore excluded from the analysis.

14/29 patients (48·3%) with thyroid sAID were positive for ThyAb at baseline (4× anti-TG, 3× anti-TPO, and 7× anti-TG + anti-TPO), whereas only 4/75 patients (5·3%) without sAID were ThyAb positive (Table 1) so far. Autoantibody titres seemed to be higher in the thyroid sAID group however not reaching statistical significance. A multivariate Cox regression for time to thyroid sAID showed no significant coefficients for sex, age, disability, number and type of previous DMT, disease duration and smoking history (Fig. 1a–c) suggesting negligible impact on thyroid sAID development. In contrast, anti-TG and anti-TPO or both combined had statistically significant coefficients in the multivariate Cox regression. We evaluated each antibody separately and then combined. Performance of anti-TPO (hazard ratio 12.52, 95% CI 5.15–30.4) suggested slightly superior performance to anti-TG (hazard ratio 7.23, 95% CI 2.88–18.1) without reaching statistical significance (Fig. 1a–c). The combination of anti-TPO and anti-TG (hazard ratio 12.15, 95% CI 4.73–31.2) yielded almost similar performance as anti-TPO only. The predictive ability of the multivariate Cox regression model with anti-TG (concordance index 0.71), was slightly lower than anti-TPO (concordance index 0.74) and the combination of both (concordance index 0.76), however indicating overall good to strong models. Comparing the survival plots of each antibody set showed a statistical significance between anti-TG and/or anti-TPO presence and the absence of both, but no statistical significance between all other possible comparisons (Fig. 1d).

ThyAb positivity was not associated with a specific disease entity of thyroid sAID, 14 patients developed autoimmune thyroiditis and 15 Graves' disease. 6/14 (42·9%) with baseline ThyAb positivity versus 4/15 (26·7%) without needed radioiodine therapy and/or thyroidectomy for successful treatment, however not reaching statistical significance. Remarkably, baseline ThyAbs were associated with shorter time to sAID (Table 2).

Table 1
Patient baseline characteristics.

	No sAID (n = 75)	*Thyroid sAID (n = 29)*	*p*
Female patients, No (%)	*46 (61.3)*	*21 (72.4)*	*0.36*[b]
Age at baseline, years, median (IQR)	*35 (29–43)*	*33 (27.5–39)*	*0.52*[a]
Disease duration, years, median (IQR)	*5 (2−10)*	*6 (4–11)*	*0.1*[a]
Previous disease-modifying therapies, No, median (IQR)	*2 (1–3)*	*2 (2–4)*	*0.15*[a]
EDSS step ≥ 3 at baseline, No (%)	*36 (48)*	*15 (51.7)*	*0.83*[b]
Months of follow-up, median (IQR)	*35 (25–44)*	*37 (32.5–52)*	*0.06*[a]
History of smoking, No (%)	*18 (24)*	*8 (27.5)*	*0.8*[b]
Last previous DMT, No (%)			*0.28*[b]
Naïve	*13 (17.3)*	*2 (6.9)*	
Natalizumab (escalation)	*29 (38.7)*	*6 (20.7)*	
Fingolimod (escalation)	*8 (10.7)*	*11 (37.9)*	
Dimethyl fumarate (basic)	*9 (12)*	*2 (6.9)*	
Interferon-beta (basic)	*8 (10.7)*	*4 (13.8)*	
Glatiramer acetate (basic)	*2 (2.7)*	*3 (10.3)*	
Teriflunomide (basic)	*3 (4)*	*0 (0)*	
Other	*3 (4)*	*1 (3.4)*	
ThyAb serostatus at baseline, No (%)			*<0.001*[b]
TPO−; TG−	*71 (94.7)*	*15 (51.7)*	
TPO+; TG−	*1 (1.3)*	*3 (10.3)*	
TPO−; TG+	*3 (4.0)*	*4 (13.8)*	
TPO+; TG+	*0 (0.0)*	*7 (24.1)*	
Titer level in ThyAb + patients, IU/mL, median (IQR)			
Anti-TPO	*61.1*	*75.3 (60–1064)*	*n/a*
Anti-TG	*47.8 (37.1–65.2)*	*84.1 (72.1–139)*	*0.09*

No: number; IQR: interquartile range; EDSS: expanded disability status scale; DMT: disease-modifying treatment.
[a] Significance evaluated using Mann-Whitney test.
[b] Significance evaluated using Fisher's exact test.

4. Discussion

sAID are common and the most relevant risk associated with alemtuzumab treatment in RRMS patients [1]. Therefore, biomarkers instructing risk-assessment for sAID are a big unmet need in clinical practice for alemtuzumab-related treatment decisions. This safety-triggered interim analysis of a multicentre, prospective cohort of alemtuzumab treated RRMS patients provides evidence that standard ThyAb-testing of anti-TPO and anti-TG antibodies at baseline is able to indicate increased risk for clinically manifest thyroid sAID. ThyAb positivity was not associated with a specific disease entity of thyroid sAID, but with shorter time to sAID.

Consistently, thyroid autoantibodies have been associated with hypothyroidism and autoimmune thyroid disease in individuals with a positive family history for these disorders [13,14]. Moreover, a previous retrospective case-control study of 522 female US military personnel described that anti-TG, anti-TPO, and anti-TSHR precede the development of spontaneous thyroid autoimmunity and reported odds ratios between 4·6 and 25 two to five years prior diagnosis [15]. However, these studies were small, retrospective in design or in case of the study on female US military personnel comprised a highly selected population. Therefore, no sufficient data exist allowing for a direct comparison of risk associations between our data and the general population. However, a large Canadian cohort comparing 4192 MS patients with 20,940 healthy controls has shown comparable incidence (422·8 vs. 407·7 per 100,000 persons/year) and prevalence (9·51% vs. 8·56%) of autoimmune thyroid disease [16]. Of note, the prevalence of thyroid autoimmunity in alemtuzumab-treated MS patients accounts for 38–41% as stated above [3]. Therefore, alemtuzumab seems to be related to a highly increased risk for thyroid autoimmunity with fundamental pathophysiological differences compared to the natural history.

Concerning alemtuzumab, two retrospective analyses evaluated the risk-indicative value of ThyAb prior to alemtuzumab treatment for the development of thyroid sAID. The analysis of the phase II CAMMS223 patient cohort (median follow-up of 57·3 months) demonstrated anti-TPO positivity in patients with thyroid sAID at baseline, in contrast to our cohort, only in 11/73 patients (15%) [17]. However, in this study only anti-TPO were measured and patients included were treatment-naïve with disease duration lower than 36 months, potentially

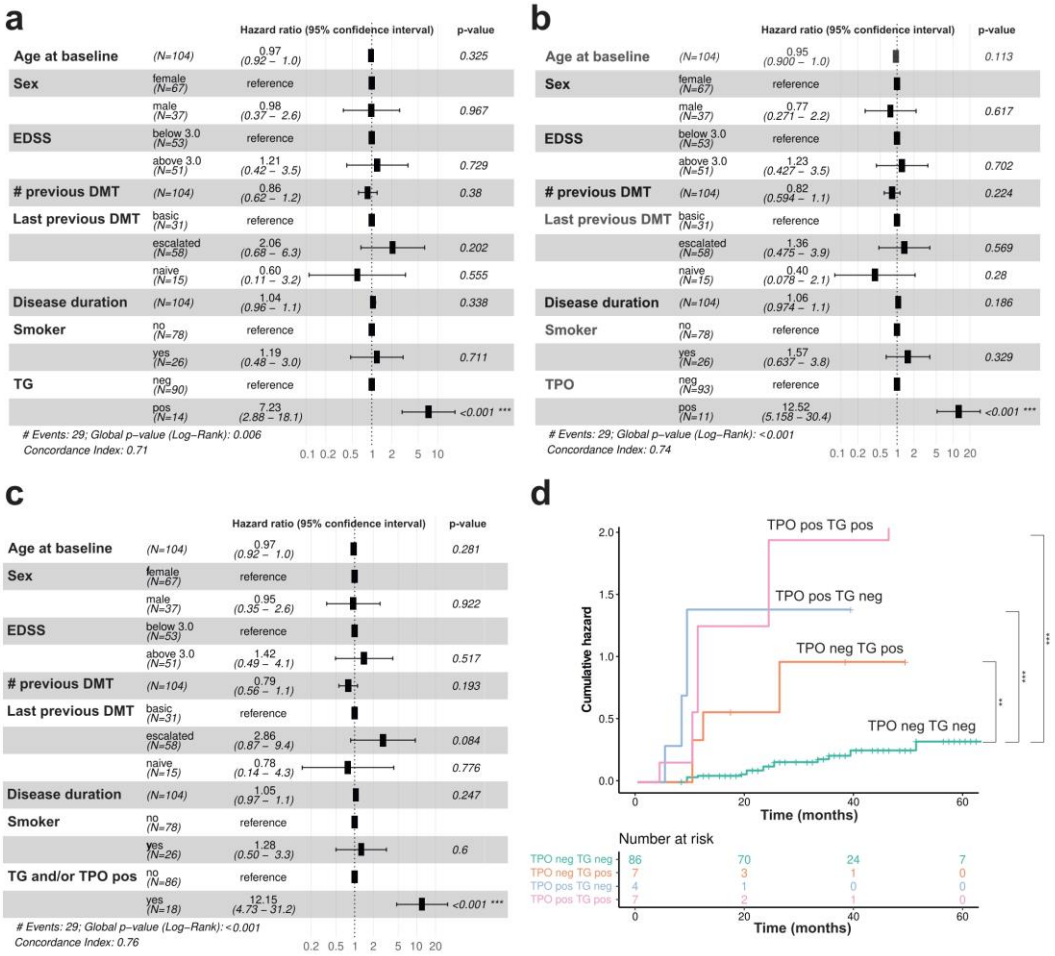

Fig. 1. Thyroid autoantibodies at baseline indicate increased risk for thyroid secondary autoimmunity following alemtuzumab treatment. Multivariate Cox proportional hazards model: forests plots of hazard ratios for time to thyroid sAID for age, sex, disability, number and type of previous disease modifying therapies (DMT), disease duration, smoking history and either (a) anti-TG (TG), (b) anti-TPO (TPO) or (c) both combined. (d) Survival plot displaying the cumulative hazard and patients at risk for time to thyroid sAID for each set of antibody. * $p < .05$, ** $p < .01$, *** $p < .001$. EDSS: expanded disability status scale.

Table 2
Thyroid sAID patient characteristics stratified according to baseline antibody status.

	ThyAb^{+}	*ThyAb*$^{-}$	*p*
Disease entity			*0.715*[b]
Autoimmune thyroiditis	*6*	*8*	
Graves' disease	*8*	*7*	
Treatment required			*0.304*[b]
Levothyroxine	*6*	*8*	
Levothyroxine + TPOI	*2*	*3*	
Levothyroxine + TPOI + RIT	*2*	*1*	
Levothyroxine + TPOI + surgery	*4*	*3*	
Onset from baseline, months (IQR)	*10 (8.75–15)*	*23 (11—33)*	*0.041*[a]

TPOI: thyroperoxidase inhibitor; RIT: radioiodine therapy.
[a] Significance evaluated using Mann-Whitney test.
[b] Significance evaluated using Fisher's exact test.

explaining the observed differences. In our cohort only 14% of patients were treatment naïve before alemtuzumab treatment, which might be a better representation of the current alemtuzumab prescription strategies in real-life [18].

In another smaller, retrospective cohort study using custom-made high-sensitivity anti-TSHR testing at baseline, anti-TSHR were present in 5/16 (31%) patients with thyroid sAID and in 0/14 (0%) without, supporting the indicative value of ThyAb at baseline [11]. In contrast, we used a standard electrochemiluminescence assay common in clinical routine. Moreover, anti-TSHR positive patients were excluded from the analysis since anti-TSHR are causatively involved in the pathogenesis of Graves' disease therefore precluding inclusion in our study [14]. However, implementation of high-sensitivity anti-TSHR testing might further improve the value of baseline ThyAb for risk-estimation.

Remarkably, a higher proportion of patients needing radioiodine therapy and/or thyroidectomy for successful treatment were ThyAb

positive at baseline potentially indicating a more severe disease course. However, differences were not statistical significant and need to be substantiated in larger studies or in the final analysis of our cohort.

Of note, thyroid disease represents particular risks in women who are pregnant and therefore prediction of thyroid sAID might especially instruct clinical decisions concerning alemtuzumab treatment in women with active family planning [19].

Strengths of our study are the prospective design and the use of standard ThyAb testing. Of note, the interim analysis, the relatively small cohort with only 29 positive thyroid sAID cases (as per the exploratory nature of the study) and potential centre effects might be limitations that should be considered for data interpretation. These limitations might also affect generalisability of our data, however real-life and trial cohorts of alemtuzumab-treated RRMS patients share many characteristics of our cohort [4,18]. Since autoimmune thyroid disorders have been diagnosed >7 years following the first alemtuzumab course our analysis might have missed further thyroid sAID cases becoming clinically manifest in the future [20].

Model performance of anti-TPO suggested slightly superior performance compared to anti-TG and the combination of anti-TPO and anti-TG yielded almost similar performance as anti-TPO only. However, since 4/29 (13·8%) patients developing thyroid autoimmunity were only anti-TG positive, we suggest a pragmatic approach testing both autoantibodies in clinical practice.

In conclusion, standard ThyAb-testing of anti-TPO and anti-TG at baseline was able to indicate increased risk for clinically manifest thyroid sAID and is therefore a valuable tool to facilitate risk-benefit considerations before alemtuzumab treatment initiation.

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Author contributions

TR: design and concept of study, analysis and interpretation of data, writing of the manuscript. ASM: analysis, and interpretation of data, writing of the manuscript. SP: analysis and interpretation of data, critical revision of manuscript for important intellectual content. MH: critical revision of manuscript for intellectual important content and further statistical analysis of data. LK: critical revision of manuscript for intellectual important content. SW + CK patient recruitment, critical revision of manuscript for important intellectual content. CCG: study concept, interpretation of data, and critical revision of manuscript for intellectual content. HW + SGM: study concept and design, study supervision and critical revision of manuscript for intellectual content. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

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4.6 Vitiligo after alemtuzumab treatment: Secondary autoimmunity is not all about B cells

ARTICLE OPEN ACCESS

Vitiligo after alemtuzumab treatment

Secondary autoimmunity is not all about B cells

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Abstract

Objective

To report 3 patients with relapsing-remitting multiple sclerosis (RRMS) showing vitiligo after treatment with alemtuzumab.

Methods

Retrospective case series including flow cytometric analyses and T-cell receptor (TCR) sequencing of peripheral blood mononuclear cells.

Results

We describe 3 cases of alemtuzumab-treated patients with RRMS developing vitiligo 52, 18, and 14 months after alemtuzumab initiation. Histopathology shows loss of epidermal pigmentation with absence of melanocytes and interface dermatitis with $CD8^+$ T-cell infiltration. Also compatible with pathophysiologic concepts of vitiligo, peripheral blood mononuclear cells of one patient showed high proportions of $CD8^+$ T cells with an activated (human leukocyte antigen–DR^+), memory ($CD45RO^+$), and type 1 cytokine (interferon-γ + tumor necrosis factor–α) phenotype at vitiligo onset compared to a control cohort of alemtuzumab-treated patients with RRMS (n = 30). Of note, analysis of CD8 TCR repertoire in this patient revealed a highly increased clonality and reduced repertoire diversity compared to healthy controls and treatment-naive patients with RRMS. We observed a predominance of single clones at baseline in this patient and alemtuzumab treatment did not substantially affect the proportions of most abundant clones over time.

Conclusion

The 3 cases represent a detailed description of vitiligo as a T-cell-mediated secondary autoimmune disease following alemtuzumab treatment. The prevailing concept of unleashed B-cell responses might therefore not cover all facets of alemtuzumab-related secondary autoimmunity. Mechanistic studies, especially on TCR repertoire, might help clarify the underlying mechanisms.

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Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article. The Article Processing Charge was funded by the University of Münster.

Glossary

EDSS = Expanded Disability Status Scale; **HLA** = human leukocyte antigen; **IFN-γ** = interferon-γ; **IL** = interleukin; **PBMC** = peripheral blood mononuclear cell; **RRMS** = relapsing-remitting multiple sclerosis; **TCR** = T-cell receptor; **TNF-α** = tumor necrosis factor–α.

Alemtuzumab is an anti-CD52 antibody leading to rapid depletion followed by differential repopulation of B and T lymphocytes approved for the treatment of active relapsing-remitting multiple sclerosis (RRMS). Secondary autoimmunity following alemtuzumab treatment represents the most relevant risk. Seven-year data from the Cambridge cohort demonstrated 41.0% of patients develop autoimmune thyroid disorders and 3.5% immune thrombocytopenia (ITP); moreover, cases of nephropathies and other autoimmune disorders have been described.[1,2] Here, we present a retrospective case series of 3 patients developing vitiligo after alemtuzumab treatment.

Methods

Patients and biomaterials

Patients were recruited at the Department of Neurology of the University Hospitals Münster and Essen, Germany. Thirty patients with RRMS prior to and under alemtuzumab (Lemtrada®, Genzyme) treatment (mean number of relapses was 2.2 ± 1.1 and mean Expanded Disability Status Scale [EDSS] progression was 1.2 ± 1.1 2 years prior to alemtuzumab initiation), 11 sex- and age-matched, treatment-naive patients with RRMS (mean number of relapses was 1.8 ± 0.7 and mean EDSS progression was 1.1 ± 0.7 in the last 2 years), and 10 sex- and age-matched healthy controls were included in the current study. Alemtuzumab patients received pretreatments including azathioprine, β-interferons (IFNs), glatiramer acetate, teriflunomide, fingolimod, natalizumab, mitoxantrone, and siponimod (within a clinical trial). Peripheral blood mononuclear cells (PBMCs) were isolated from ethylenediaminetetraacetic acid blood drawn from alemtuzumab-treated patients at baseline, 6, 12, and 18 months after standard treatment regimen and cryopreserved as previously described.[3]

Standard protocol approvals, registrations, and patient consents

This study was performed according to the Declaration of Helsinki and approved by the local ethics committees (Münster: 2014-398-f-S, Essen: 16-7290-BO). All patients gave written informed consent.

Flow cytometry

Flow cytometry of thawed PBMCs was performed as previously described[3] using fluorochrome-conjugated antibodies for CD3, CD4, CD8, CD14, CD19, CD45RO, CD56, human leukocyte antigen (HLA)–DR, IFN-γ, tumor necrosis factor–α (TNF-α), and perforin (all purchased from BioLegend [San Diego, CA]). Intracellular staining for cytokines (IFN-γ and TNF-α) and perforin was performed using the intracellular staining kit (eBioscience [San Diego, CA]) following the manufacturer's instructions. Samples were acquired on a 10-color Navios (Beckman Coulter [Sharon Hill, PA]) or FACSCanto II (BD Biosciences [East Rutherford, NJ]) flow cytometer and analyzed by FlowJo v10 and Kaluza 1.3.

T-cell receptor sequencing

T-cell receptor (TCR) sequencing and analysis was performed as previously described.[4] TCRβ chain sequencing of magnetic-activated cell sorted $CD8^+$ T cells ($CD8^+$ T Cell Isolation Kit, human; Miltenyi Biotec [Bergisch Gladbach, Germany]) was performed at Adaptive Biotechnologies (Seattle, WA) using the ImmunoSEQ platform with primers specific for all 54 known expressed Vβ and all 13 Jβ regions.

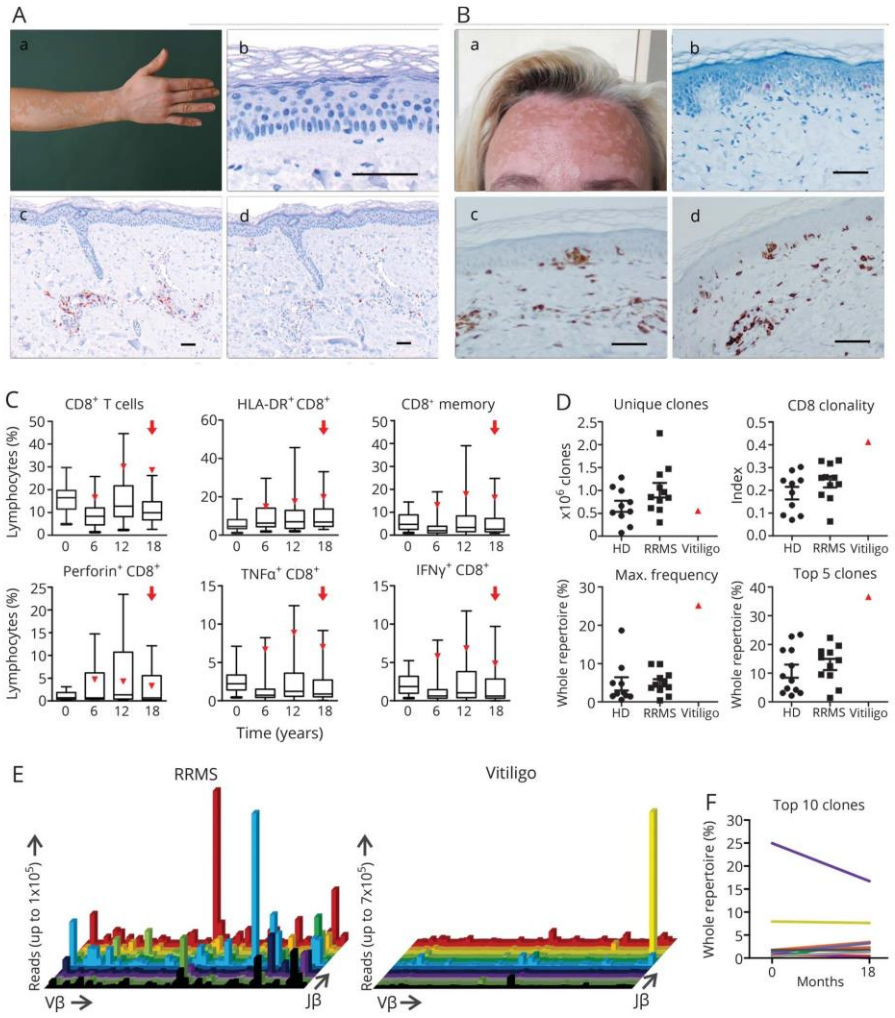
Data availability statement

Any data not published within the article will be shared anonymized upon request from any qualified investigator.

Results

In September 2016, a 31-year-old woman presented with depigmentation of her skin following therapy with alemtuzumab. She had been diagnosed with RRMS in 2004 with typical findings on brain MRI and positive oligoclonal bands in the CSF. Despite receiving different immunomodulatory treatments including IFN-β-1a and natalizumab, she demonstrated ongoing disease activity on MRI and several relapses with an EDSS progression to 3.5. In terms of compassionate use, a first course of alemtuzumab was given in June 2012. In March 2013, the patient presented with a worsening of preexisting right-sided sensorimotor hemiparesis responsive to IV methylprednisolone. Since alemtuzumab was retained from the market at this time, she received 2 courses of rituximab 1,000 mg in August 2013 and April 2014. However, she experienced 3 further relapses within 1 year and was therefore switched back to alemtuzumab, receiving the second course in October 2015. Since then, clinical and MRI disease course remained stable. Skin depigmentation started in September 2016 with circumscribed, symmetrical white macules and patches typical for vitiligo (figure, A), which slowly progressed, now involving 30% of the body surface. Skin biopsy confirmed diagnosis showing complete loss of epidermal pigmentation with absence of melanocytes at the basal layer and a subtle perivascular dermatitis consisting of $CD3^+$ and $CD8^+$ T cells

Figure CD8+ T-cell-driven pathology in alemtuzumab-treated patients with relapsing-remitting multiple sclerosis (RRMS) developing vitiligo



(A) Patient 1: circumscribed, symmetrical white macules and patches typical for vitiligo (A.a). Exemplary skin biopsy from the same patient: melanocyte staining (A.b), panMel antibody cocktail directed to tyrosine, melanA, HMB45. Positive stainings for CD3 (A.c) and CD8 (A.d). (B) Patient 3: typical skin lesions for vitiligo (B.a). Exemplary skin biopsy from the same patient: melanocyte staining (B.b). Positive stainings for CD3 (B.c) and CD8 (B.d). Scale bars represent 100 μM in A and B. (C) Proportions of $CD8^+$ T cells and $CD8^+$ T-cell subsets (defined by the displayed markers) within peripheral blood mononuclear cells (PBMC) isolated from alemtuzumab-treated patients with RRMS (n = 30 for baseline, n = 29 at 6 months, n = 25 for 12 months, n = 17 for 18 months) and patient 2 (red triangle) were determined by flow cytometry. For the analysis of cytokine production, cells were treated with leukocyte activation cocktail (BD) for 4 hours. The red arrow indicates the timepoint of vitiligo onset. Boxplots show median, 25% and 75% percentile, whiskers represent 5% and 95% percentile. (D) Unique clones, clonality, as well as maximum frequency of distinct clones and top 5 clones in the CD8 T-cell receptor (TCR) repertoire was analyzed by deep sequencing. Prior to analysis, $CD8^+$ T cells were magnetic-activated cell sorted from PBMC of healthy controls (ctrl, n = 10), treatment-naive patients with RRMS (MS naive, n = 11), and patient 2 at baseline (vitiligo). RNA of at least 2×10^6 $CD8^+$ T cells from patient 2 at baseline was purified and cDNA was sequenced by Adaptive Biotechnologies, resulting in a range from 1.5 to 2.5×10^6 total reads in each analyzed sample. (E) 3D histograms show expansion of single clones in patient 2 in comparison to a representative treatment-naive patient with RRMS (i.e., sex- and age-matched and exhibiting a similar mean number of relapses and mean EDSS progression in the last 2 years as compared to patient 2). The x axis lists all Vβ genes, the z axis the Jβ genes, and the column height indicates the total reads of this specific V/J combination. (F) Clone tracking of the top 10 expanded clones (highest number of total reads) of patient 2 before and 18 months after alemtuzumab treatment. The proportion of clones in the whole TCR repertoire is depicted. HLA = human leukocyte antigen; IFN-γ = interferon-γ; TNF-α = tumor necrosis factor–α.

(figure, A), the latter expressing T-cell-restricted intracellular antigen-1, while $CD20^+$ B cells were almost absent (data not shown).

A second patient, a 34-year-old man, was referred to our clinic with skin lesions suspicious for vitiligo in February 2017 after having received courses of alemtuzumab in August 2015 and 2016. He was diagnosed with MS in 2001, fulfilling McDonald criteria with typical findings on MRI and in the CSF. Several immunomodulatory treatments including IFN-β-1a, natalizumab, fingolimod, and dimethyl fumarate were ineffective. Since alemtuzumab initiation the patient has demonstrated clinical and radiologic stable disease. However, in February 2017, he reported patchy, well-defined depigmentation of the skin progressively affecting the whole integument in September 2017. Dermatologic examination established the diagnosis of vitiligo due to characteristic clinical presentation and disease course; the patient refused a skin biopsy.

A third patient, a 42-year-old woman diagnosed with MS in August 2015, was treated with alemtuzumab in the University Hospital Essen in February 2017 and 2018 due to ongoing disease activity with dimethyl fumarate and fingolimod. Fourteen months after treatment initiation, she reported vitiligo-characteristic depigmentation of the skin starting from the extremities and then affecting the whole body including the face (figure, B). Skin biopsy confirmed the diagnosis, showing interface dermatitis with predominant $CD3^+CD8^+$ lymphocyte infiltration along the basal lamina and missing melanocytes in central parts of the biopsy specimen (figure, B).

The pathophysiologic mechanisms underlying autoimmunity secondary to alemtuzumab remain insufficiently understood. However, currently it is assumed that B cells are the central drivers of these autoimmune conditions due to predominance of antibody-mediated autoimmune disorders.[5,6] In contrast, vitiligo is a T-cell-driven autoimmune disorder, where autoreactive, melanocyte-specific $CD8^+$ T cells are recruited to the skin and target melanocytes.[7]

As patient 2 is part of a prospective biobanking cohort, we were able to analyze PBMCs for vitiligo-related immune patterns. Interestingly, he showed high proportions of $CD8^+$ T cells with an activated ($HLA\text{-}DR^+$), memory ($CD45RO^+$), and type 1 cytokine ($IFN\text{-}\gamma^+$ and $TNF\text{-}\alpha^+$) phenotype at vitiligo onset compared to a control cohort of alemtuzumab-treated patients with RRMS (figure, C). Analysis of CD8 TCR repertoire in patient 2 at baseline revealed a highly increased clonality and reduced repertoire diversity (figure, D) as compared to healthy controls and treatment-naive patients with RRMS. Furthermore, a predominance of single clones could be observed at baseline in this patient (figure, E), illustrated in a 3D histogram, which provides an overview of the clonal distribution within the TCR repertoire. Of note, alemtuzumab treatment did not substantially affect the proportions of the top 10 clones over time (figure, F).

Discussion

Previous mechanistic studies support a pivotal role of interleukin (IL)–21-driven homeostatic proliferation of chronically activated, oligoclonal, effector memory T cells in autoimmunity following alemtuzumab.[8] Of note, IL-21 has also been implicated in the pathogenesis of vitiligo, therefore it can be speculated that increased IL-21 levels might contribute to vitiligo development in these patients.[9] Shared HLA haplotypes between MS and vitiligo conferring increased disease risk such as HLA-DRB1 or HLA-DQB1 might further facilitate coincidence of both diseases in susceptible individuals.[7,10] Interestingly, all 3 patients expressed at least 2 vitiligo risk alleles (HLA-A*02, HLA-DRB1*03, HLA-DRB1*04, and HLA-DQB1*03) (table).

The 3 cases represent a detailed description of vitiligo as an autoimmune complication after alemtuzumab; however, they teach something more important: T cells also can be drivers of secondary autoimmunity after alemtuzumab. Hence, the prevailing concept of unleashed B-cell autoimmunity due to faster reconstitution kinetics might not cover all facets of secondary autoimmunity in this context. More mechanistic studies, especially on TCR repertoire, as well as comprehensive monitoring for various autoimmune conditions are warranted to improve our knowledge of autoimmunity in the context of alemtuzumab treatment in MS.

Author contributions

T. Ruck: design and concept of study, analysis and interpretation of data, writing of the manuscript. S. Pfeuffer: analysis and interpretation of data, critical revision of manuscript for important intellectual content. A. Schulte-Mecklenbeck: acquisition of data, critical revision of manuscript for intellectual important content. C.C. Gross: analysis and interpretation of data, critical revision of manuscript for intellectual important

Table Human leukocyte antigen (HLA) class I and II haplotypes of alemtuzumab-treated vitiligo patients

	Patient 1	Patient 2	Patient 3
HLA class I			
HLA-A[a]	**02**/68	01/11	11/24
HLA-B[a]	07/35	08/51	15/51
HLA-C[a]	04/07	03/07	03/03
HLA class II			
HLA-DRB1[a]	**03**/11	**03/04**	**04**/13
HLA-DQB1[a]	02/**03**	02/**03**	**03**/06

DNA was extracted from peripheral blood mononuclear cells and HLA genotyping was performed following standard procedures. HLA haplotypes for the indicated HLA class I and II genes are displayed for the different patients. Vitiligo risk alleles are in bold font.
[a] Risk haplotypes.

content. M. Lindner: acquisition, analysis, and interpretation of data, critical revision of manuscript for intellectual important content. J. Ehrchen: acquisition, analysis, and interpretation of data, critical revision of manuscript for intellectual important content. D. Metze, W. Sondermann: acquisition and analysis of data, critical revision of manuscript for intellectual important content. R. Pul, C. Kleinschnitz: acquisition and analysis of data, critical revision of manuscript for intellectual important content. H. Wiendl: critical revision of manuscript for intellectual important content. S.G. Meuth, L. Klotz: study concept and design, study supervision and critical revision of manuscript for intellectual content.

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4.7 Sarcoidosis following alemtuzumab treatment: Autoimmunity mediated by T cells and interferon-γ

Sarcoidosis following alemtuzumab treatment: Autoimmunity mediated by T cells and interferon-γ

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Among the therapeutic options for relapsing-remitting multiple sclerosis (RRMS), alemtuzumab certainly stands out as it can induce a long-lasting remission in the absence of further treatment in formerly active patients. It rapidly depletes most lymphocytes from the peripheral blood and each treatment course is followed by differential repopulation of the affected cell lines. The exact mechanisms controlling this so-called immune reconstitution are still only partially understood.[1]

However, the long-lasting effects of the drug and the profound changes in patients' disease courses towards a less active or even quiescent form of the disease in the absence of re-treatment led to the hypothesis that alemtuzumab treatment not only suppresses but also 'reprograms' the immune system.[2] Both ongoing or even increased disease activity and occurrence of secondary autoimmunity after treatment were hence interpreted as 'glitches' during reprogramming.

They were thought to be primarily mediated by dysregulated B cells for some reasons: First, B cells repopulate within few months whereas T cell numbers can remain depressed for several years.[3] Second, the three most abundant autoimmune events (thyroid dysfunction, thrombocytopenia and glomerulonephritis) involve development of autoantibodies. Finally, paradoxical disease exacerbation was supposed to be B cell-dependent because of the presence of ring-enhancing lesions and sufficient response to plasma exchange and rituximab treatment.[4]

Now, after more than 5 years of alemtuzumab administration in real-world cohorts, novel entities of autoimmune disorders, such as sarcoidosis, expand the spectrum of adverse events and subject our previous explanatory models to debate.

Willis et al. present three cases having developed sarcoidosis following alemtuzumab treatment.[5] These patients were healthy apart from RRMS and confidence regarding the diagnosis was high. Pre-existing autoimmunity was excluded. Fortunately, their clinical courses were mild in terms of sarcoidosis and required no treatment apart from corticosteroids in one case. Interestingly, alemtuzumab was mostly successful for RRMS treatment in these cases as only one patient required an additional course due to disease activity.

Graf et al. add another case which is slightly different as the presented patient suffered from Löfgren's syndrome, an acute form of sarcoidosis.[6] Multisystemic manifestation and symptom severity rendered intravenous corticosteroid treatment necessary. Of note, the patient displayed some features suggestive of B cell-influenced pathology such as ring-enhancing lesions and hyper-repopulated B cells after treatment. Nonetheless, RRMS itself remained well controlled clinically by alemtuzumab and no additional autoimmunity developed.

These cases contradict our view of organ-specific and primarily B cell-mediated autoimmunity after alemtuzumab. Sarcoidosis is mainly driven by type-1 T helper (Th1) cells and macrophages and cytokines such as interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α).[7]

Recent publications introduced further autoimmune events preferably involving other than B cells such as hemophagocytic syndrome, alopecia and vitiligo.[8–10] These diseases are all mediated by T cells (predominantly $CD4^+$ Th1 cells in hemophagocytic syndrome and $CD8^+$ type-1 cytotoxic T cells (Tc1) in vitiligo and alopecia). Their pathophysiology also involves cytokines such as IFN-γ and TNF-α.[11]

Previous studies indicated a substantial role for interleukin-21 (IL-21) in autoimmunity following alemtuzumab. Besides other effects, IL-21 positively influences maturation from B to plasma cells.[12] Now in the novel disorders depicted above, the involvement of IL-21 is less clear but in general appears to be minor.

Taken together, we have now hints towards a deregulation in the Th1/Tc1 cell/IFN-γ network inducing autoimmunity besides the established B cell/IL-21 network. However, the low incidence of reported adverse events attributable to the first network indicates a susceptibility to alemtuzumab-induced autoimmunity far lower than in the second one. What we still don't know is the extent to which

alemtuzumab treatment unmasks pre-existing, maybe genetically determined, preponderances to autoimmunity or to which treatment induces autoimmunity de novo.

Now what is important for the clinician? Generally, the use of alemtuzumab forces us to keep our eyes open for autoimmune disorders more than ever before. Notably, the mandatory routine blood-testing no longer covers the multitude of autoimmune adverse events entirely. Patients need to be aware of the potential risks of treatment and clinical and paraclinical follow-up must be performed thoroughly. In the context of these case reports, physicians have to keep in mind that TNF-α blockade, which can be a reasonable option in refractory sarcoidosis, can aggravate multiple sclerosis (MS).[13] Consequently, 'atypical' autoimmunity following alemtuzumab treatment requires multiprofessional treatment and intensive communication between physicians.

Declaration of Conflicting Interests

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4.8 Effect of Previous Disease-Modifying Therapy on Treatment Effectiveness for Patients Treated With Ocrelizumab

RESEARCH ARTICLE OPEN ACCESS

Effect of Previous Disease-Modifying Therapy on Treatment Effectiveness for Patients Treated With Ocrelizumab

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Abstract

Background and Objectives

B cell–depleting antibodies were proven as effective strategy for the treatment of relapsing multiple sclerosis (RMS). The monoclonal antibody ocrelizumab was approved in 2017 in the United States and in 2018 in the European Union, but despite proven efficacy in randomized, controlled clinical trials, its effectiveness in the real-world setting remains to be fully elucidated. In particular, most study patients were treatment naive or switched from injectable therapies, whereas oral substances or monoclonal antibodies made up >1% of previous treatments.

Methods

We evaluated ocrelizumab-treated patients with RMS enrolled in the prospective cohorts at the University Hospitals Duesseldorf and Essen, Germany. Epidemiologic data at baseline were compared, and Cox proportional hazard models were applied to evaluate outcomes.

Results

Two hundred eighty patients were included (median age: 37 years, 35% male patients). Compared with using ocrelizumab as a first-line treatment, its use as a third-line therapy increased hazard ratios (HRs) for relapse and disability progression, whereas differences between first- vs second-line and second- vs third-line remained smaller. We stratified patients according to their last previous disease-modifying treatment and here identified fingolimod (FTY) (45 patients, median age 40 years, 33% male patients) as a relevant risk factor for ongoing relapse activity despite 2nd-line (HR: 3.417 [1.007–11.600]) or 3rd-line (HR: 5.903 [2.489–13.999]) ocrelizumab treatment, disability worsening (2nd line: HR: 3.571 [1.013–12.589]; 3rd line: HR: 4.502 [1.728–11.729]), and occurrence of new/enlarging MRI lesions (2nd line: HR: 1.939 [0.604–6.228]; 3rd line: HR: 4.627 [1.982–10.802]). Effects were persistent throughout the whole follow-up. Neither peripheral B-cell repopulation nor immunoglobulin G levels were associated with rekindling disease activity.

Discussion

Our prospectively collected observational data suggest suboptimal effectiveness of ocrelizumab in patients switching from FTY compared with those switching from other substances or having been treatment naive. These findings support previous studies indicating abated effectiveness of immune cell–depleting therapies following FTY treatment in patients with RMS.

MORE ONLINE

Class of Evidence
Criteria for rating therapeutic and diagnostic studies
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Glossary

aHR = adjusted hazard ratio; **DMF** = dimethyl fumarate; **DMT** = disease-modifying therapy; **EID** = extension of the dosing interval; **FTY** = fingolimod; **GLAT** = glatiramer acetate; **IFN** = interferon; **IgG** = immunoglobulin G; **NEDA-3** = no evidence of disease activity 3; **NTZ** = natalizumab; **OCR** = ocrelizumab; **PML** = progressive multifocal leukoencephalopathy; **RMS** = relapsing multiple sclerosis; **SID** = standard interval of dosing; **SPMS** = secondary progressive MS.

Classification of Evidence

This study provides Class IV evidence that for patients with RMS, previous treatment with FTY compared with previous treatment with other immunomodulating therapies decreases the effectiveness of ocrelizumab.

Treatment of active forms of relapsing multiple sclerosis (RMS) remains challenging despite approval of numerous therapies over the past decade. Treatment options currently comprise oral substances such as cladribine or sphingosine-1-phosphate receptor blockers (e.g., fingolimod [FTY]) and monoclonal antibodies such as natalizumab (NTZ) or alemtuzumab.[1] B-cell depletion using anti-CD20 antibodies such as rituximab also has proved to be highly effective[2] yet remained an off-label treatment until approval of ocrelizumab (OCR) in 2018.[3]

These depletion therapies can be administered as first-line treatment, but their use more commonly follows the use of platform therapies in patients in whom sufficient disease control has not been achieved. However, evidence regarding the use of these treatments following previous therapy remains scarce. In the respective phase 3 clinical trials, B cell–depleting therapies were either tested against placebo or injectable substances such as beta-interferons (IFNs).[4]

During the OPERA trials, 827 patients were administered to ocrelizumab treatment. Of those, 605 were treatment naive (73%), and 220 were previously (≤2 years prior screening) treated with disease-modifying therapy (DMT). However, dimethyl fumarate (DMF), FTY, or NTZ was only administered in 7 patients (>1% of the study population), whereas injectable substances were used in 238 cases (the number of patients having received more than 1 injectable substance was not published in further detail).[5]

Nonetheless, both effectiveness and safety concerns can arise with the subsequent use of different immunotherapies. Regarding B-cell depletion, the use of rituximab appeared safe in patients stopping NTZ because of increased risk for progressive multifocal leukoencephalopathy (PML) and was more effective than FTY in those 256 patients.[6] Another study comprising 55 patients compared subsequent use of alemtuzumab and rituximab in patients stopping FTY and found comparable outcomes regarding safety and effectiveness.[7] However, we have previously shown that safety and effectiveness profiles of alemtuzumab treatment are compromised in patients switching from FTY compared with patients having been previously naive or been switched from other substances,[8] but for ocrelizumab, such analysis is absent. Hence, we conducted a large prospective multicenter analysis of ocrelizumab patients and evaluated effectiveness outcomes to assess whether the last previous DMT should become a variable that needs to be considered in treatment decisions, in light of the growing armory of available therapies and thereby patients having increasing numbers of pretreatments.

Methods

Patients

Between February 2018 and November 2020, adult patients with active RMS according to the 2017 revised McDonald criteria,[9] who were eligible for treatment with ocrelizumab according to national and international guidelines and to the summary of product characteristics, were enrolled in 2 connected, local prospective cohorts sharing a prespecified examination plan as described below.

Based on these prospective cohorts, patients were selected for this study depending on the following inclusion criteria: (1) disease duration since onset <10 years, (2) age at baseline <55 years, (3) previous treatment with either basic substances (here summarizing injectable treatments, teriflunomide, and DMF), FTY, or NTZ for at least 6 months, and (4) a minimal follow-up duration of 6 months following ocrelizumab baseline infusion. Patients were excluded according to the following exclusion criteria: (1) presence of any progressive form of multiple sclerosis at baseline (patients were reevaluated for the presence of SPMS according to Lorscheider criteria at baseline[10]) and (2) presence of further autoimmune disorders.

Outcome Measurements

Epidemiologic data were first recorded as part of the preparation of treatment and validated at the time of baseline infusion (relapses within past year from baseline were recorded retrospectively in patients not having been known the respective center previously). Baseline MRI was conducted no earlier than 6 weeks before treatment initiation.

The washout duration from the last previous DMT was defined as time from the last intake of medication to the first infusion of ocrelizumab. Ocrelizumab treatment was administered according to the most recent summary of product characteristics.

Patients undergoing an extension of the dosing interval (EID) of more than 4 weeks were censored until the last follow-up, representing a standard interval of dosing (SID). Similarly, patients having shortened their dosing interval for more than 4 weeks were also censored until completion of the last SID.

Patients were evaluated with standardized neurologic examinations on sites at least semiannually, including determination of the EDSS score. The occurrence of relapses, including their date of onset, their severity, and the relapse treatment given, was recorded. Follow-up MRI scans were evaluated regarding abundance of new and/or enlarging T2 hyperintense lesions (all MRI scans were reevaluated by the authors). For determination of new or enlarging T2 hyperintense lesions at baseline, baseline MRI was compared with a scan obtained 6 months earlier (±2 months).

Follow-up MRI was conducted no earlier than 4 weeks in advance of readministration of ocrelizumab. Worsening of disability was considered clinically relevant if 2 independent clinical assessments 6 months apart indicated an increase of the EDSS score as follows: +1.5 points (baseline = 0.0), +1.0 point (baseline = 1.0–4.0), and +0.5 points (baseline ≥4.5). To determine the progression to secondary progressive MS (SPMS) during follow-up, Lorscheider[10] criteria were applied (using a 6-month confirmation of disability worsening; applying only to patients with ≤12 months of follow-up). No evidence of disease activity 3 (NEDA-3) was defined as the absence of relapses, worsening disability, and new or enlarging T2 hyperintense MRI lesions. $CD19^+$ B-cell levels and immunoglobulin G (IgG) serum levels were measured immediately before each ocrelizumab infusion as a part of clinical routine.

Statistical Analysis

Epidemiologic parameters at baseline were assessed using descriptive statistics. Patients receiving basic treatments (beta-IFN formulations, glatiramer acetate [GLAT], or DMF) were merged into one group (referred to as the basic group). For analysis of effectiveness outcomes, including time to first relapse, time to first 6-month confirmed worsening of disability, time to abundance of new/enlarging T2 hyperintense MRI lesions, and time to loss of NEDA-3, we used the Kaplan-Meier method and Cox proportional hazards models.

Regression models included the following covariates with an enter method: sex, age, annualized relapse rate (ARR) at baseline, baseline EDSS score, disease duration since onset, and last previous DMT. Results are given as adjusted hazard ratios (aHRs) including the abovementioned covariates throughout the article.

Further analyses were performed using the Fisher exact test or the χ^2-test for categorical variables, where appropriate, and the Wilcoxon paired rank-sum test for continuous variables. A p value of less than 0.05 was considered statistically significant. Analyses were considered exploratory throughout and were performed using SPSS Statistics 29 (IBM, NY).

Standard Protocol Approvals, Registrations, and Patient Consents

Ethical approval was given by local authorities (Institutional Review Board of the Heinrich-Heine-University Dusseldorf, Germany [5951R], and Institutional Review Board of the University Duisburg-Essen [20-9510-BO]). All patients gave written consent for participation and data acquisition.

Data Availability

Data are available on reasonable request. Anonymized data will be shared on reasonable request from qualified investigators.

Data Access Statement

All authors were given unrestricted access to data published herein during manuscript preparation.

Results

Patients

In total, 358 patients were subjected to ocrelizumab treatment for RMS. Of those, 42 patients were excluded as the follow-up duration was less than 6 months. All non-naive patients had received their last previous DMT for at least 6 months. Twelve patients were excluded as they switched from alemtuzumab (4), daclizumab (6), or cladribine (2) and those subgroups appeared too small for reasonable evaluation. Two patients started ocrelizumab early following pregnancy and hence were also excluded from the analysis. Twenty-four patients underwent an EID within the first maintenance interval and were excluded.

The study population therefore consisted of 280 patients, representative of 486.5 patient-years. Forty-one patients underwent EID in their later treatment course. Thirty-two patients were lost to follow-up during the observation period.

Seventy-three patients were switched from NTZ, 45 previously received FTY, and 76 patients were treatment naive. Among 86 patients who previously received platform treatment, 43 were last treated with DMF, 23 with GLAT, and 20 with beta-IFNs. Most patients were subjected to ocrelizumab treatment due to ongoing disease activity, with the exception of previously NTZ-treated patients. Here, 71 of 73 patients were switched due to an increased risk of developing PML. The remaining 2 patients were switched due to ongoing disease activity, and this is reflected by a significantly lower baseline ARR in the NTZ group compared with other patient groups ($p > 0.001$ for NTZ vs other groups and $p = 1.000$ for all other comparisons).

Epidemiologic data at baseline were well balanced, and statistical significance was absent for all comparisons except for disease duration, which was of course relevantly shorter in previously naive patients, and washout duration (median washout duration [days]: NTZ: 58; FTY: 48; basic: 15),

Table 1 Baseline Data of Our Cohort

		Last previous DMT			
	Whole cohort	NTZ	FTY	Basic	Naive
Patients, no.	280	73	45	86	76
Age at baseline 1st OCR infusion, y, median (IQR)	37 (29–47)	37 (29–45)	40 (30–49)	37 (29–47)	34 (28–46)
Male patients, no. (%)	98 (35)	30 (41)	15 (33)	26 (31)	27 (36)
Baseline ARR, median (IQR)	1 (0–1)	0 (0–0)	1 (1–1)	1 (0–1)	1 (1–2)
Baseline EDSS score, median (IQR)	2 (1–3)	2.5 (2–3.5)	2.5 (2–3.5)	2 (1–3)	2 (1–3)
Disease duration since onset, mo, median (IQR)	43 (17–72)	70 (38–104)	55 (38–77)	43 (21–71)	8 (4–35)
Previous DMT					—
0 (no. [%])	76 (27)	0 (0)	0 (0)	0 (0)	
1 (no. [%])	68 (24)	12 (16)	10 (22)	46 (54)	
2 (no. [%])	57 (20)	25 (34)	13 (29)	19 (22)	
≥3 (no. [%])	79 (29)	36 (50)	22 (49)	21 (24)	
Washout duration of last previous DMT, (non-naive pat), d, median (IQR)	41 (19–60)	58 (42–82)	48 (40–66)	15 (7–26)	—
Follow-up duration, mo, median (IQR)	28 (20–35)	29 (22–35)	28 (21–35)	28 (21–34)	25 (18–33)

Abbreviations: ARR = annualized relapse rate; DMT = disease-modifying treatment; EDSS = Expanded Disability Status Scale; FTY = fingolimod; basic includes glatiramer acetate (23), beta-interferon (20), and dimethyl fumarate (43); IQR = interquartile range; NTZ = natalizumab; OCR = ocrelizumab.

which was determined by national and international recommendations based on the respective treatment sequence. An overview is given in Table 1. Of note, 15 patients experienced a clinical relapse during washout (DMF: 1 patient; FTY: 4 patients; NTZ: 10 patients).

Effectiveness Outcomes

During follow-up, we documented 73 relapses in our cohort compared with 271 relapses during the year before ocrelizumab induction. Regarding the relapses on ocrelizumab treatment, 19 took place within first 6 months. Annualized relapse rates per treatment epoch declined rapidly following ocrelizumab initiation from 0.97 to 0.07 and generally remained low. In line with this, median EDSS scores were mostly stable within follow-up (Figure 1, A and C).

Relapse-associated worsening was observed after 41 relapses (in 34 patients [12%]), whereas 32 relapses recovered without persistent worsening of disability. Because 15 cases of confirmed progression of disability independent of relapse activity were noted (5%), we documented disability worsening in 49 (17%) patients during the observation period compared with 154 patients (55%) during the year before ocrelizumab induction. Six patients experienced a conversion to SPMS according to Lorscheider[10] criteria during follow-up (naive: 1 patient; IFN: 1 patient; DMF: 2 patients; NTZ: 2 patients).

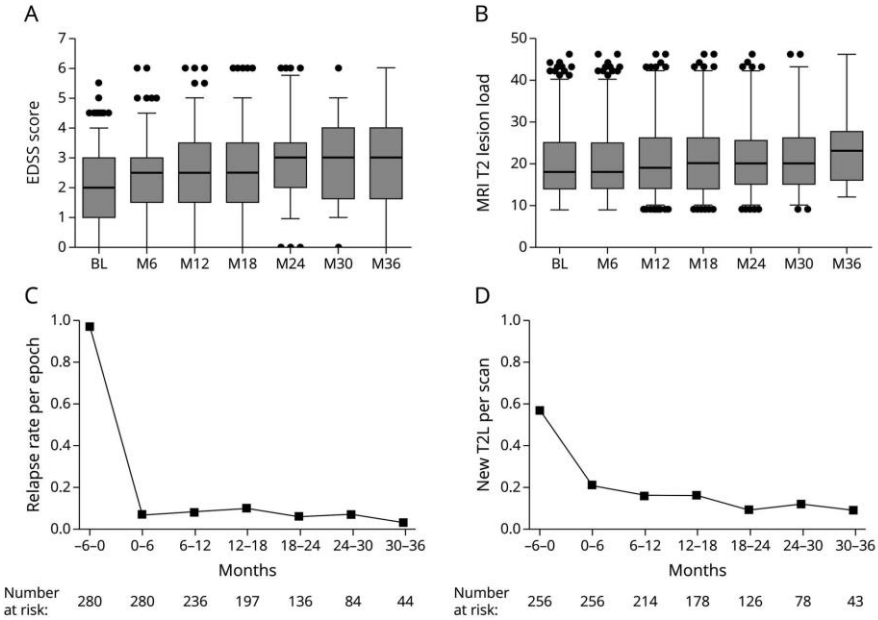
Overall, ocrelizumab treatment also appeared effective in terms of the abundance of new or enlarging T2 hyperintense MRI lesions. Although 146/256 patients (57%) with available scans exhibited new lesions in their baseline MRI, this fraction rapidly declined throughout the treatment epochs; details are shown in Figure 1, B and D.

We next assessed whether the effectiveness of ocrelizumab treatment depended on its use as a first-, second- or third-line treatment and evaluated the HR for time to first relapse in a multivariate regression model. Here, we found that the use of ocrelizumab as third-line treatment was associated with an increased HR compared with its use as first-line substance (aHR: 2.738; 95% CI: 1.219–6.151; $p = 0.015$). Neither the use of ocrelizumab as a second-line substance vs a first-line treatment was associated with an increased hazard for relapses (aHR: 1.810; 95% CI: 0.792–4.138; $p = 0.160$), nor was the use of ocrelizumab as third-line vs second-line treatment (aHR: 1.520; 95% CI: 0.781–2.960; $p = 0.218$).

Among covariates from the abovementioned model, ARR at baseline (calculated from 1 year before baseline ocrelizumab infusion) was also identified as a risk factor with an aHR of 1.492 (95% CI: 1.098–2.026; $p = 0.010$; eTable 1, links.lww.com/NXI/A818). None of the further covariates were selected; in addition, we found no interaction between covariates.

The use of ocrelizumab as third-line treatment was, however, not associated with an increased HR for time to first confirmed worsening of disability (aHR: 1.971; 95% CI: 0.823–4.722; $p = 0.128$) compared with use as a first-line

Figure 1 Efficacy Outcomes of Our Cohort



(A) Expanded Disability Status Scale (EDSS) scores at baseline and within further treatment epochs (whiskers comprise 5%–95% of data). (B) Total T2 hyperintense MRI lesion load at baseline and within further treatment epochs. (C) Development of mean relapse rates treatment epoch compared with last 6 months before ocrelizumab induction. (D) Rate of new or enlarging T2 hyperintense MRI lesions per scan conducted at the end of the respective treatment epoch. Numbers at risk listed below 1C and 1D also refer to 1A and 1B, respectively.

treatment. Notably baseline ARR again was a significant covariate here with an aHR of 1.617 (95% CI: 1.152–2.269, $p = 0.005$; eTable 2, links.lww.com/NXI/A818).

Because 19/73 relapses happened during the first 6 months, for which carryover activity appeared to be a likely explanation, we decided to perform rebaselining and selected data from month 6 as the earliest available follow-up visit. However, models remained largely unaltered (eTables 3 and 4, links.lww.com/NXI/A818).

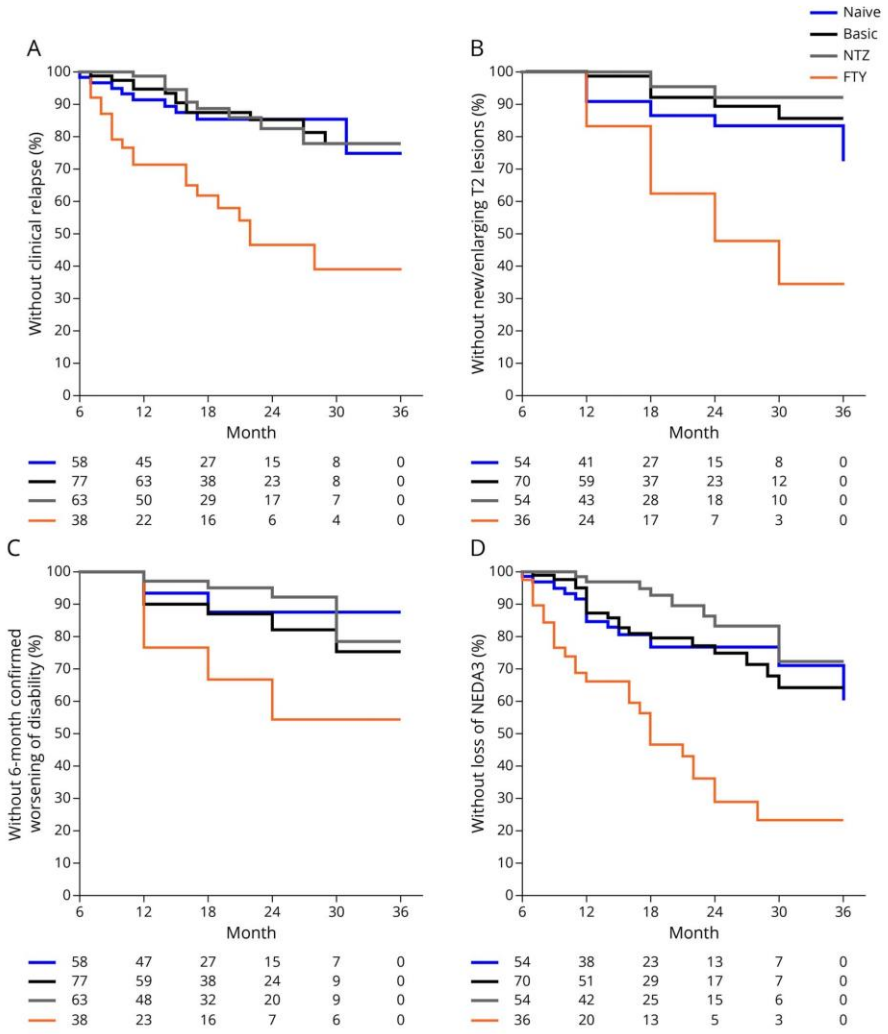
Next, we were interested in the effect of different last previous DMT on effectiveness outcomes. Because position of ocrelizumab within the treatment line was significantly associated with the last previously administered DMT (p <0.001), interactive covariates (last previous DMT*treatment line) were used. Rebaselining to month 6 was again performed (13/19 of the relapses within first 6 months of follow-up were observed in patients switching from NTZ or FTY).

Using time to first clinical relapse as the dependent variable, we again identified ARR at baseline as relevant covariate (aHR: 1.640; 95% CI: 1.099–2.446; $p = 0.015$). In addition, FTY as last previous DMT substantially increased the hazard, too, and this applied to 2nd-line OCR (aHR: 3.417; 95% CI: 1.007–11.600; $p = 0.049$) and to 3rd-line OCR (aHR: 5.903; 95% CI: 2.489–13.999; $p < 0.001$; Figure 2A and Table 2).

Similar observations were made regarding time to first confirmed worsening of disability as the dependent variable. Here, FTY as last previous DMT increased the hazard to 3.571 (95% CI: 1.013–12.589; $p = 0.048$) in 2nd-line OCR and to 4.502 (95% CI: 1.728–11.729; $p = 0.002$) in 3rd-line OCR (Figure 2B and Table 2). None of the further covariates were selected.

Furthermore, we evaluated whether the risk for development of new or enlarging T2 hyperintense MRI lesions was associated with the last previous DMT as well. Previous exposure to FTY increased the hazard for time to first abundance of new or enlarging T2 lesions in 3rd-line OCR patients to 4.627 (95% CI: 1.982–10.802; $p < 0.001$), with none of the further covariates being selected (Figure 2C and Table 2). Finally, the hazard for time to loss of NEDA-3 was increased by FTY as last previous DMT in 2nd-line OCR to 3.069 (95% CI: 1.217–7.739; $p = 0.017$) and in 3rd-line OCR to 3.642 (95% CI: 1.753–7.564; $p = 0.001$; Figure 2D and Table 2).

Figure 2 Kaplan-Meier Plots Depicting the 4 Effectiveness Outcomes Evaluated in This Study With Stratification According to the Last Previous Data Were Rebaselined Following Month 6



Numbers at risk are displayed below the respective graphs. (A) Proportion of patients without a clinical relapse over time. (B) Proportion of patients without confirmed worsening of disability over time. (C) Proportion of patients without new or enlarging T2 hyperintense MRI lesions over time. (D) Proportion of patients with loss of NEDA-3 over time. FTY = fingolimod; NTZ = natalizumab; NEDA-3 = no evidence of disease activity 3.

Given our findings, we (retrospectively) reevaluated the patients switching from FTY but found no differences regarding quantity or type of disease activity in the year before switch compared with other treatment subgroups (neither were FTY-treated patients prone to more frequent or more severe relapses, nor did they show pronounced disability worsening of MRI activity compared with other subgroups).

We also evaluated the baseline lymphocyte counts in all patients switching from FTY to ocrelizumab. Median lymphocyte levels were 917/μL (interquartile range: 759–1,063). Sixteen of 45 patients (36%) presented with lymphocyte levels below 800/μL at baseline. Similar to previous studies, baseline lymphocyte levels were not predictive of impaired treatment response.

Table 2 Cox Proportional Hazards Models for Multivariate Analysis of Effectiveness Outcomes

			95% CI		
	A (relapses)	**HR**	**Lower**	**Upper**	***p* Value**
	Age at baseline (<37 [ref] vs ≥37)	0.906	0.495	1.659	0.750
	Female (ref) vs male	1.061	0.587	1.919	0.845
	ARR at baseline	**1.640**	**1.099**	**2.446**	**0.015**
	EDSS score at baseline (<2 [ref.] vs ≥2)	0.842	0.455	1.558	0.584
	Last previous DMT (naive*1st-line OCR = ref.)				
2nd-line OCR	Basic (IFN/GLAT/DMF)	1.329	0.459	3.850	0.600
	NTZ	1.546	0.179	13.356	0.692
	FTY	**3.417**	**1.007**	**11.600**	**0.049**
3rd-line OCR	Basic (IFN/GLAT/DMF)	1.290	0.445	3.741	0.639
	NTZ	1.950	0.622	6.120	0.252
	FTY	**5.903**	**2.489**	**13.999**	**<0.001**
			95% CI		
	B (disability worsening)	**HR**	**Lower**	**Upper**	***p* Value**
	Age at baseline (<37 [ref] vs ≥37)	0.771	0.406	1.466	0.428
	Female (ref) vs male	1.283	0.694	2.372	0.427
	ARR at baseline	1.270	0.831	1.941	0.268
	EDSS score at baseline (<2 [ref.] vs ≥2)	1.164	0.600	2.257	0.653
	Last previous DMT (naive*1st-line OCR = ref.)				
2nd-line OCR	Basic (IFN/GLAT/DMF)	1.486	0.488	4.531	0.486
	NTZ	1.388	0.158	12.233	0.768
	FTY	**3.571**	**1.013**	**12.589**	**0.048**
3rd-line OCR	Basic (IFN/GLAT/DMF)	2.155	0.759	6.120	0.150
	NTZ	1.269	0.362	4.444	0.709
	FTY	**4.502**	**1.728**	**11.729**	**0.002**
			95% CI		
	C (new MRI lesions)	**HR**	**Lower**	**Upper**	***p* Value**
	Age at baseline (<37 [ref] vs ≥37)	0.834	0.423	1.643	0.600
	Female (ref) vs male	0.564	0.618	1.136	0.086
	ARR at baseline	0.823	0.494	1.371	0.455
	EDSS score at baseline (<2 [ref.] vs ≥2)	0.793	0.394	1.598	0.517
	Last previous DMT (naive*1st-line-OCR = ref.)				
2nd-line OCR	Basic (IFN/GLAT/DMF)	0.290	0.063	1.335	0.112
	NTZ	0.593	0.071	4.934	0.629
	FTY	1.939	0.604	6.228	0.266

Continued

Table 2 Cox Proportional Hazards Models for Multivariate Analysis of Effectiveness Outcomes *(continued)*

			95% CI		
	C (new MRI lesions)	**HR**	**Lower**	**Upper**	***p* Value**
3rd-line OCR	Basic (IFN/GLAT/DMF)	0.579	0.180	1.859	0.358
	NTZ	0.243	0.139	1.261	0.105
	FTY	**4.627**	**1.982**	**10.802**	**<0.001**
			95% CI		
	D (loss of NEDA-3)	**HR**	**Lower**	**Upper**	***p* Value**
	Age at baseline (<37 [ref] vs ≥37)	0.872	0.506	1.501	0.620
	Female (ref) vs male	1.104	0.648	1.882	0.716
	ARR at baseline	1.185	0.828	1.694	0.353
	EDSS score at baseline (<2 [ref.] vs ≥2)	1.110	0.640	1.923	0.711
	Last previous DMT (naive*1st-line-OCR = ref.)				
2nd-line OCR	Basic (IFN/GLAT/DMF)	0.865	0.342	2.184	0.759
	NTZ	0.551	0.069	4.400	0.574
	FTY	**3.069**	**1.217**	**7.739**	**0.017**
3rd-line OCR	Basic (IFN/GLAT/DMF)	1.223	0.539	2.774	0.630
	NTZ	0.489	0.159	1.509	0.214
	FTY	**3.642**	**1.753**	**7.564**	**0.001**

Abbreviations: ARR = annualized relapse rate; DMF = dimethyl fumarate; FTY = fingolimod; GLAT = glatiramer acetate; HR = hazard ratio; IFN = interferon beta; NTZ = natalizumab; OCR = ocrelizumab.
A: time to first relapse as the dependent variable. B: time to first 6-month confirmed worsening of disability as the dependent variable C: time to abundance of first new or enlarging T2 MRI lesion as the dependent variable. D: time to first loss of NEDA-3 as the dependent variable. Bold values represent significant covariates. Data were rebaselined to month 6 before calculation. Reference categories for categorical variables are indicated.

Persistence of CD19$^+$ B-Cell Depletion Among Patients

Next, we investigated the absolute numbers of peripheral blood CD19$^+$ B cells. Before treatment induction, we found no significant differences among groups with the exception of patients switching from NTZ who expectedly showed increased numbers of B cells in the blood (NTZ: 432 ± 230 cells/μL vs naive: 312 ± 194 cells/μL; $p < 0.001$; $p > 0.05$ for other comparisons among treatment groups at baseline). Notably, the spread of peripheral B cells was greatest in patients switching from FTY, yet increased (or decreased) baseline levels of CD19$^+$ B cells were not associated with ongoing disease activity here ($p = 0.247$). Following ocrelizumab infusion, we observed persistent depletion of B cells (<10 CD19$^+$ B cells/μL) at the next follow-up in 235/280 (84%) patients (Figure 3A). Because that threshold was repeatedly associated with reemerging disease activity,[11] we evaluated whether the fraction of patients showing this degree of repopulation was different among patients switching from a different DMT. However, this phenomenon was equally distributed.

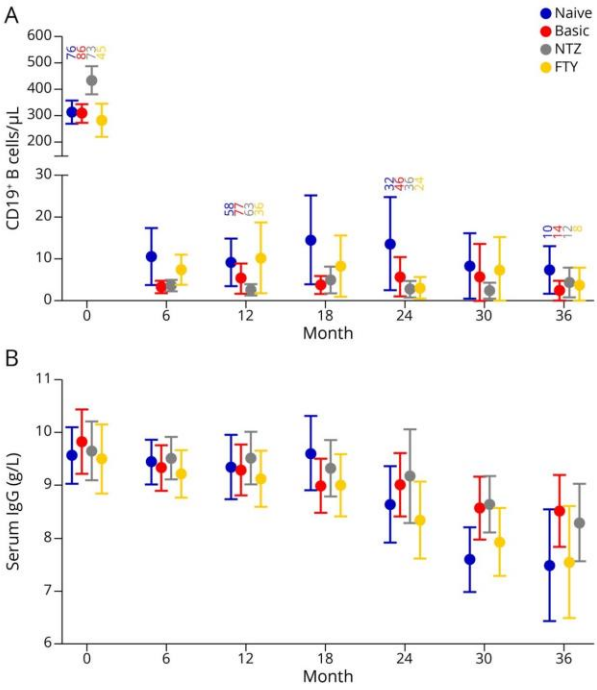
In addition, we checked whether the evidence of disease activity (loss of NEDA-3) was accompanied by early repopulation of B cells using the data set for which rebaselining was performed. First, we evaluated the proportion of patients with persistent peripheral B-cell depletion at the end of a treatment epoch, in whom disease activity was experienced. Overall, 12/74 relapses (16%) were followed by repletion at the next visit. This fraction was equally distributed among different pretreatments ($p = 0.945$). Similar results were obtained regarding the fraction of patients with repletion throughout the last previous treatment epoch ($p = 0.711$) and the fraction of patients with repletion at either of these time points ($p = 0.822$).

We also performed a sensitivity analysis including a definition of ≥5 CD19$^+$ B cells/μL. However, despite the expected increase of the fraction of active patients with repopulation of B cells, the abovementioned pattern did not change.

Serum Immunoglobulin G Levels During Ocrelizumab Therapy

Because previous studies suggest a dose-dependent decrease of serum IgG levels in patients receiving anti-CD20 therapy, we evaluated whether such effects were visible in our cohort. Among 233 available data sets, we found 20 patients with hypogammaglobulinemia at baseline defined as serum IgG levels <7 g/L, with none of the patients presenting with a

Figure 3 CD19+ B Cells and Immunoglobulin Levels Following Ocrelizumab Treatment



(A) Development of peripheral blood CD19⁺ B cells over time, stratified according to the last previous disease-modifying therapy (DMT). Data are shown as mean ± 95% CI. The color scheme indicated in the legend applies to the whole figure. Numbers above error bars indicate sample numbers at respective time points (numbers apply to 3B-C as well). (B) Development of serum immunoglobulin G levels within follow-up among patient subgroups. Data are shown as mean ± 95% CI. IgG = immunoglobulin G.

serum IgG level <5 g/L. Hypogammaglobulinemia at baseline was not associated with a specific pretreatment.

Following ocrelizumab treatment, IgG levels demonstrated the well-described[12] time-dependent decrease during follow-up (9.67 ± 2.23 g/L at baseline vs 8.04 ± 1.32 g/L at month 36; $p < 0.001$; Figure 3D). This decline was not associated with a specific pretreatment, and we did not observe any case of serum IgG reduction <5 g/L.

Classification of Evidence

This study provides Class IV evidence that for patients with RRMS, previous treatment with FTY compared with previous treatment with other immunomodulating therapies decreases the effectiveness of ocrelizumab.

Discussion

In this prospective real-world cohort comprising 280 patients from 2 centers, we compared the effectiveness of ocrelizumab therapy among patients with different previous DMTs. Patients coming from injectable therapies and naive patients demonstrated outcomes that were comparable to results from the clinical trials and previous real-world studies on B cell–depleting therapies in RMS.[13,14] Furthermore, we were able to confirm previous results regarding the effectiveness of anti-CD20 therapy in patients stopping NTZ due to increased risk of the development of PML.[6]

Previous reports indicated profound disease reactivation following the switch from FTY to rituximab, but disease exacerbation was restricted to the first weeks of treatment, and rituximab was only administered as single-shot treatment at baseline.[15] In addition, it is well known that FTY withdrawal can result in severe multiple sclerosis relapses.[16]

Recently, a retrospective analysis confirmed worse clinical outcomes in patients switching from FTY to ocrelizumab compared with patients switching from NTZ.[17] However, this study again evaluated only a short-term follow-up of 6 months and hence did not distinguish between rebound activity and persistent suboptimal disease control. Data from the MSBase registry previously indicated that among patients switched to ocrelizumab, those having previously received FTY performed worse compared with other treatment groups. However, these data were also limited to a shorter follow-up duration of 1 year.[18]

In contrast, impairment in disease control in patients switching from FTY to ocrelizumab was persistent during

follow-up and thus far beyond the previously reported timespans. Whereas the abovementioned publications repeatedly identified the duration of washout as a risk factor for disease reactivation and/or disability worsening,[17,19] and were restricted to rather short follow-up, we here document impaired effectiveness of ocrelizumab in patients switching from FTY even after rebaselining to month 6 of ocrelizumab treatment.

Notably, we have recently demonstrated rather similar effects in patients switching from FTY to alemtuzumab.[8] Alemtuzumab is a monoclonal antibody directed against CD52, which is expressed on a wider variety on lymphocytes, particularly T cells. Because alemtuzumab mainly exerts its effects in the peripheral blood and—to a lesser extent—the lymph nodes,[20] sequestration of lymphocytes in the deeper lymphoid tissues by sphingosine-1-phosphate receptor blockade could contribute to incomplete depletion and, subsequently, suboptimal disease control.

Anti-CD20 antibodies are thought to penetrate lymphoid tissues more effectively than alemtuzumab,[3] but recent studies indicated persistence of B cells in lymphoid tissues following ocrelizumab or ofatumumab.[21] Although the peripheral $CD19^+$ B-cell levels were not different in stable or relapsing patients having received FTY as last previous DMT, we were of course unable to evaluate whether this B-cell reservoir has been substantially altered. Generally, these compartment-specific effects might render development of durable biomarkers determined from peripheral blood difficult.

Performing treatment switch in patients with active RMS from FTY to other substances is associated with particular difficulties as has been reviewed by our group before.[22] In terms of the switch from FTY to ocrelizumab, several mechanisms beyond sequestration and incomplete depletion are likely to contribute to suboptimal disease control. FTY modulates the B-cell compartment toward higher abundance of regulatory B cells[23] and also reduces proinflammatory subsets such as memory B cells.[24] Both of these findings are likely to underlie the observed relative increase of anti-inflammatory cytokines in blood samples from FTY-treated patients.

On the other hand, plasma cells become more abundant following FTY treatment,[25] and these cells are generally spared from depletion due to absence of CD20.[25] It is possible that patients having received FTY before ocrelizumab develop a deleterious imbalance between earlier and later, with regard to progeny, B-cell subgroups such as has been associated within paradoxical disease worsening in patients following administration of atacicept.[26]

In contrast, changes in the B-cell compartment induced by NTZ treatment could account for the beneficial outcomes of patients having switched to ocrelizumab. NTZ mobilizes B cells toward the peripheral blood rendering them prone to depletion and moreover triggers a more proinflammatory phenotype in these cells,[27] which are then easily removed on ocrelizumab infusion. Because these B cells are thought to be a prerequisite to development of Th17, which can trigger breakthrough activity in patients receiving NTZ,[28] ocrelizumab appears a reasonable choice as subsequent DMT. In addition, different subsets of T cells express CD20 and hence are a potential target of ocrelizumab therapy.[29,30]

Although the complex mechanisms underlying the observed effects remain unclear, our findings underline that ocrelizumab has features of an immune reconstitution therapy. This is supported by the recent observation that EID in previously stable patients can be performed with only minor cutbacks on effectiveness,[31] suggesting extensive, durable changes in the immune system as have been described for various peripheral autoimmune disorders following rituximab treatment.[32] Such changes appear not to be limited to the B-cell compartment but also involve reduction of effector T cells and increased frequencies of regulatory T cells in patients with multiple sclerosis.[33,34]

Hence, our results indicate that initial anti-CD20 therapy can induce long-lasting changes in the immune system. Because differences in effectiveness of ocrelizumab remained visible even following additional treatment courses, they consequently cannot be explained by pure sequestration effects as proposed previously.[17] We also evaluated serum immunoglobulin G levels throughout the study and found the well-known pattern of a dose-dependent decline during ocrelizumab therapy.

Due to being collected from real-world patients, our data have some limitations. The recent approval of ocrelizumab in 2018 limits the available duration of follow-up, but despite this sample size remains large. Due to the COVID-19 pandemic, some patients entered the extended interval dosing and thus were censored until then skewing the cohort toward patients with the standard dosing interval that indeed might have been more active compared with patients having been allowed an extended dosing interval. Furthermore, it remains unclear whether patients having received FTY here shared other unknown traits rendering them more susceptible to ineffective ocrelizumab treatment.

Of course, optimal assessment of these effects would require a randomized and controlled clinical trial. Although the need for such studies has been discussed before, conduction remains unlikely.[35]

What indeed remains a potential sample bias among our cohort is that substantial differences exist regarding the reason for treatment switch among patients coming from FTY compared with those switching from NTZ. Latter mostly were previously stable yet developed an increased risk for PML, whereas the majority of FTY pretreated patients switched due to active RMS. Previous data indicate that patients switching from NTZ show similar disease courses following immune cell–depleting treatment by alemtuzumab irrespective of previous disease activity.[8] However, obtaining a profound cohort of patients irresponsive to NTZ and thus switching to ocrelizumab remain

difficult because such patients are rare. Nonetheless, future studies should further evaluate whether the reason for cessation of NTZ eventually distinguishes outcome groups in patients switched to ocrelizumab.

In our study, previous exposure to FTY was a risk factor for suboptimal disease control in patients receiving ocrelizumab. Although it remains unclear whether these effects will fade following repeated courses of ocrelizumab treatment beyond month 36, patients should be informed that their clinical outcome may be worse compared with what would have been expected from the clinical trials. Future studies should be conducted to elucidate whether this effect is restricted to FTY or also applies to second-generation sphingosine-1-phosphate receptor modulators substances such as ozanimod, siponimod, or ponesimod.

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Leoni Rolfes, MD	Department of Neurology, University Hospital Duesseldorf, Germany	Data acquisition, data analysis, and revision of the manuscript for intellectual content
Jens Ingwersen, MD	Department of Neurology, University Hospital Duesseldorf, Germany	Data acquisition and revision of the manuscript for intellectual content
Refik Pul, MD	Department of Neurology and Centre for Translational Neuro- and Behavioural Sciences (C-TNBS), University Hospital Essen, Germany	Data acquisition and revision of the manuscript for intellectual content
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Continued

Appendix *(continued)*

Name	Location	Contribution
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Saskia Räuber, MD	Department of Neurology, University Hospital Duesseldorf, Germany	Data acquisition and revision of the manuscript for intellectual content
Tobias Ruck, MD	Department of Neurology, University Hospital Duesseldorf, Germany	Revision of the manuscript for intellectual content
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Christoph Kleinschnitz, MD	Department of Neurology and Centre for Translational Neuro- and Behavioural Sciences (C-TNBS), University Hospital Essen, Germany	Revision of the manuscript for intellectual content
Hans-Peter Hartung, MD	Department of Neurology, University Hospital Duesseldorf, Germany; Brain and Mind Center, University of Sydney, NSW, Australia; Department of Neurology, Palacky University, Olomouc, Czech Republic; Department of Neurology, Medical University of Vienna, Austria	Revision of the manuscript for intellectual content
Ludwig Kappos, MD	Neurologic clinic and Policlinic, Departments of Medicine, Biomedicine and clinical research, University Hospital Basel, Switzerland	Revision of the manuscript for intellectual content
Sven G. Meuth, MD, PhD	Department of Neurology, University Hospital Duesseldorf, Germany	Study concept and design, study supervision, and revision of the manuscript for intellectual content

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4.9 Ocrelizumab Extended Interval Dosing in Multiple Sclerosis in Times of COVID-19

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Ocrelizumab Extended Interval Dosing in Multiple Sclerosis in Times of COVID-19

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Abstract

Objective
To evaluate the clinical consequences of extended interval dosing (EID) of ocrelizumab in relapsing-remitting multiple sclerosis (RRMS) during the coronavirus disease 2019 (COVID-19) pandemic.

Methods
In our retrospective, multicenter cohort study, we compared patients with RRMS on EID (defined as ≥4-week delay of dose interval) with a control group on standard interval dosing (SID) at the same period (January to December 2020).

Results
Three hundred eighteen patients with RRMS were longitudinally evaluated in 5 German centers. One hundred sixteen patients received ocrelizumab on EID (median delay [interquartile range 8.68 [5.09–13.07] weeks). Three months after the last ocrelizumab infusion, 182 (90.1%) patients following SID and 105 (90.5%) EID patients remained relapse free (p = 0.903). Three-month confirmed progression of disability was observed in 18 SID patients (8.9%) and 11 EID patients (9.5%, p = 0.433). MRI progression was documented in 9 SID patients (4.5%) and 8 EID patients (6.9%) at 3-month follow-up (p = 0.232). Multivariate logistic regression showed no association between treatment regimen and no evidence of disease activity status at follow-up (OR: 1.266 [95% CI: 0.695–2.305]; p = 0.441). Clinical stability was accompanied by persistent peripheral $CD19^+$ B-cell depletion in both groups (SID vs EID: 82.6% vs 83.3%, p = 0.463). Disease activity in our cohort was not associated with $CD19^+$ B-cell repopulation.

Conclusion
Our data support EID of ocrelizumab as potential risk mitigation strategy in times of the COVID-19 pandemic.

Classification of Evidence
This study provides Class IV evidence that for patients with RRMS, an EID of at least 4 weeks does not diminish effectiveness of ocrelizumab.

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Glossary

COVID-19 = coronavirus disease 2019; **CPD** = confirmed progression of disability; **EDSS** = Expanded Disability Status Scale; **EID** = extended interval dosing; **IQR** = interquartile range; **NEDA-3** = no evidence of disease activity; **RRMS** = relapsing-remitting multiple sclerosis; **SID** = standard interval dosing.

Immunotherapy for relapsing-remitting multiple sclerosis (RRMS) is critical for maintaining disease stability, but potentially increases the risk of infection. This is of particular importance in light of the ongoing coronavirus disease 2019 (COVID-19) pandemic. In general, pulsed depletion of CD20-expressing B cells by ocrelizumab or rituximab can increase the risk of respiratory infections for several months.[1-3] Regarding COVID-19 disease, it has recently been discussed that B cell–depleting therapies may not only be accompanied with higher rates of infection, but could also influence the severity and mortality,[4,5] albeit well-controlled data are still lacking.

Drug-free intervals are long between 2 courses of ocrelizumab as its treatment effect is determined by long-lasting (selective) immune suppression eventually appraisable by peripheral B-cell reconstitution.[6] This provides the opportunity to individually delay therapy during the pandemic.[7,8] In addition, extended interval dosing (EID) might also be favorable in terms of severe acute respiratory syndrome coronavirus 2 vaccine response, which is probably reduced under therapeutic approaches with B-cell depletion.[9-11] During the first peak of the COVID-19 outbreak between January 2020 and September 2020 in Germany, several treatment courses of ocrelizumab were delayed due to safety concerns. Although some smaller studies suggest longer treatment-free intervals of B cell–depleting therapies in RRMS without lack of efficacy,[8,12,13] real-world data on EID in a larger cohort of ocrelizumab-treated patients with RRMS are still lacking.

We here report clinical outcomes of delayed ocrelizumab infusions during the COVID-19 pandemic in 116 patients on EID compared with 202 patients on standard interval dosing (SID).

Methods

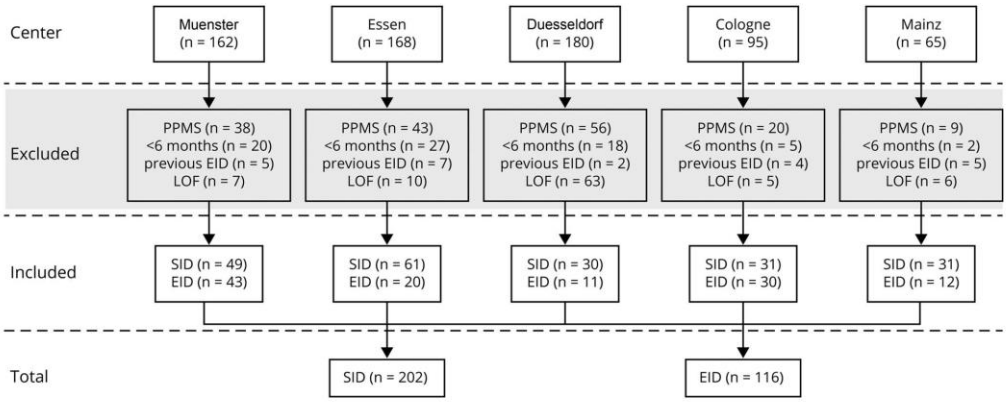
We performed an ad hoc analysis of our observational, multicentric cohort of adult patients with RRMS undergoing ocrelizumab treatment. Ocrelizumab therapy was performed at the German University Hospitals Muenster, Mainz, Essen, Duesseldorf, and Cologne in accordance with national and international guidelines. We included patients with RRMS who received at least both initial treatment cycles of ocrelizumab (2 × 300 mg with a 2-week interval) before experiencing SID or EID during the observation period. In other words, the observation period in which either the SID or EID took place always related to maintenance cycle (600 mg). The SID was defined as regular maintenance interval of ocrelizumab infusion after 6 months, whereas the EID group included patients with an ocrelizumab infusion delay of at least 4 weeks (6 months + ≥4 weeks delay). Patients were excluded if (1) they were treated with ocrelizumab for primary progressive MS, (2) if only the 2 induction cycles with 300 mg were administered (treatment duration with ocrelizumab <6 months), (3) experienced EID before the observation period (before January 2020), (4) or if no follow-up data were available (Figure 1).

Looking at the period between January 2020 and September 2020, patients receiving ocrelizumab EID were compared with patients receiving ocrelizumab on SID (Figure 2). The 2 infusions defining SID vs EID (Infusions B and C, Figure 2) were defined as follows: Infusion B was the last ocrelizumab infusion (second 300 mg cycle or 600 mg maintenance infusion) before January 2020, and Infusion C (always 600 mg standard maintenance dose) was the subsequent infusion, administered between January 2020 and September 2020. A relapse was defined as a neurologic deficit related with an acute inflammatory demyelinating event that lasts at least 24 hours in the absence of infection or fever.

Confirmed progression of disability (CPD) was determined by standardized neurologic examinations 3 months following Infusion C, further referred to as follow-up (3 months ± 10 days after the last ocrelizumab infusion). Clinical and MRI outcomes were collected at the end of the observation interval (Figure 2). MRI progression was defined as new or enlarged T2-weighted or T1-weighted gadolinium-enhancing lesions. Expanded Disability Status Scale (EDSS) progression was considered clinically relevant if 2 independent clinical assessments 3 months apart (at Infusion C and follow-up) indicated an increase of the EDSS as follows: +1.5 points (baseline = 0.0), +1.0 point (baseline = 1.0–4.0), and +0.5 points (baseline ≥ 4.5). Treatment success was further classified with the concept of no evidence of disease activity (NEDA-3). While at Infusion B, NEDA-3 status was related to the time period of 6 months before this infusion B (in other words between Infusion A and Infusion B), NEDA-3 status during the observation period (between Infusion B and follow-up) was calculated based on the time period between Infusion B to follow-up (including Infusion C, Figure 2).[14] Peripheral blood $CD19^+$ B-cell depletion was defined as < 10 cells/μL.

The Mann-Whitney *U* test (ordinal) or χ^2 test (categorical) was used for comparison of demographic and clinical features where appropriate. Binary logistic regression was performed, using loss of NEDA-3 status as the dependent variable and sex, age (above vs below median), reason for ocrelizumab

Figure 1 Flowchart of Case Ascertainment



This flowchart depicts how the 318 ocrelizumab-treated patients with relapsing-remitting multiple sclerosis (RRMS) were identified. The source population was all patients with multiple sclerosis (MS) treated with ocrelizumab in 5 German centers during the period between January 2020 and September 2020. We excluded patients with MS who were treated with ocrelizumab due to a primary progressive disease course (PPMS), if only the 2 induction cycles with 300 mg were administered (treatment duration with ocrelizumab <6 months (Mo) during the observational period). SID and EID do not refer to the first 2 half doses with 300 mg, respectively, if patients experienced an extended interval dosing (EID) before Infusion C (the infusion administered between January 2020 and September 2020 that led to the division of the 2 groups–standard interval dosing [SID] and EID), or if the patient for any other reason did not satisfy the inclusion criteria (e.g., loss of follow-up [LOF]).

initiation (treatment-naive patients, disease progression, adverse events, or risk of progressive multifocal leukoencephalopathy number of previous disease-modifying therapies, loss of NEDA-3 before the observational period, and $CD19^+$ B-cell depletion at Infusion C as covariates in an enter method. Statistical analysis was conducted using SPSS Statistics 26 (IBM, NY).

Standard Protocol Approvals, Registrations, and Patient Consents

Ethical approval was obtained from local authorities (2016-002937-31; 2019-712-f-S; 2017044238), and patients gave informed consent.

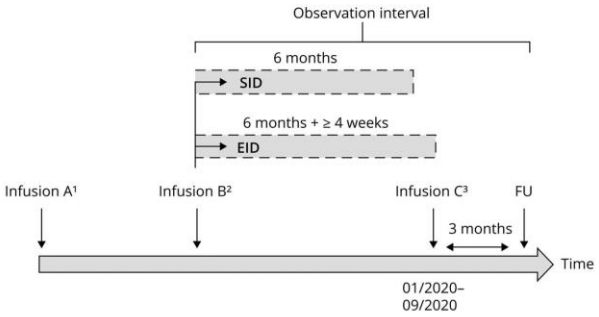
Data Availability

Data will be shared with qualified investigators on request; please contact meuth@uni-duesseldorf.de.

Results

Three hundred eighteen patients with RRMS treated with ocrelizumab between January 2020 and September 2020 were included in our study (Figure 1). One hundred sixteen patients received ocrelizumab on EID (median delay [interquartile range, IQR] 8.68 [5.09–13.07] weeks), and 202 patients received ocrelizumab on SID (median delay [IQR]

Figure 2 Flowchart of the Study Procedure



[1]Infusion A was defined as the second last ocrelizumab infusion (300 mg or 600 mg cycles) before January 2020 (before the coronavirus disease 2019 pandemic started in Germany). [2]Infusion B was defined as the last ocrelizumab infusion (second 300 mg infusion or 600 mg dose) before January 2020 and as the beginning of the observation interval. [3]The infusion that followed on, further referred to as Infusion C, was the infusion administered between January 2020 and September 2020 (always 600 mg maintenance cycle). EID = extended interval dosing; FU = follow-up; SID = standard interval dosing.

Table 1 Baseline Characteristics of the Ocrelizumab Cohort and Subgroups (Total N = 318)

	Patients on SID	Patients on EID	*p* Value
Patients, n	202	116	
Age, yrs, median (IQR)	43.0 (33.0–51.8)	40.0 (32.0–49.0)	0.217[a]
Male patients, n (%)	85 (40.5)	48 (41.0)	1.0[b]
Disease duration in yrs, median (IQR)	8.8 (3.8–15.9)	8.6 (3.4–15.2)	0.995[a]
Total number of previous DMTs, median (IQR)	2 (1–3)	2 (1–3)	0.786[a]
Last previous DMT, n (%)			0.899[b]
Treatment naive	38 (20.1)	24 (21.3)	
Basic	72 (35.2)	43 (36.7)	
Escalation	92 (43.8)	49 (41.0)	
Reason for switch to OCR, n (%)			0.639[b]
Treatment initiation	38 (20.9)	24 (21.3)	
Progression	113 (54.3)	62 (52.9)	
Adverse events	28 (13.3)	21 (17.9)	
PML risk	22 (7.1)	9 (7.7)	
Previous OCR courses to Infusion C, n (%)			0.439[b]
2 (2 × 300 mg)	74 (36.6)	34 (29.3)	
3 (2 × 300 mg + 1 × 600 mg)	43 (20.9)	22 (18.9)	
4 (2 × 300 mg + 2 × 600 mg)	50 (24.7)	31 (26.7)	
5 (2 × 300 mg + 3 × 600 mg)	35 (17.6)	29 (25.0)	
EDSS at OCR initiation, median (IQR)	3.3 (2.0–6.0)	3.5 (2.0–6.0)	0.671[a]
ARR the year before OCR initiation, median (IQR)	1 (0–1)	1 (0–1)	0.600[a]

Abbreviations: EID = extended interval dosing; IQR = interquartile range; PML = progressive multifocal leukoencephalopathy; SID = standard interval dosing.
Age refers to the first ocrelizumab infusion. Disease duration was defined as the time between symptom onset and last follow-up date (January 12, 2020). Basic includes the following disease-modifying therapies (DMTs): interferon-beta, glatiramer acetate, teriflunomide, fumaric acid, and dimethyl fumarate. Escalation includes fingolimod, natalizumab, alemtuzumab, and cladribine. The Expanded Disability Status Scale (EDSS) and the annualized relapse rate (ARR) refer to the time before ocrelizumab initiation. Progression was defined as ongoing disease activity under previous therapy (either persistent relapses, EDSS progression, and/or new or enlarged T2-weighted or T1-weighted gadolinium-enhancing lesions).
Statistics: patients with ocrelizumab on EID were compared with controls receiving ocrelizumab at regular intervals (SID) by applying the Mann-Whitney *U* Test ([a]) or χ^2 test ([b]). Significance levels are indicated; not significant: $p \geq 0.05$.

−0.07 [−1.07 to 1.07] weeks). Baseline parameters were evenly balanced between groups (Table 1). Moreover, no significant differences between the SID and EID group in terms of disease activity before the observation period (before Infusion B) were evident (number of patients with relapses [SID vs EID]: 14 [6.9%] vs 9 [7.8%], $p = 0.783$; with CPD: 10 [5.0%] vs 6 [5.2%], $p = 0.466$; with MRI progression: 24 [11.9%] vs 11 [9.5%], $p = 0.943$; with loss of NEDA-3 Infusion B: 39 patients [19.3%] vs 17 patients [14.6%], $p = 0.860$).

Regarding the interval between Infusion B and follow-up, no significant differences in clinical and radiologic measurements of disease progression between SID and EID were visible. In total, 29 patients (9.1%) showed 3-month CPD at follow-up, with 18 patients on SID (8.9%) and 11 (9.5%) on EID ($p = 0.433$). Moreover, 20 patients (9.9%) on SID experienced a relapse since Infusion B vs 11 patients (9.5%) on EID ($p = 0.903$). MRI progression was evident in 9 patients (4.5%) on SID vs 8 patients (6.9%) on EID ($p = 0.232$). Of note, 39 patients (19.3%) on SID experienced loss of NEDA-3 at follow-up, compared with 25 patients (21.6%) on EID ($p = 0.312$). The adjusted OR for loss of NEDA-3 since Infusion B was 1.266 (95% CI: 0.695–2.305; $p = 0.441$), with no selection of further covariates (Table 2). Of note, NEDA-3 status at follow-up ($p = 0.262$) as well as the 3-month CPD rate ($p = 0.814$), the relapse rate ($p = 0.086$), and MRI activity ($p = 0.754$) since Infusion B were not related to the duration of EID.

Next, we analyzed the available longitudinal B-cell levels of our cohort (also data that were available before the COVID-

Table 2 Regression Model for Analyzing NEDA-3 Since Infusion B

Covariate	OR	95% CI	*p* Value
Age ≥40 vs <40 y (ref.)	0.693	0.365–1.314	0.262
Female vs male sex (ref.)	0.909	0.488–1.694	0.765
Reason for OCR induction (PML risk = ref.)			
Treatment initiation	0.827	0.194–3.520	0.797
Progression	1.559	0.476–5.105	0.463
Adverse events	1.151	0.291–4.558	0.841
No. of previous DMTs	0.932	0.701–1.237	0.624
Loss of NEDA-3 between infusion A and infusion B[2] (no = ref.)	1.686	0.810–3.508	0.162
CD19⁺ cell depletion at Infusion C[1] (no = ref.)			
Yes	2.067	0.760–5.624	0.155
Unknown	1.432	0.361–5.678	0.609
OCR Infusion Center (Muenster = ref.)			
Duesseldorf	0.920	0.331–2.560	0.873
Essen	1.794	0.784–4.105	0.166
Cologne	0.896	0.284–2.822	0.851
Mainz	1.039	0.383–2.823	0.940
EID vs SID (ref.)	1.266	0.695–2.305	0.441

Abbreviations: EID = extended interval dosing; NEDA-3 = no evidence of disease activity; OCR = ocrelizumab; OR = odd ratio; PML = progressive multifocal leukoencephalopathy; SID = standard interval dosing.
Results from our binary logistic regression analysis using an enter method to integrate all the covariates in the final analysis. Loss of NEDA-3 was assessed as a dependent variable. For analysis of age as a covariate, we split our group according to the median. Reference categories are indicated for categorical covariates (ref.). Significance levels are indicated; not significant: $p \geq 0.05$.

19 pandemic, Figure 3A) to illustrate the dynamic of B-cell depletion and repopulation. The absolute B-cell counts decreased after the first ocrelizumab infusion and remained low over the entire treatment period. No differences in longitudinal B-cell counts between the SID and the EID cohort were visible. At Infusion C, absolute peripheral CD19+ B-cell counts were available in 278 of 318 patients (87.4%). Of note, $CD19^+$ B-cell depletion was widely persistent (Figure 3B), with a percentage of patients depleted at Infusion C of 82.6% (150/176) on SID vs 83.3% (85/102) on EID ($p = 0.463$). Moreover, CD 19^+ B-cell depletion at Infusion C was not related to the duration of EID ($p = 0.337$).

With regard to Infusion C, we did not observe a significant difference in re-emerging disease activity between the patients with persistent B-cell depletion (n = 235) and those with evidence of B-cell repopulation (n = 43, relapse: $p = 0.616$, MRI progression: $p = 0.828$, CPD: $p = 0.671$, graphical illustration of individual B-cell counts at Infusion C and disease activity is shown in Figure 3C).

Of interest, 4 patients (1.3%) of our multicentric cohort had COVID-19 disease during the observation period. Two of them were in the EID cohort (1.7%), and the other 2 received ocrelizumab on SID (0.9%). Two patients were female, and they were aged 46, 33, 23, and 40 years at the time of COVID-19 disease. Apart from RRMS and the associated ocrelizumab treatment, none of these patients had other existing chronic conditions or an otherwise compromised immune system. All of them experienced a mild to moderate disease course and had classical symptoms of fever, dry cough, and tiredness. Two patients reported loss of taste and smell and headache, and 1 had diarrhea during the infection. Only 1 patient (from the SID group) required hospitalization; however, not for COVID-19 symptoms, but rather due to acute but short-lasting clinical deterioration of RRMS. All patients recovered from COVID-19 without sequelae.

Discussion

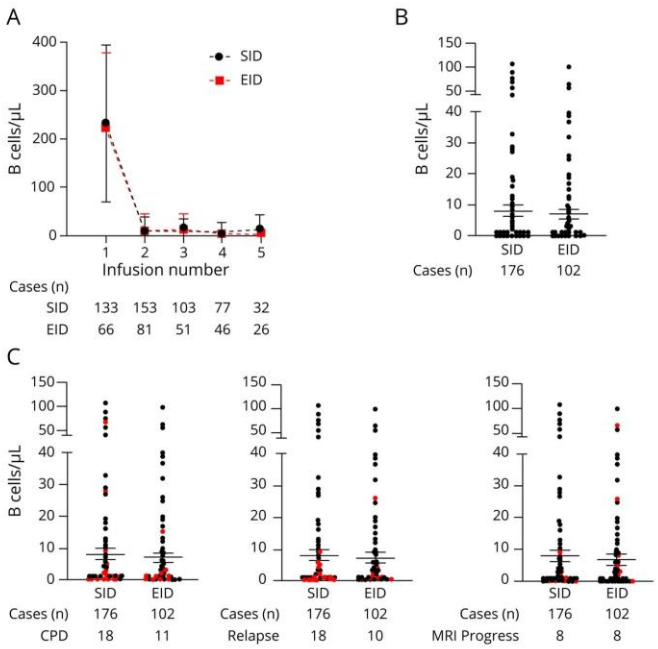
Considering the potential infection risks in times of COVID-19 and the future vaccine response, it is crucial to evaluate whether dosing intervals of immune cell–depleting therapies can be extended.[10,15] Furthermore, as general infection risks may increase with treatment duration and age while benefits may decrease,[16] long-term B cell–depleting treatment strategy studies are needed.

Here, we show real-world data of patients who received ocrelizumab on EID (median delay [IQR] 8.68 [5.09–13.07] weeks) compared with patients treated at regular intervals. The rate of patients reaching NEDA-3 did not differ significantly between both groups, suggesting that EID of at least 4 weeks did not diminish effectiveness of ocrelizumab, at least after short-term evaluation. Although a substantial proportion of our cohort had an aggressive disease course and had been on highly active immunotherapies before ocrelizumab initiation, our EID results are consistent with the high NEDA-3 rates observed in phase III clinical trials.[14]

Our findings support previous results from smaller studies in patients with RRMS receiving ocrelizumab[12,13] or rituximab,[8] indicating long-term disease stability after few treatment cycles. Albeit most of our patients showed persistent B-cell depletion on EID, recurrence of $CD19^+$ B cells may occur in the absence of disease activity.[8] Although our data did not reveal an association between absolute peripheral $CD19^+$ B-cell number and re-emerging disease activity, low levels have been discussed to serve as surrogate marker to justify delaying B cell–depleting infusions,[17] in particular in other disease entities.[18,19]

Considering that the incidence of upper respiratory tract infection was increased in ocrelizumab phase III clinical trials in

Figure 3 B-Cell Levels Before and During Ocrelizumab Treatment in Patients With Relapsing-Remitting Multiple Sclerosis



(A) $CD19^+$ B-cell levels at sampling immediately before ocrelizumab infusions 1 to 5 in the standard interval dosing (SID) group compared with the extended interval dosing (EID) group. Infusion number 1 represents both baseline ocrelizumab infusions (2 × 300 mg with a 2-week interval). The numbers of cases used to estimate the means and SDs are shown below the figure. B-cell counts are presented as mean absolute counts of $CD19^+$ cells/µL of blood. (B) The CD19+ B-cell levels immediately before ocrelizumab Infusion C (the infusion administered between January 2020 and September 2020 that led to the division of the 2 groups—SID and EID). B-cell counts are presented as absolute numbers of $CD19^+$ cells/µL of peripheral blood. The number of patients with absolute $CD19^+$ B-cell counts available at Infusion C is shown below the figure. (C) CD19+ B-cell counts immediately before ocrelizumab Infusion C are depicted. CD19+ B-cell numbers of patients who experienced either a 3-month confirmed progression of disability (CPD, left), a relapse (center), or MRI progression (either new or enlarged T2-weighted lesions or T1-weighted gadolinium enhancement, right) between Infusion B and follow-up are highlighted in red. The number of patients with absolute $CD19^+$ B-cell counts available at Infusion C as well as those with disease activity is shown below the figure.

RRMS,[3] there are some concerns about the infection risk and severity of COVID-19 in patients with MS treated with ocrelizumab. In our cohort, 4 patients had COVID-19 disease regardless of the dosing interval. Besides the severe B-cell impairment (documented B-cell counts in 3 patients at COVID-19 infection were 0, 0, and 4 cells/µL, respectively) and partly higher disability (EDSS in the affected patients was 2.0, 2.5, 4.0, and 8.0, respectively), representing an additional risk factor for COVID-19 severity,[20,21] clinical presentation was mild to moderate in all patients. Of note, it is currently still unclear whether the $CD19^+$ B-cell level in the peripheral blood correlates with the severity of a COVID-19 disease.[22,23] Although our study was not designed to identify the effect of EID on the clinical outcomes of a COVID-19 disease, the fact that B-cell depletion is maintained in our EID cohort suggests that this strategy might not mitigate the risk of severe COVID-19 disease. However, we cannot formally rule out differential effects in tissues like spleen, lymph nodes, bone marrow, or the CNS. Thus, an EID might lead to an earlier B-cell repopulation simultaneously in the bone marrow and spleen before the B cells reappear in the peripheral blood, resulting in an earlier immunity.[24,25]

Our findings of a favorable outcome in the absence of severe complications reflect the preliminary results of an Italian study in patients with MS, in which only 5% of 232 cases of COVID-19 disease were defined as severe or critical,[21] and those of several case series on COVID-19–related pneumonia in patients with MS under ocrelizumab treatment.[26-28] Contrastingly, other authors reported a more severe, even fatal, COVID-19 disease course in RRMS cases treated with ocrelizumab.[4,5,29] As such, the data published are conflicting, possibly explained by selection bias and confounding factors (e.g., age, EDSS) not sufficiently controlled for in the mostly retrospective cohort studies available so far.

In addition, given the effect of ocrelizumab in compromising the immune system, an impact on immunization responses cannot be ruled out, introducing new challenges in the rapid pandemic outbreak of COVID-19. Although the B-cell response to a variety of different vaccines is markedly inhibited by CD20 depletion,[11,30,31] an EID might probably increase the likelihood of repopulation of naive B cells and thus the response to the current COVID-19 vaccines.[10,13] However, in our study, we did not observe a difference in $CD19^+$ B-cell repopulation rates between the SID and the EID group, probably due to a relatively short EID interval (median delay 8.68 weeks). Thus, the immunogenicity of SARS-CoV2 vaccines in patients with RRMS during treatment with ocrelizumab and whether the immune response mounted by

antigenic stimulation of these vaccines is enhanced in EID need to be investigated in future studies.

As a limitation, we would like to stress the short observation period and possible selection bias of our study, including individual physician and/or patient decisions to potentially delay ocrelizumab infusion irrespective of disease activity in the light of the infection risk during the COVID-19 pandemic. These preclude a general recommendation for EID in patients treated with ocrelizumab. In particular, it might be important to determine whether the extension of a single infusion interval has a significant impact on disease progression over a longer period of time. For this purpose, follow-up of the cohort over a period of more than 12 months might be useful. Moreover, future prospective, noninferiority studies should investigate the long-term approach of continuous EID in terms of clinical outcomes and safety concerns. In addition, other outcome parameters such as Multiple Sclerosis Functional Composite score or sub scores as well as neurofilament light chain levels should be considered to evaluate disease progression.[3,32]

Nevertheless, in light of the current COVID-19 pandemic, clinicians can benefit from our results obtained from a well-characterized, large, multicenter cohort, when evaluating risk-based treatment strategies on an individual level. Given the successful transfer of real-world retrospective data[33,34] into the considerations of a prospective clinical trial of natalizumab in RRMS (NCT03689972), our findings may help when designing future studies for long-term therapy with B cell–depleting agents.

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Marc Pawlitzki, MD	University Hospital Münster, Münster, Germany	Study concept and design, acquisition and interpretation of data; and drafted the manuscript
Steffen Pfeuffer, MD	University Hospital Münster, Münster, Germany	Analysis of data and critical revision of the manuscript for intellectual content
Christopher Nelke, MD	University Hospital Münster, Münster, Germany	Analysis of data and critical revision of the manuscript for intellectual content
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Sven G. Meuth, MD, PhD	Heinrich-Heine University, Duesseldorf, Germany	Study concept and design; interpretation of data; and drafted the manuscript

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4.10 Effectiveness and safety of cladribine in MS: Real-world experience from two tertiary centres

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Original Research Paper

Effectiveness and safety of cladribine in MS: Real-world experience from two tertiary centres

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Abstract

Background: Oral cladribine has been approved for the treatment of relapsing multiple sclerosis (MS) yet real-world evidence regarding its effectiveness and safety remains scarce.
Objective: To evaluate efficacy and safety outcomes of MS patients following induction of cladribine.
Methods: We evaluated our prospective cohort of cladribine-treated MS patients from two tertiary centres in Germany. Relapses, disability worsening and occurrence of new or enlarging T2-hyperintense magnetic resonance imaging (MRI) lesions were assessed as well as lymphocyte counts and herpes virus infections.
Results: Among 270 patients treated with cladribine, we observed a profound reduction of both relapses and new or enlarging MRI lesions. Treatment appeared more efficacious, especially in patients without previous therapy or following platform substances. Patients switching from natalizumab were prone to re-emerging disease activity. Among patients following dimethyl fumarate pre-treatment, severe lymphopenia was common and associated with increased rates of herpes virus manifestations.
Conclusion: Overall, we observed an efficacy and safety profile of cladribine consistent with data from the phase 3 clinical trial. However, patients switching from natalizumab experienced suboptimal disease control beyond rebound activity following cessation of natalizumab. Furthermore, dimethyl fumarate pre-treatment was associated with a profound risk of developing severe lymphopenia and subsequent herpes virus infections.

Keywords: Cladribine, treatment response, real-world evidence

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Introduction

Cladribine is a synthetic purine analogue and induces lymphocyte depletion by accumulation of intracellular chloro-deoxyadenosine triphosphate resulting in apoptosis of B and T lymphocytes.[1,2]

Initially established for the treatment of haematologic malignancies such as hairy cell leukaemia, histiocytosis or acute myeloid leukaemia, cladribine was first approved for the treatment of active relapsing multiple sclerosis (RMS) in 2017 in Europe and subsequently in 2019 in the United States after having been positively evaluated in placebo-controlled randomized clinical trials.[3,4]

As so-called *immune reconstitution therapy*,[5] it offers the advantage of only few treatment days per year yet providing durably efficacy in absence of treatment. It is usually administered in two courses of 5 days being 4 weeks apart in year 1 and 2, respectively.

Although finally proven safe within the highly controlled pivotal trial and its extension, little is known about the safety and efficacy of cladribine under diversified real-world conditions, especially when used following immunomodulatory treatment different from injectable substances.

Thus, we here analysed our large dual-centre cohort of cladribine-treated RMS patients with a focus on the impact of previous immunomodulatory treatments on safety and efficacy outcomes of cladribine treatment.

Methods

Adult patients diagnosed with RMS according to 2017 revised McDonald criteria[6] who underwent treatment with cladribine were longitudinally evaluated at our two tertiary referral centres from November 2017 to March 2021. Patients with a minimum follow-up of 6 months were included. Exclusion criteria were any progressive form of MS, inability to undergo magnetic resonance imaging (MRI) examination, presence of other autoimmune disorders than MS and a history of malignant disorders or previous exposition to cytostatic substances. Patients having delayed their second course of cladribine treatment beyond month 13 due to the ongoing coronavirus disease 2019 (COVID-19) pandemic were only evaluated until then.

Administration of cladribine was performed according to national and international guidelines as well as to the most recent summary of product characteristics. Following treatment induction, patients were evaluated every 3 months involving standardized neurologic examination. During the second peak of COVID-19 pandemic in Germany since autumn 2020, the follow-up interval in clinically stable patients was eventually expanded to 6 months. Relapses were evaluated either at unscheduled visits or at least within the next scheduled visit. Baseline MRI was performed no earlier than 3 months prior to treatment induction. Follow-up MRI was conducted every 6 months. MRI data were independently evaluated regarding abundance of new or enlarging T2-hyperintense lesions since gadolinium was not administered regularly during follow-up (but was administered at baseline MRI in all cases). 'No evidence of disease activity-3' (NEDA3) status was assumed in patients without clinical relapses, 6-month confirmed worsening of disability and new or enlarging T2-hyperintense MRI lesions.

Epidemiological data at baseline were evaluated using descriptive statistics. Kaplan–Meier plots were generated for efficacy outcomes, and multivariate analysis was conducted using the Cox proportional hazards model. 'Sex', 'age at baseline', 'last previous disease-modifying treatment (DMT)', 'baseline expanded disability status scale (EDSS) score', 'baseline annualized relapse rate' (refers to the patient's relapse rate during the last 12 months prior to cladribine induction) and 'disease duration since MS onset' were used as covariates in an enter method. Lymphocyte levels were transformed into lymphopenia severity grades according to the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Binary logistic regression for evaluation of meaningful covariates of development of severe lymphopenia was conducted using an enter method with the abovementioned covariates excluding 'annualized relapse rate at baseline'.

Worsening of disability was considered clinically relevant if two independent clinical assessments 6 months apart indicated an increase of the EDSS as follows: +1.5 points (baseline = 0.0), +1.0 point (baseline = 1.0–4.0) and +0.5 points (baseline ⩾4.5). To determine progression to secondary progressive multiple sclerosis (SPMS), patient datasets were analysed according to Lorscheider criteria.[7] In addition, the fraction of patients having undergone confirmed worsening of disability in absence of a clinical relapse during the last 3 months ('progession independent of relapse activity' (PIRA)) was evaluated.

Further analyses were carried out using Fisher's exact test for categorical variables and Kruskal–Wallis test including Dunn's post-test for continuous variables where appropriate. A value of $p < 0.05$ was considered significant. All analyses were considered exploratory. Statistical analysis was conducted using SPSS Statistics 27 (IBM, NY, USA). All patients gave consent to data conduction, and ethical approval was given by local authorities (Ethical board of the Medical Council Westphalia-Lippe and the University of Münster; 2020-459-f-S).

Data availability statement

Anonymized patient data will be shared with qualified investigators upon reasonable request.

Results

Patients

During observation period, 313 patients were treated at both centres. Of those, 43 patients were excluded: 39 had a follow-up shorter than 6 months, 2 patients retrospectively fulfilled Lorscheider criteria for SPMS conversion at baseline and 1 patient was exposed to mitoxantrone earlier in their disease course.

In total, 270 patients were included. Notably, we lost two patients to follow-up and hence only evaluated their datasets until month 12. Ninety-seven (36%) patients were treatment-naïve, whereas the other patients had previously been treated with different DMTs. Among those pre-treated patients, 74 patients received one previous DMT (27%), 49 received two previous DMTs (18%) and 50 patients received three or more previous DMTs (19%).

Our patients had a median age of 39 years and a median disease course of 6 years since RMS onset, and the median EDSS score was 2.0 indicating a low disability burden. None of the patients fulfilled the Lorscheider criteria for SPMS conversion at baseline. Table 1 shows baseline epidemiological data of our cohort. Median follow-up duration was 25 months. Follow-up duration did not differ significantly between patient subgroups stratified according to the last previous DMT ($p = 0.253$). A total of 234 patients passed month 12 (and hence received the dose of 3.5 mg/kg) and 142 patients passed month 24. In total, data are representative of 6496 patient-months. The recommended treatment interval between first and second course was usually maintained with the exception of 17 patients who received their second course 14–17 months following induction as a consequence of the COVID-19 pandemic. Notably, 11 patients decided to postpone their second course in the hopes of upcoming vaccination at the time of data analysis.

Of 142 patients having passed month 24, 5 patients received additional courses of cladribine (months 24, 25, 28, 34 and 36, respectively) within the observation period due to ongoing disease activity. In addition, three patients were switched to treatment with ocrelizumab due to ongoing disease activity in month 12 instead of undergoing the second course of cladribine.

Clinical efficacy

We observed 85 relapses in 69 patients in our cohort following cladribine induction in contrast to 279 relapses in 191 patients in the year prior to induction (132 relapses within 6 months prior to cladribine induction). Median time to first relapse was 9 months and 40 patients had a relapse within the first year of treatment. Sixty-five patients experienced confirmed worsening of disability during the observation period. Conversely, EDSS scores as well as the total cranial T2-hyperintense lesion load remained stable in the majority of patients (Figure 1(a) and (b)). Evaluation of cranial MRI data (1194 follow-up scans available in 259 patients (96%)) showed 218 new or enlarging T2-hyperintense lesions in 104 patients. Overall, the relapse rate per treatment epoch substantially declined compared to the last 6-month epoch prior to induction and similar findings were made for detection of new or enlarging T2-hyperintense MRI lesions per epoch compared to baseline (Figure 1(c) and (d)).

Baseline T2 lesion load was equally distributed among patients except for patients having previously been treated with natalizumab ($p < 0.001$ for all comparisons to other DMT). All patients coming from natalizumab ($n = 23$) were switched due to increased risk for development of progressive multifocal leukoencephalopathy while having been clinically stable before. Thus, only four patients following natalizumab exposed new MRI lesions in their baseline MRI (all lesions were contrast-enhancing attributing their development to natalizumab cessation). One of these patients developed a clinical relapse during the washout period. Among other patients who stopped their previous immunomodulatory treatment, three further relapses were identified within the washout period. All these relapses resolved quickly following the administration of intravenous methylprednisolone, and none of the patients experienced confirmed worsening of disability because of these relapses. Of those, two relapses were observed in patients having stopped treatment with daclizumab due to market withdrawal, whereas one relapse was documented in a patient who stopped fingolimod treatment due to ongoing disease activity (indicated by multiple contrast-enhancing lesions in their last previous MRI).

We next aimed for investigation of potential risk factors for suboptimal disease control by cladribine treatment and stratified patients according to their last previous DMT:

1. Since no relevant differences in both baseline and follow-up parameters were observed between patients previously exposed to beta-interferons, glatiramer acetate or teriflunomide, these patients were combined to a group termed ‘platform’ treatment.
2. We excluded the subgroup of previously daclizumab-treated patients since patient numbers were low and this substance is no longer approved for treatment of RMS patients. However, we did not observe notable differences in efficacy or – eventually more important – safety outcomes in patients coming from daclizumab (data not shown).

Generally, follow-up duration was equally distributed among treatment groups (Supplemental Figure S1).

Univariate analysis using the Kaplan–Meier method suggested an impact of the previous immunotherapy (Supplemental Figure S2). However, since we aimed to rule out disease activity predominantly driven by rebound following cessation of the last previous DMT, we performed re-baselining to month 6 parameters following cladribine induction (Figure 2).

Table 1. Baseline characteristics of our cohort.

	Whole cohort	Naïve	'Platform'		DMF	FTY	NTZ	DAC
			IFN/GLAT	TRF				
Patients	270	97	59	21	42	18	23	10
Age, years, median (IQR)	39 (32–44)	38 (29–44.5)	39 (32–44)	37 (29–48)	40 (34–48)	37 (31–48)	40 (35–44)	43 (37–50)
Male sex, no. (%)	104 (39)	38 (39)	19 (32)	8 (38)	17 (41)	8 (44)	9 (39)	5 (50)
Disease duration, years, median (IQR)								
Since onset	6 (2–12)	1 (1–6.5)	8 (4–13)	7 (3.5–11)	5.5 (4–13.5)	10 (7–16.5)	8 (6–14)	14 (10–15)
Since diagnosis	4 (1–9)	0 (0–1)	5 (2–9)	4 (2–9)	4.5 (2–12)	7.5 (6–11.5)	6 (4–9)	12 (9–15)
EDSS at baseline, median (IQR)	2.0 (1.5–3.0)	2.0 (1.0–3.0)	2.0 (1.5–2.5)	2.0 (1.5–3.0)	2.0 (1.0–3.0)	3.0 (2.0–3.5)	2.5 (1.5–3.0)	3.5 (2.0–4.0)
ARR at baseline, median (IQR)	1 (0–2)	1 (1–2)	1 (0–2)	1 (1–2)	1 (0–1)	1 (0–1.5)	0 (0–1)	0 (0–1)
Number of previous DMT, median (IQR)	1 (0–2)	x	1 (1–2)	1 (1–2)	2 (1–3)	3 (2–4)	2 (1–3)	3 (2–4)
Number of last previous DMT, no. (%)								
0	97 (36)	97 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1	74 (27)	0 (0)	39 (66)	11 (52)	16 (38)	0 (0)	7 (30)	1 (10)
2	49 (18)	0 (0)	11(19)	6 (29)	14 (33)	8 (44)	7 (30)	3 (30)
⩾3	50 (19)	0 (0)	9 (15)	4 (19)	12 (29)	10 (56)	9 (40)	6 (60)
Washout duration of last previous DMT, days, median (IQR)	39 (13.5–72)	x	11 (3–23)	65 (44–92)	32.5 (13–56)	82 (56–117)	66 (49–81)	115 (75–190)
Number of baseline T2 MRI lesions, median (IQR)	15 (11–20)	13 (9–18)	14 (11–18)	14 (12–17)	15 (11–19)	21 (13.5–24)	25 (19–28)	20 (13–25)

IQR: interquartile range; EDSS: expanded disability status scale; ARR: annualized relapse rate; DMT: disease-modifying treatment; IFN: beta-interferon; GLAT: glatiramer acetate; DMF: dimethyl fumarate; TRF: terriflunomide; FTY: fingolimod; NTZ: natalizumab; DAC: daclizumab.

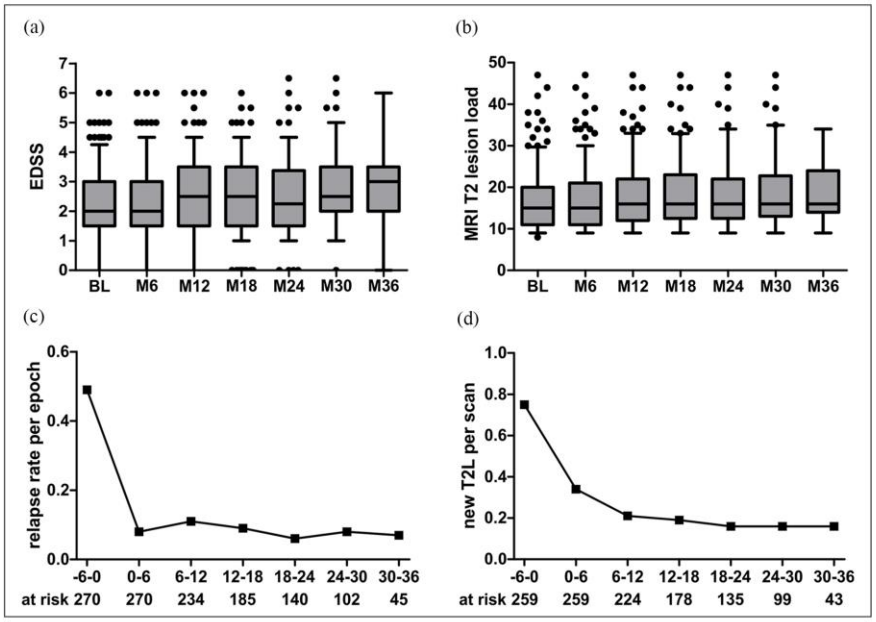

Figure 1. Efficacy outcomes of our cladribine cohort. (a) Expanded disability status scale (EDSS) scores at baseline and within follow-up. Boxes indicate 25%–75% interquartile ranges; lines indicate the median. Whiskers include 5%–95% of patients. (b) Total T2-hyperintense MRI lesion load at baseline and within follow-up. (c) Development of mean relapse rates per 6-month treatment epoch. (d) Development of MRI T2-hyperintense lesion load calculated as new T2-hyperintense lesion per scan conducted within end of the indicated treatment epoch. Numbers at risk are listed below (c) and (d) and also refer to (a) and (b), respectively.
MRI: magnetic resonance imaging.

Here, patients following natalizumab appeared to be substantially prone to clinical and paraclinical disease activity following induction of cladribine. Accordingly, 18 of 23 patients exhibited disease activity following cladribine induction. Of those, 12 experienced this already within 6 months following treatment induction, which of course could have also been driven by rebound activity following natalizumab cessation.

Multivariate regression analyses were performed using the Cox-proportional hazards model following re-baselining of patient data to month 6. A first model confirmed natalizumab pre-treatment as a relevant risk factor for relapses following cladribine induction (Table 2A). Adjusted hazard ratio (HR) was 4.771 (95% confidence interval (CI): 2.074–10.972; $p < 0.001$) against natalizumab (reference: naïve patients). Notably, male patients were less prone to relapses (adjusted HR: 0.473 (95% CI: 0.251–0.889; $p = 0.020$)).

Previous exposition to natalizumab was confirmed as a risk factor in a model using 'time to confirmed worsening of disability' as a dependent variable with an adjusted HR of 8.582 (95% CI: 3.583–20.555; $p < 0.001$)) with none of the further covariates being selected (Table 2B).

Regarding abundance of new or enlarging T2-hyperintense MRI lesions following cladribine induction, natalizumab was again identified as a relevant risk factor for development of new or enlarging T2-lesions with an adjusted HR of 5.168 (95% CI: 2.406–11.102; $p < 0.001$; Table 2C).

Consequently, patients switching from natalizumab to cladribine were also more prone to lose their status of NEDA3 with an adjusted HR of 5.162 (95% CI: 2.646–10.070; $p < 0.001$; Table 2D).

Progression independent of relapse activity (PIRA) was evaluated in 21 patients. Of those, two patients fulfilled Lorscheider criteria for SPMS progression.

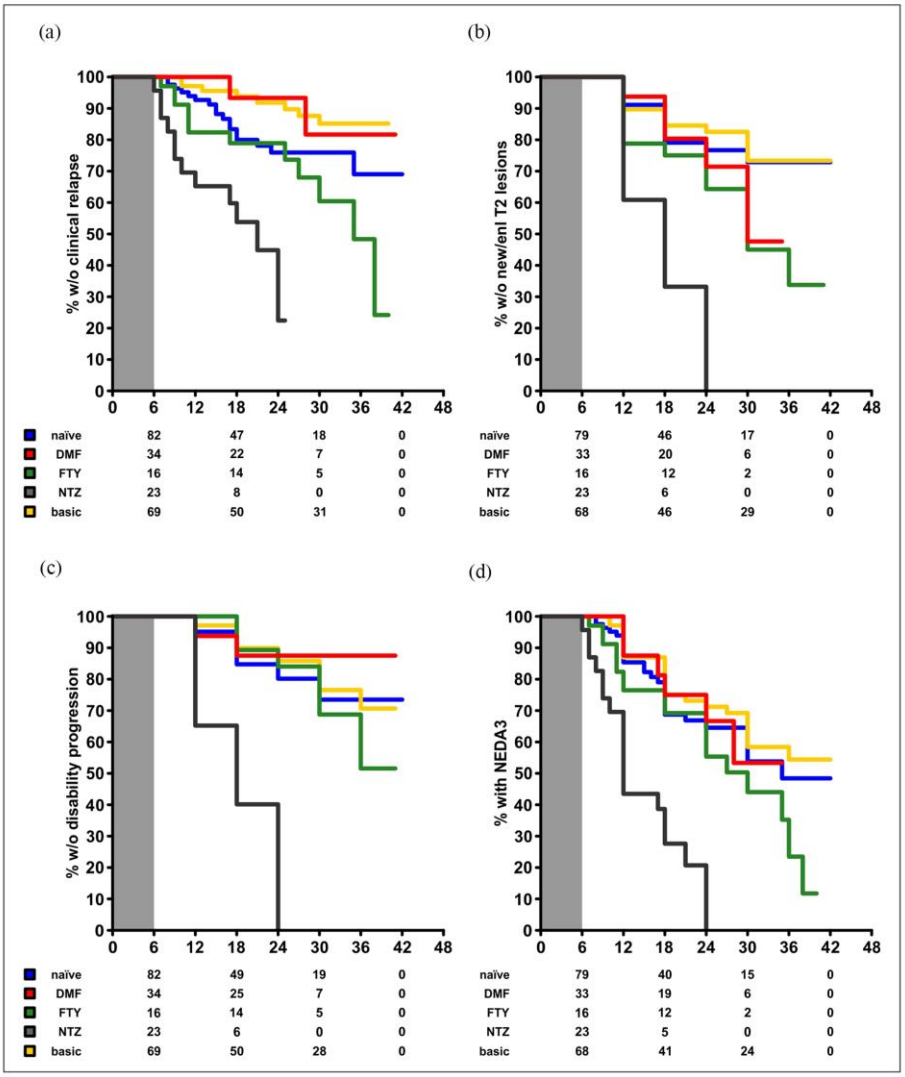

Figure 2. Efficacy outcomes of our cohort stratified according to the last previous DMT using the Kaplan–Meier method. Data are re-baselined towards month 6. Numbers at risk are displayed below the respective graphs. (a) Proportion of patients without a clinical relapse over time. (b) Proportion of patients without confirmed worsening of disability over time. (c) Proportion of patients without new or enlarging T2-hyperintense MRI lesions. (d) Proportion of patients with persistent NEDA3 status over time.
DMF: dimethyl fumarate; FTY: fingolimod; NTZ: natalizumab; NEDA3: no evidence of disease activity-3; MRI: magnetic resonance imaging; DMT: disease-modifying treatment.

Among patients having experienced PIRA, no significant difference among last previously administered DMT became visible ($p = 0.572$).

Lymphopenia and herpes virus infections

Complete longitudinal data on blood lymphocyte levels were available in 226 of 243 (93%) patients.

Table 2. Cox proportional hazards models using the outcome parameters depicted in Figure 1 as dependent variables.

	HR	95% CI	*p* value
A (time to first clinical relapse)			
Last previous DMT (naïve = ref.)			
Platform (IFN/GLAT/TRF)	0.588	0.161–1.338	0.436
Dimethyl fumarate	1.825	0.833–3.997	0.133
Fingolimod	0.476	0.105–2.155	0.335
Natalizumab	**4.771**	**2.074–10.972**	**<0.001**
Male vs. female sex (ref.)	**0.473**	**0.251–0.889**	**0.020**
Age at baseline (years)	0.999	0.965–1.034	0.961
MS duration since onset (years)	0.998	0.953–1.045	0.919
ARR at baseline	1.042	0.741–1.464	0.815
EDSS at baseline (<3.0 = ref.)	0.870	0.417–1.816	0.710
B (time to first confirmed worsening of disability)			
Last previous DMT (naïve = ref.)			
Platform (IFN/GLAT/TRF)	1.075	0.484–2.386	0.859
Dimethyl fumarate	1.293	0.503–3.320	0.594
Fingolimod	0.725	0.153–3.432	0.685
Natalizumab	8.582	**3.583–20.555**	**<0.001**
Male vs. female sex (ref.)	1.009	0.571–1.781	0.976
Age at baseline (years)	0.996	0.963–1.031	0.824
MS duration since onset (years)	0.940	0.819–1.064	0.129
ARR at baseline	0.864	0.655–1.168	0.233
EDSS at baseline (<3.0 = ref.)	1.085	0.539–2.186	0.819
C (time to first new/enlarging T2 MRI lesion)			
Last previous DMT (naïve = ref.)			
Platform (IFN/GLAT/TRF)	0.939	0.455–1.939	0.866
Dimethyl fumarate	2.011	0.933–4.331	0.074
Fingolimod	1.432	0.489–4.189	0.512
Natalizumab	**5.168**	**2.406–11.102**	**<0.001**
Male vs. female sex (ref.)	0.809	0.487–1.344	0.413
Age at baseline (years)	0.987	0.958–1.016	0.369
MS duration since onset (years)	1.009	0.968–1.050	0.680
ARR at baseline	0.928	0.684–1.258	0.629
EDSS at baseline (<3.0 = ref.)	0.836	0.442–1.578	0.580
D (time to first loss of NEDA3)			
Last previous DMT (naïve = ref.)			
Platform (IFN/GLAT/TRF)	0.889	0.508–1.554	0.679
Dimethyl fumarate	1.655	0.885–3.093	0.114
Fingolimod	1.069	0.423–2.699	0.888
Natalizumab	**5.162**	**2.646–10.070**	**<0.001**
Male vs. female sex (ref.)	0.720	0.468–1.108	0.135
Age at baseline (years)	0.987	0.963–1.011	0.287
MS duration since onset (years)	0.982	0.947–1.018	0.314
ARR at baseline	0.898	0.693–1.163	0.415
EDSS at baseline (<3.0 = ref.)	1.281	0.781–2.101	0.326

HR: hazard ratio; CI: confidence interval; DMT: disease-modifying treatment; IFN: beta-interferon; GLAT: glatiramer acetate; TRF: teriflunomide; MS: multiple sclerosis; ARR: annualized relapse rate; EDSS: expanded disability status scale; MRI: magnetic resonance imaging; NEDA3: no evidence of disease activity-3. A: Proportion of patients without a clinical relapse over time. B: Proportion of patients without confirmed worsening of disability over time. C: Proportion of patients without new or enlarging T2-hyperintense MRI lesions. D: Proportion of patients without loss of NEDA3 over time. Bold values represent significant covariates.

Baseline lymphocyte counts were >1200/mm³ in all patients prior to first treatment course and >800/mm³ prior to re-exposition. Datasets were censored beyond month 24 since the number of available datasets decreased substantially afterwards. Patients in whom the second course of cladribine was delayed due to COVID-19 pandemic were also excluded from this analysis (17 patients).

Overall, our patients largely reflected the well-known pattern of lymphocyte kinetics following cladribine exposition with two peaks of lymphopenia in months 3 and 14, respectively.

Stratified according to CTCAE v5.0, 2 patients (0.7%) were spared from this phenomenon, 216 patients (80.0%) developed grade I–II lymphopenia, whereas 48 patients (17.8%) suffered from grade III and 3 patients (1.1%) from grade IV lymphopenia.

Compared to previously naïve patients, individuals having previously received DMT experienced slightly pronounced decreases of lymphocyte counts. However, this did not lead to an increase of the respective CTCAE severity grade in the majority of patients. Patients following glatiramer acetate, beta-interferons or teriflunomide again showed similar trends and were again merged to one group (Figure 3(a)–(c)).

Contrasting this, patients following dimethyl fumarate (DMF) were substantially prone to development of severe lymphopenia already in year 1, although their baseline lymphocyte counts were not lower compared to other treatment groups (DMF: median 1930 (interquartile range (IQR): 1540–2270) vs. other: median 2000 (IQR: 1650–2390); $p = 0.2695$; Figure 3(d)). Even more surprisingly, baseline lymphocyte counts between previously DMF-exposed patients who developed grade III–IV lymphopenia were not lower compared to DMF-exposed patients without this phenomenon (I–II: median 1970 (IQR: 1590–2325) vs. III–IV: median 1850 (IQR: 1475–2210); $p = 0.3651$). In total, 21 patients coming from DMF (50%) were affected with grade III–IV compared to 29 patients with a different last previous DMT (13%) (Figure 3(e)).

Nine patients (21%) required a delay of cladribine re-exposition in month 12 (range: 2- to 8-month delay) due to ongoing lymphopenia compared to one previously beta-interferon-exposed and one naïve patient (4- and 7-month delay).

Multivariate binary logistic regression confirmed DMF pre-treatment as a risk factor for development of grade III–IV lymphopenia with an adjusted odds ratio of 5.037 (95% CI: 2.108–12.034; $p < 0.001$). Of note, patients with an EDSS score above 3.0 at cladribine induction were also more likely to experience severe lymphopenia (adjusted OR: 2.761 (95% CI: 1.255–6.075; $p = 0.007$)). None of the further covariates were selected (Table 3).

We also evaluated the occurrence of herpes infections in our cohort and identified 33 patients who suffered thereof at least once. Of those, 23 patients suffered from herpes simplex infections, whereas 22 developed herpes zoster manifestations (two cases of cranial nerve involvement with one case of zoster ophthalmicus and one case of zoster oticus were noted). Whereas herpes simplex infections usually resolved following local treatment (apart from three cases that received intravenous acyclovir due to symptom persistence and concomitant lymphopenia), all herpes zoster patients received intravenous acyclovir treatment. Despite this, nine cases of postherpetic neuralgia were observed and this involves the patient with previous zoster ophthalmicus who continues to suffer from trigeminal neuropathy. A transient Ramsay Hunt syndrome in the patient with zoster oticus resolved completely within 2 months. No case of herpes virus-associated encephalitis was observed.

Notably, all patients presented with sufficient anti-varicella-zoster virus titres at baseline. Lymphopenia was present in all cases of herpes zoster, and in 11 patients, lymphocyte counts were <500/mm³ at zoster manifestation. Herpes simplex manifested also in 13 patients with lymphocyte counts >1000/mm³ (Figure 3(f)). Remarkably, herpes infections mostly occurred in year 1 (34 of 45 patients, Figure 3(g)), although 14 patients experienced further episodes of herpes simplex manifestation in year 2. There were no cases of recurrent zoster manifestation. Since lymphopenia appears as major risk factor for development of zoster manifestation, it was not surprising to see the majority of patients coming from the previously DMF-exposed group, whereas most herpes simplex cases were observed in patients following platform treatment (as this was simply the largest subgroup; Figure 3(h)).

Unfortunately, data on conduction of oral acyclovir prophylaxis within lymphopenia are only incomplete in our cohort (although there is documentation of such prescription in 21 of 50 patients). Therefore, no assessment of efficacy of this measure can be deducted from our cohort.

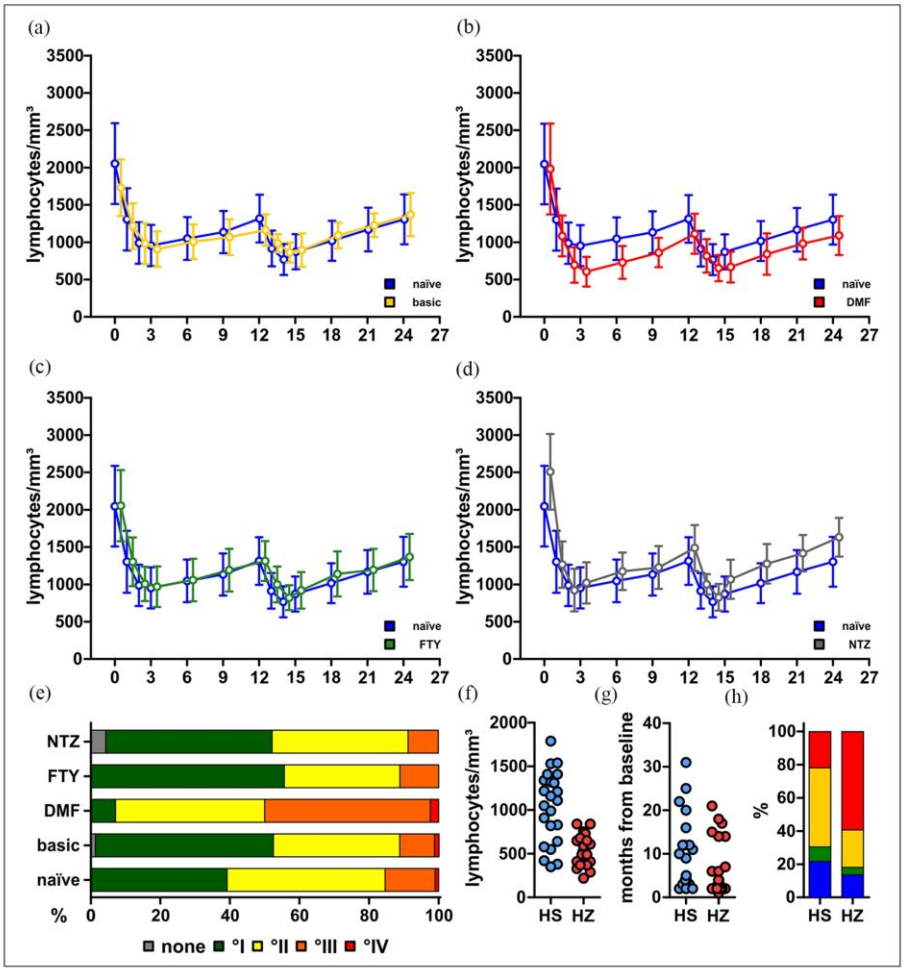

Figure 3. Lymphocyte levels and safety outcomes of our cohort. (a–d) Absolute lymphocyte counts over time since baseline in different treatment groups compared to naïve patients. (a) Naïve (n = 73) vs. platform (IFN/GLAT/TRF; n = 69); (b) naïve vs. DMF (n = 35); (c) naïve vs. FTY (n = 17); (d) naïve vs. NTZ (n = 23). Data are expressed as mean ± standard deviance. Data are censored at month 24. (e) Proportions of patients with the respective lymphopenia severity grade according to the CTCAE at their nadir. (f) Absolute lymphocyte counts at disease manifestation in patients with herpes simplex (HS) and herpes zoster (HZ). (g) Treatment months since baseline at disease manifestation in patients with HS and HZ. (h) Patients with HS and HZ according to their last previous DMT (blue: naïve; green: FTY; yellow: IFN/TRF/GLAT; red: DMF).
IFN: beta-interferon; GLAT: glatiramer acetate; TRF: teriflunomide; DMF: dimethyl fumarate; FTY: fingolimod; NTZ: natalizumab; CTCAE: Common Terminology Criteria for Adverse Events; DMT: disease-modifying treatment.

Discussion

Here, we present a large longitudinal real-world dataset on 243 RMS patients following cladribine treatment from two tertiary centres. Overall, our cohort closely resembled those in the pivotal CLARITY trial as median age at baseline, sex distribution and the proportion of previously treatment-naïve patients are fairly comparable. However, disease duration (mean: 7.9 vs.

Table 3. Binary logistic regression model with 'development of grade III/IV lymphopenia' as a dependent variable.

	OR	95% CI	*p* value
Last previous DMT (naïve = ref.)			
Platform (IFN/GLAT/TRF)	0.621	0.244–1.578	0.317
Dimethyl fumarate	**5.037**	**2.108–12.034**	**<0.001**
Fingolimod	0.500	0.098–2.547	0.404
Natalizumab	0.406	0.082–2.016	0.270
Male vs. female sex (ref.)	0.508	0.238–1.082	0.079
Age at baseline (years)	1.009	0.970–1.050	0.653
MS duration since onset (years)	1.032	0.983–1.084	0.209
EDSS at baseline (<3.0 = ref.)	**2.761**	**1.255–6.075**	**0.007**

DMT: disease modifying treatment; IFN: beta-interferon; GLAT: glatiramer acetate; TRF: teriflunomide; EDSS: expanded disability status scale; OR: odds ratio; CI: confidence interval; MS: multiple sclerosis.
Bold values indicate significant covariates.

8.9 years among the CLARITY study population) and median EDSS score at baseline (mean: 2.3 vs. 2.9) were lower in our cohort.[4] Our data underline that cladribine treatment results in a profound reduction of relapse rates per treatment epoch as well as abundance of new or enlarging T2-hyperintense MRI lesions.

Since the amount of DMT approved for RMS has tremendously increased since completion of the clinical development programme, the exact position of cladribine within the therapeutic armamentarium is of interest. Two previous studies suggested that cladribine was more efficacious than beta-interferons, comparable to fingolimod and less efficacious to natalizumab.[8,9] Generally, our data corroborate these findings. Patients coming from platform therapies and previously naïve patients experienced sufficient control of disease activity in the majority of cases. Patients following fingolimod pre-treatment experienced predominantly paraclinical disease activity following treatment switch yet most patients experienced disease stability after having passed month 6. However, patients following natalizumab pre-treatment were not only prone to natalizumab cessation-related disease reactivation but also experienced disease activity throughout the whole follow-up time contrasting data from previous case series.[10] Disease reactivation following natalizumab is a well-known phenomenon and is observed in patients switching to fingolimod[11] but can be controlled by subsequent use of high-efficacy treatment including alemtuzumab or rituximab.[12,13]

We were unable to demonstrate superiority or inferiority of cladribine or fingolimod to each other since disease activity in this group mostly restricted to the early period following switch.

We also evaluated lymphocyte counts in our cohort and were again able to reproduce kinetics known from previous clinical trials.[4,14] Yet, relevant lymphopenia was more common compared to data from clinical trials. Furthermore, we found that patients with DMF as last previous DMT were susceptible to development of severe lymphopenia. DMF exerts profound and long-lasting changes of the lymphocyte repertoire and mainly targets T cells.[15,16] Certain risk factors for development of lymphopenia among DMF-treated patients are described involving increased age and low baseline lymphocyte counts.[17,18] Among our patients, only few patients developed this phenomenon within DMF treatment and baseline lymphocyte counts were normal prior to cladribine induction. We can only speculate about the synergistic effect of previous DMF exposure and cladribine induction on lymphocyte counts. A previous hypothesis was that circulating T cells following DMF exposure represent DMF-insensitive cells as they were not more susceptible to induction of apoptosis than untreated cells in vitro.[19] Our clinical observations however contradict this hypothesis since pronounced lymphopenia in year 1 compared to year 2 indeed indicates prolonged susceptibility of lymphocytes. We also observed several relapses in cladribine patients previously treated with DMF, even in patients with lymphopenia. However, relapses during DMF-related lymphopenia have been observed before.[20]

Notably, we did observe neither prolonged lymphopenia nor increased relapse rates compared to naïve patients following teriflunomide as last previous DMT, despite the known property to interfere with T cell proliferation via alteration of T cell metabolic properties.[21]

Switching from DMF to cladribine seems to be problematic and requires a closer clinical monitoring since lymphopenia affected the majority of patients and subsequent zoster manifestations were substantially more common than was expected from previous studies.[14] The question arises whether patients should definitely be immunized with the newly available herpes zoster vaccine before switching from DMF to cladribine.[22]

Our study faces some limitations. These include of course the non-controlled real-world setting and unknown existence of confounders in our patient subgroups. In addition, we can currently finally comment neither on long-term outcomes nor on the proportion of patients requiring additional treatment courses. Nonetheless, this cohort represents a high number of patient years considering the time passed since approval of cladribine. Furthermore, follow-up density is high including thorough follow-up of MRI and lymphocyte count data.

Taken together, the efficacy and safety profile of cladribine appears consistent with previously published data. However, lymphopenia and subsequent herpes virus infections appear more abundant than has been suggested from the clinical development programme. Our data furthermore indicate that DMF might represent a risk factor for development of lymphopenia, and therefore, a decision towards cladribine as escalation treatment should be weighted carefully in those patients.

Authors' Note

All authors have read and agreed to the submitted version of the manuscript. This manuscript is not submitted to or under revision at another journal. The submitting author hereby declares that he takes responsibility for conduction of the study and analysis of the data and that he had full access to all study data. The submitting author furthermore declares that there are no competing interests concerning these data and that the authors have all rights to publish the data. The submitted manuscript does not contain data that have been published in any other journal. The authors have no related articles under submission.

Declaration of Conflicting Interests

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The local institutional review board (IRB) has approved the conduction of this trial. Further details are listed in the 'Methods' chapter.

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4.11 Immunological consequences of cladribine treatment in multiple sclerosis: A real-world study

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Original Article

Immunological consequences of cladribine treatment in multiple sclerosis: A real-world study☆

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ABSTRACT

Background: Cladribine is a synthetic deoxyadenosine analogue approved for the treatment of highly active relapsing multiple sclerosis (RMS). Cladribine is considered to be a semi-selective immune-reconstitution therapy (IRT) that induces long-term remission following short course of treatment. Here, we evaluated the effect of cladribine on immune cell reduction and reconstitution during the first two years of treatment.

Methods: We analyzed our longitudinal, prospective, real-world cohort of 80 cladribine-treated RMS patients from two tertiary centers in Germany. Laboratory testing was conducted monthly and included evaluation of cellular as well as soluble parameters. Laboratory outcomes were correlated with infectious adverse events (AEs) and clinical or paraclinical disease activity.

Results: Selective alterations in immune cell populations occurred following cladribine treatment, with the most marked effects observed in year two of treatment. Specifically, a rapid reduction in $CD56^+$ natural killer cells (nadir: month 1 (year 1) and 14 (year 2); -37 and -41% from baseline) was followed by a greater reduction in $CD19^+$ B cells (nadir: month 2 and 14; -81 and -82%); a moderate effect on $CD4^+$ (nadir: month 3 and 15; -48 and -61%) and $CD8^+$ T cells (nadir: month 5 and 18; -40 and -48%). Despite the marked effect on B cells, immunoglobulin levels were unaffected. There was no or minimal effect on thrombocytes and innate immune cells. Clinical and paraclinical disease activity was unrelated to the observed immune alterations. Lymphopenia was the most commonly observed AE (86.3% of patients; grade III-IV lymphopenia: 38.8%). The cumulative incidence of infections was 55% with cladribine treatment, which were mostly mild or moderate. In total, 19 herpes infections developed in 8 (10%) cladribine-treated patients; all cases were dermatomal and 94.7% of the herpetic infections occurred during a period of lymphopenia.

Conclusions: The immunophenotyping data obtained in our real-world setting are comparable to those demonstrated in pivotal clinical trials and provide further evidence that cladribine may represent a form of IRT.

Abbreviations: AE, adverse event; CTCAE, Criteria for adverse events; DCK, deoxycytidine kinase; DMT, disease modifying treatment; IRT, Immune reconstitution therapy; MRI, magnetic resonance imaging; NK cells, Natural killer cells; RCT, randomized clinical study; RMS, Relapsing multiple sclerosis; SD, standard deviation; SmPC, summary of product characteristics.

☆ All authors qualify for authorship according to current guidelines of Multiple Sclerosis and Related Disorders. All authors have read and agreed to the submitted version of the manuscript. The current manuscript is not submitted to or under revision at another journal.All authors have read and agreed to the conditions noted on the Authorship Agreement Form.The submitting author hereby declares that she takes responsibility for conduction and analysis of the data and that she had full access to all study data. The submitting author furthermore declares that there are no competing interests concerning these data and that the authors have all rights to publish the data. The submitted manuscript does not contain data that have been published in any other journal. The authors have no related articles under submission.

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However, regarding the side-effect profile of cladribine, severe lymphopenia (exceeding grade II CTCAE) was more frequent, which may have prompted the development of herpes infections. Of note, lymphocyte dynamics did not correlate with clinical and paraclinical measures of disease activity in the two-year follow-up period.

1. Introduction

Immune reconstitution therapy (IRT) is an emerging concept for the treatment of highly active relapsing multiple sclerosis (RMS) (Lunemann et al., 2020). Cladribine is considered the first short-course oral IRT. Immunological changes induced by cladribine in patients with RMS have been investigated in several clinical trials (Baker et al., 2017, Comi et al., 2019, Stuve et al., 2019) and the findings are generally supported by studies evaluating subcutaneous cladribine (Ceronie et al., 2018, Mitosek-Szewczyk et al., 2013). Here, cladribine had a pronounced effect on lymphocytes but minimal effect on innate immune function. While natural killer (NK) and B cell reduction was rapid, with pronounced B cell reduction, cladribine only modestly affected T cells; however, they were depleted in the long term after a course of cladribine.

Safety outcomes from randomized clinical studies (RCTs) have been reported previously (Cook et al., 2019, Cook et al., 2011, Leist et al., 2014, Giovannoni et al., 2010, Giovannoni et al., 2018). Lymphopenia was the most commonly reported adverse event (AE) in clinical trials and cladribine was not associated with an increased risk of infection, except for herpes zoster infections (Cook et al., 2019, Cook et al., 2011).

Unfortunately, RCTs supporting the approval of cladribine were initiated in 2010 and included patients who were either treatment naïve or previously given injectable treatments. However, sequential drug use in the real-world setting may affect efficacy, AE and long-term immunocompetence. Consequently, a comprehensive risk-benefit assessment critically depends on the evaluation of real-world safety (Rolfes et al., 2021) and efficacy data (Pfeuffer et al., 2021) in addition to those obtained from RCTs (Sormani and Laroni, 2017).

Therefore, the objective of the present study was to examine the reduction and reconstitution dynamics of immune cells and humoral factors after the two treatment courses of cladribine in a real-world setting. We further examined whether lymphocyte repopulation kinetics offer insights into the efficacy and safety-profile of cladribine, focusing on hematologic abnormalities and infections.

2. Methods

2.1. Study population

Adult, cladribine-treated patients diagnosed with RMS according to the 2017 revised McDonald criteria (Thompson et al., 2018) were prospectively evaluated at two tertiary referral centers from November 2017 to June 2021. Administration of cladribine was performed according to the most recent summary of product characteristics (SmPC). Laboratory testing occurred monthly. Patients were evaluated clinically at baseline and every six months. Follow-up magnetic resonance imaging (MRI) was performed every six months. Safety assessment included monitoring for AEs, physical examination and laboratory parameters. Ethical approval was obtained from local authorities (5794R, 5951R and 20-9510-BO) and patients provided informed consent.

2.2. Laboratory measurements

The cell counts of leukocytes, lymphocytes, neutrophils, monocytes, and thrombocytes were assessed via standard hematology laboratory measures. Lymphocyte subsets were assessed in a central laboratory using flow cytometry. Total amount of primary immunoglobulin classes (IgA, IgM and IgG) and immunoglobulin subclasses (IgG1, IgG2, IgG3, and IgG4) were analyzed via latex-enhanced assay by kinetic nephelometry according to manufacturer guidelines (Mohn et al., 2020). Data were presented as absolute numbers (cells/µl) or unit volumes (immunoglobulins, g/l).

For each cell type, the mean absolute cell counts (± standard deviation (SD)) at each time point are summarized descriptively. The nadir for each cell type was defined as the lowest mean absolute cell count in each treatment year. The maximum (absolute or relative) change from baseline was calculated for each patient, defined as the lowest post-baseline value by the end of year 1 and year 2, and presented as the mean.

The grade of hematologic abnormalities was based on standardized definitions of the Common Terminology Criteria for adverse events version 5.0 (CTCAE, severe events were defined as grade III or IV). Threshold counts (indicating the lower level of normal) were defined as 4.1×10^3 cells/µl for leukocytes, 1×10^3 cells/µl for lymphocytes, 2.0×10^3 cells/µl for neutrophils, 300 cells/µl for monocytes, 140×10^3 cells/µl for thrombocytes, and 100 cells/µl, 500 cells/µl, 350 cells/µl, 200 cells/µl, 18 cells/µl and 75 cells/µl for $CD19^+$ B cells, $CD3^+$, $CD4^+$, $CD8^+$ and activated ($HLA\text{-}DR^+$) T cells, and NK cells, respectively. Moreover, a CD4/CD8 ratio ≥ 1.5 was defined as normal. For immunoglobulin levels, normal values were set as follows: IgG >7 g/l, IgA >0.8 g/l and IgM >0.4 g/l.

2.3. Data analysis

Epidemiological data at baseline were evaluated using descriptive statistics. All analyses of changes in hematological components for the year 1 and year 2 data were summarized using descriptive statistics, including number of patients, mean and SD.

2.4. Data availability statement

Anonymized patient data will be shared with qualified investigators upon reasonable request.

3. Results

3.1. Patients

80 patients were included in our prospective study, with demographic data given in Table 1.

All 80 patients provided full laboratory data for treatment year 1, and 51 patients (63.8%) completed their dosing scheme of 3.5 mg/kg in year 2 with full laboratory datasets until month 24. Of the scheduled 262 clinical and radiological visits, 251 (95.8%) and 233 (88.9%) datasets were available, respectively. Data are representative of 1,572 patient-months.

3.2. In vivo effect of cladribine on hematological components

Rapid depletion of the total leukocyte population occurred after each treatment course, reaching the nadirs at months 4 and 13 (maximum change from baseline: -28 and -40%, respectively; Fig. 1A, Supplemental Table 1). Thereafter, leukocyte counts slowly recovered yet remained below baseline (-14 and -19% at months 12 and 24, respectively).

Lymphocytes rapidly declined from baseline until months 2 and 14 during years 1 and 2 (-50 and -58%), respectively, which was followed by gradual recovery (Fig. 1A, Table 2). At the end of each treatment year, mean absolute lymphocyte counts still remained below the baseline level (-26% and -37% in months 12 and 24, respectively).

Table 1
Baseline characteristics of the cladribine cohort; total n = 80 patients.

Cladribine cohort	Year 1	Year 2
Patients, No. (%)	80 (100.0)	51 (63.8)
Age, years, median (IQR)	41 (31-50)	42 (31-49)
Male patients, No. (%)	20 (25.0)	13 (25.5)
Disease duration, years, median (IQR)	6.1 (1.8-13.3)	5.6 (1.8-11.3)
EDSS Baseline, median (IQR)	2.5 (1.5-3.0)	2.0 (1.5-3.5)
ARR Baseline, median (IQR)	1 (0-2)	1 (0-2)
Total number of previous DMT, median (IQR)	1 (0-2)	1 (0-2)
Last previous DMT, No. (%)	30 (37.5)	
- treatment-naïve	40 (50.0)	17 (33.3)
- platform treatment	10 (12.5)	27 (52.9)
- escalation treatment		7 (13.7)
Reason for switch to cladribine, No. (%)	30 (37.5)	
- treatment initiation	42 (52.5)	17 (33.3)
- disease progression	8 (10.0)	28 (54.9)
- adverse events		6 (11.8)
Wash out duration, days, median (IQR)	62 (20-179)	64 (20-220)

Disease duration was defined as the time period between symptom onset and first cladribine administration. *Platform* includes the following disease modifying therapies (DMT): interferon-beta, glatiramer acetate, teriflunomide, and dimethyl fumarate. *Escalation* includes fingolimod, natalizumab and daclizumab. The Expanded Disability Status Scale (EDSS) and the annualized relapse rate (ARR) refer to the time before cladribine initiation. IQR: interquartile range; No.: number

Reductions in neutrophils and monocytes were substantially smaller (Fig. 1A, Supplemental Table 1). Mean neutrophil counts transiently reduced at month 1 during year 1 and month 13 during year 2, but remained above the lower limit of normal at all follow-up time points and, in both years, recovered to baseline levels. Monocytes, however, demonstrated a slight and gradual reduction, with lowest mean counts at months 11 and 19 (-20% and -22%, respectively, Fig. 1A, Supplemental Table 1). They did not reach baseline levels again during the 24 months of follow-up (-13 and -18% in months 12 and 24, respectively).

Mean thrombocyte counts remained close to baseline levels at all time points (Fig. 1A, Supplemental Table 1).

Among different lymphocyte subsets, the first course of cladribine induced a gradual decline in T cells, reaching a maximal 43% reduction from baseline at month 5 after treatment initiation (Fig. 1B, Table 2). T cell counts then stabilized at a lower level than baseline before the second course of cladribine at month 12 was given (-31%). After the second course, maximum depletion was achieved (-58% in month 15) and maintained, with only a minimal continuous increase until month 24 (-41%).

Regarding T cell subpopulations, kinetics of $CD4^+$ and $CD8^+$ T cell depletion were similar to one another: a slow decline from baseline was followed by stabilization with little sign of recovery until months 12 and 24 (Fig. 1B). However, $CD4^+$ T cell depletion was faster and more pronounced (Table 2, $CD4^+$: months 3 (-48%) and 15 (-61%); $CD8^+$: 5 (-40%) and 18 (-48%), respectively). Those changes were reflected in the CD4/CD8 ratio, showing an early decline, especially in treatment year 2, without recovery to baseline values (Fig. 1B). Interestingly, the number of activated ($HLA\text{-}DR^+$) T cells transiently increased early after cladribine administration (+47%), followed by a gradual decline until the second course of cladribine at month 12 (-20%; Fig. 1B, Table 2). In treatment year 2 we observed a similar dynamic, without recovery of cells until the last follow-up (-34%).

Contrasting the findings in T cells, NK cells were rapidly depleted, reaching nadir counts at month 1 (-37%), and then recovered, approaching baseline levels at month 12 (+3%). Following the second course of cladribine, they followed a similar pattern of depletion and repopulation (Fig. 1B, Table 2).

Of note, cladribine had the greatest effect on B cells, rapidly reducing and nearly completely depleting B cell counts at month 2 after treatment initiation (-81%; Fig. 1C, Table 2). Mean B cell counts then recovered, reaching levels about -3% below baseline before the second course, after which a similar but more pronounced pattern of depletion and repopulation was observed (Fig. 1C, Table 2). Interestingly, mean serum levels of IgG, IgG subtypes, IgA, and IgM remained above the lower limit of the normal throughout the 24-month follow-up period (Fig. 1C). Of note, the aforementioned immune cell kinetics were independent of baseline demographic characteristics, except for a more pronounced lymphocyte depletion after previous escalation treatment in year 2 (Supplemental Fig. S1).

3.3. Comparison of immune cell kinetics in patients with a stable disease course versus those exhibiting disease activity

Following cladribine induction we observed in total 25 relapses in 18 patients (22.5%). Moreover, evaluation of cranial MRI data showed new or enlarging T2-hyperintense lesions in 16 patients (20.0%, Supplemental Fig. S2).

Of note, we did not detect a specific pattern in immune cell counts at the time of clinical or paraclinical disease manifestation (Fig. 2). No differences in long-term immune cell counts were apparent between the patient cohort experiencing disease activity and those who remained free of relapses and MRI activity (Supplemental Fig. S3).

3.4. Adverse events of special interest: hematologic events

Leukopenia was observed in 38 patients (47.5%, CTCAE grade I: 24 patients, 63.2%; grade II: 12 patients, 31.5%; grade III: 2 patients, 5.3%) at any follow-up time point. Leukopenia was more frequent in the first months following each cladribine course and showed a trend to be more pronounced in treatment year 2 (Fig. 3A). Of note, previous DMTs appeared not to be a risk factor for the development of leukopenia (Fig. 3B).

Lymphopenia occurred more frequently and was observed in 69 patients (86.3%). 38 patients (47.5%) developed grade I-II lymphopenia, whereas grade III-IV lymphopenias occurred in 31 patients (38.8%, Table 3). Moreover, lymphopenia was more frequent and pronounced after re-dosing in year 2 (Fig. 3C).

Of note, only one patient had persistent severe lymphopenia that had not returned to at least grade II at the last study visit. However, 23 patients (28.8%) in year 1 and 16 patients (31.3%) in year 2 did not demonstrate recovered lymphocyte counts at months 12 and 24 (Fig. 3C). In line with this, lymphopenia was the most common AE leading to treatment discontinuation (6 patients, 7.5%; Table 3) and in 2 patients (2.5%) the second course was postponed because of persistent lymphopenia at month 12. Patients previously treated with escalation immunotherapies were more prone to develop severe lymphopenia following cladribine treatment compared to naïve patients or those treated with platform immunotherapies (Fig. 3D).

In addition, neutropenia occurred in 16 patients (20%). Grade III neutropenia occurred in 3 patients (3.8%), while grade IV neutropenia was not observed. Finally, no cases of thrombocytopenia were associated with cladribine treatment.

3.5. Adverse events of special interest – Infections and infestations

Infections occurred in 44 patients (55%). In treatment year 1, 38 patients (47.5%) demonstrated at least one infection, compared to 17 patients (33.3%) in treatment year 2. 94.6% of the infections were rated as mild or moderate. Only 3 patients experienced a severe infection: sepsis requiring hospitalization; herpes zoster requiring hospitalization; and a dental abscess requiring surgical intervention. Infections that occurred in greater than 2% of patients at any time point are listed in Table 4 and infections with an incidence of ≥5% were analyzed in more detail (Supplemental Fig. S4).

In our cohort 8 patients (10%) developed a total of 19 episodes of herpes infection (Table 4). Herpetic events frequently occurred shortly

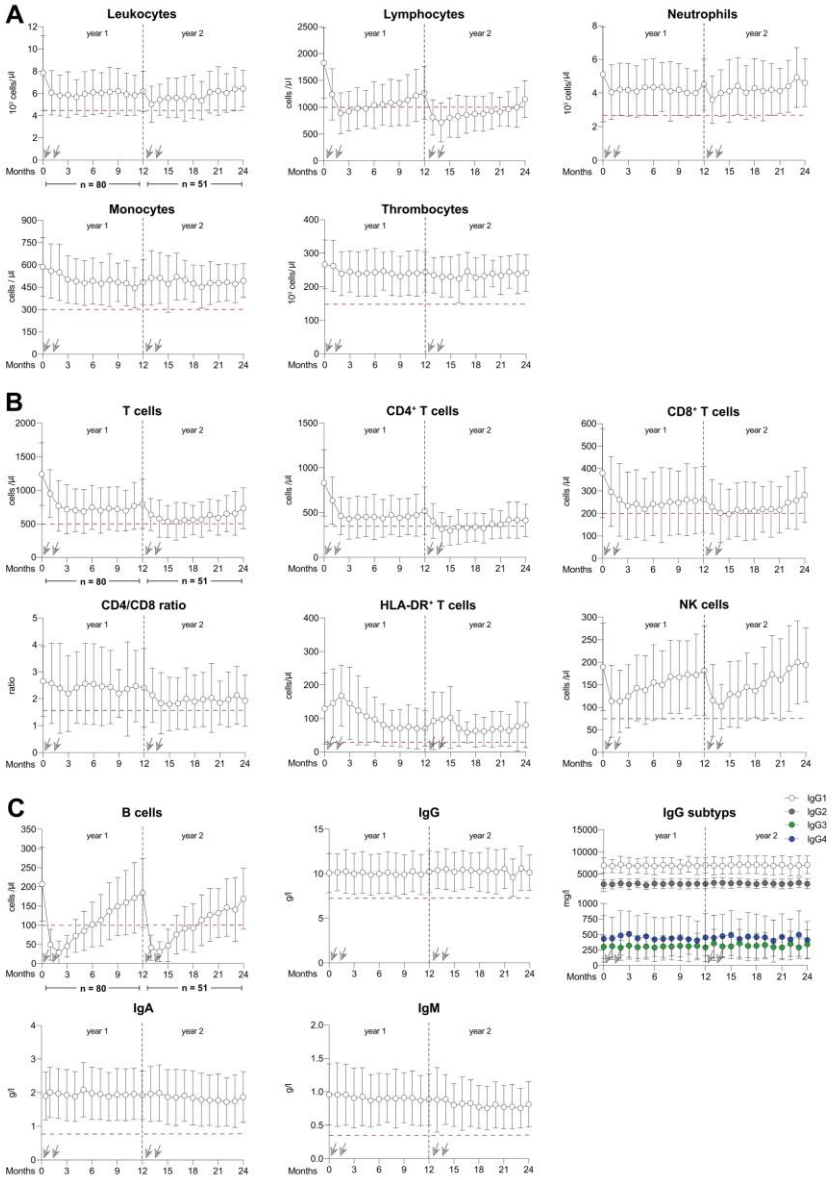

Fig. 1. ***In vivo*** **effect of oral cladribine on hematological components**. (**A-C**): Depletion and repopulation dynamics of peripheral blood cells over time in patients treated with cladribine in year 1 (n=80) and year 2 (n=51). Peripheral blood was taken and analyzed on a monthly basis. The results represent the mean absolute cell counts ± standard deviation or unit volumes (immunoglobulins (Ig), g/l). Cladribine was administered with a cumulative dose of 3.5 mg/kg in monthly courses (gray arrows) at baseline (month 0) and months 1, 12, and 13. Results show the numbers of (**A**) leukocyte (subsets) and thrombocytes, (**B**) $CD3^+$ T lymphocytes and $CD4^+$ T-helper and $CD8^+$ cytotoxic T cells subsets (including the CD4/CD8 ratio), as well as activated HLA-DR^+ T cells and natural killer (NK) cells, and (**C**) $CD19^+$ B cells and humoral factors, including secreted immunoglobulins (Ig), their primary Ig-classes (IgG, IgA, and IgM), as well as IgG subtypes. The lower limit of normal for each cell type or humoral factor is indicated by a horizontal line.

after cladribine dosing (Supplemental Fig. S4A). No clear association with patient age or sex was evident (Supplemental Fig. S4B). Interestingly, most herpes cases were observed in patients who had previously received a platform treatment, with the majority of patients coming from the previously dimethyl-fumarate-exposed group (62.5%; Supplemental Fig. S4C). All cases resolved without complication.

Upper respiratory tract infections were the most common reported infections (43 infections occurring in 31 patients, 38.8%, Supplemental Fig. S4A). In addition, 10 urinary tract infections developed in 7 patients (8.8%) and affected only women (Supplemental Fig. S4B). 'Other infections' comprised a total of 16 infections in 13 patients (20.0%): 4 cases of dental infections, 3 cases of lymphadenitis, 3 of conjunctivitis, and 3 again of unspecified febrile infection, as well as 2 cases of vaginal infection and 1 case of campylobacter sepsis. Of note, within these subgroups, we observed 3 cases of opportunistic infections (3.8%). All were mucocutaneous and cutaneous fungal infections (two cases of vaginitis and one of fungal urinary tract infection), which resolved with standard treatment.

3.6. Relationship between immunological alterations and infection risk following cladribine

94.8% of the patients who developed (recurrent) herpes infections had concomitant lymphopenia at the approximate time of infection (grade I, II and III: 31.6%, 42.1% and 21.1 %, respectively; Fig. 4A). Baseline lymphocyte counts of patients with herpes infections were lower than the 72 patients who did not suffer a herpes infection (Fig. 4A). Regarding lymphocyte subtypes, T cells (especially $CD4^+$ T

Table 2
Mean (standard deviation) counts of lymphocyte (subsets) in patients treated with cladribine.

Lymphocyte subset	Baseline	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11	Month 12
Year 1 (n = 80)													
ALC (10^3 cells/μl)	1811 (585)	1229 (434)	876 (381)	903 (347)	978 (390)	972 (336)	1029 (438)	1041 (381)	1077 (439)	1073 (424)	1136 (478)	1218 (493)	1263 (494)
Change *versus* BL		-587	-923	-916	-861	-871	-832	-778	-741	-796	-651	-622	-549
% Change *versus* BL		-32%	-50%	-49%	-45%	-45%	-44%	-41%	-38%	-39%	-33%	-31%	-26%
$CD16^+/56^+$ NK cells (cells/μl)	189 (98)	113 (80)	112 (69)	122 (64)	143 (73)	139 (76)	156 (84)	152 (80)	168 (82)	164 (80)	168 (73)	169 (89)	184 (100)
Change *versus* BL		-86	-84	-69	-49	-53	-41	-29	-11	-28	-20	-23	+5
-% Change *versus* BL		-37%	-36%	-28%	-19%	-21%	-16%	-6%	+7%	-3%	-2%	-2%	+3%
$CD19^+$ B cells (cells/μl)	207 (97)	50 (41)	29 (31)	45 (28)	72 (43)	83 (52)	103 (58)	114 (67)	136 (75)	151 (77)	159 (85)	171 (92)	184 (91)
Change *versus* BL		-158	-178	-160	-139	-127	-101	-92	-70	-69	-44	-41	-23
% Change *versus* BL		-75%	-81%	-75%	-61%	-54%	-40%	-32%	-18%	-17%	-7%	-9%	-3%
$CD3^+$ T cells (cells/μl)	1241 (468)	951 (359)	763 (374)	713 (365)	712 (321)	676 (335)	745 (333)	699 (338)	730 (321)	719 (329)	700 (302)	768 (351)	795 (366)
Change *versus* BL		-310	-510	-546	-560	-599	-589	-513	-546	-544	-500	-457	-434
% Change *versus* BL		-21%	-36%	-41%	-40%	-43%	-42%	-39%	-39%	-39%	-36%	-32%	-31%
$CD4^+$ T cells (cells/μl)	833 (374)	636 (267)	463 (214)	431 (231)	450 (235)	449 (223)	455 (254)	430 (212)	472 (220)	442 (212)	459 (204)	468 (236)	521 (267)
Change *versus* BL		-209	-374	-427	-411	-399	-417	-397	-396	-421	-356	-364	-303
% Change *versus* BL		-19%	-40%	-48%	-43%	-41%	-44%	-42%	-40%	-43%	-37%	-37%	-30%
$CD8^+$ T cells (cells/μl)	380 (200)	297 (156)	259 (162)	236 (151)	243 (152)	216 (139)	243 (142)	238 (169)	251 (151)	248 (141)	262 (162)	261 (152)	263 (148)
Change *versus* BL		-85	-117	-145	-153	-157	-154	-136	-148	-156	-117	-119	-117
% Change *versus* BL		-19%	-29%	-36%	-37%	-40%	-36%	-36%	-36%	-35%	-28%	-25%	-27%
Lymphocyte subset		Month 13	Month 14	Month 15	Month 16	Month 17	Month 18	Month 19	Month 20	Month 21	Month 22	Month 23	Month 24
Year 2 (n = 51)													
ALC (10^3 cells/μl)		810 (331)	741 (378)	803 (354)	827 (403)	850 (352)	878 (326)	892 (335)	951 (363)	918 (265)	966 (325)	998 (383)	1148 (349)
Change *versus* BL		-1098	-1097	-1041	-1082	-1088	-1040	-1010	-937	-1057	-884	-914	-781
% Change *versus* BL		-55%	-58%	-56%	-57%	-55%	-54%	-51%	-47%	-51%	-45%	-45%	-37%
$CD16^+/56^+$ NK cells (cells/μl)		115 (79)	104 (50)	131 (73)	128 (74)	143 (78)	137 (60)	156 (83)	172 (88)	157 (90)	186 (96)	200 (93)	194 (83)
Change *versus* BL		-91	-98	-61	-92	-60	-71	-39	-53	-56	-23	+10	+7
% Change *versus* BL		-34%	-41%	-18%	-37%	-23%	-32%	-9%	-14%	-14%	-1%	+9%	+8%
$CD19^+$ B cells (cells/μl)		42 (34)	32 (26)	47 (43)	74 (52)	93 (58)	93 (64)	113 (67)	124 (68)	132 (69)	150 (73)	141 (86)	169 (80)
Change *versus* BL		-165	-179	-146	-139	-123	-106	-93	-86	-70	-64	-61	-33
% Change *versus* BL		-79%	-82%	-76%	-63%	-53%	-53%	-42%	-37%	-32%	-27%	-30%	-13%
$CD3^+$ T cells (cells/μl)		637 (241)	577 (281)	526 (239)	528 (286)	557 (262)	558 (224)	578 (273)	614 (230)	591 (260)	652 (271)	653 (327)	731 (308)
Change *versus* BL		-680	-745	-825	-770	-708	-748	-725	-776	-712	-749	-709	-623
% Change *versus* BL		-47%	-55%	-58%	-57%	-54%	-54%	-53%	-52%	-52%	-52%	-47%	-41%
$CD4^+$ T cells (cells/μl)		405 (196)	313 (189)	299 (164)	331 (193)	332 (161)	336 (158)	337 (176)	362 (160)	375 (161)	418 (204)	416 (203)	413 (184)
Change *versus* BL		-463	-542	-521	-511	-520	-476	-502	-519	-470	-437	-458	-441
% Change *versus* BL		-49%	-61%	-61%	-58%	-60%	-54%	-57%	-59%	-51%	-49%	-47%	-46%
$CD8^+$ T cells (cells/μl)		229 (123)	202 (131)	201 (112)	209 (122)	210 (133)	211 (130)	219 (103)	215 (126)	231 (120)	238 (104)	259 (131)	282 (124)
Change *versus* BL		-205	-215	-207	-197	-199	-217	-209	-195	-187	-180	-173	-148
% Change *versus* BL		-42%	-47%	-47%	-44%	-46%	-48%	-43%	-45%	-39%	-37%	-34%	-25%

Shaded cells indicate the time points at which the largest changes from baseline (BL) occurred in each treatment year. Outlined in red are the largest changes from BL over the entire 2-year observation period for each cell type. Changes versus BL and percentage changes versus BL are the means of the individual values from each patient. ALC: absolute lymphocyte count

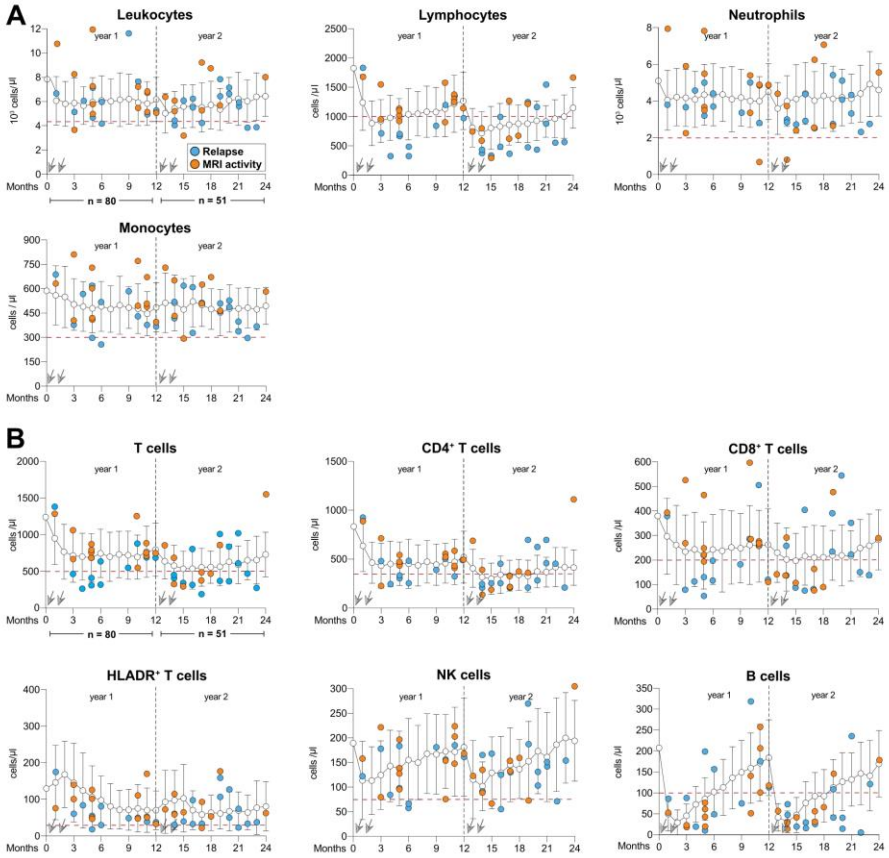

Fig. 2. Demyelinating CNS activity is not related to immune cell levels in peripheral blood. (**A-B**) Cladribine was administered as monthly courses (gray arrows) at baseline (month 0) and months 1, 12, and 13. The results of treatment year 1 (n=80) and year 2 (n=51) represent the mean absolute cell counts ± standard deviation of (**A**) leucocyte (subsets) and (**B**) lymphocyte (subsets), including $CD3^+$ T cells, $CD4^+$ T-helper and $CD8^+$ cytotoxic T cells, HLA-DR^+ activated T cells, $CD16^+/CD56^+$ natural killer (NK) and $CD19^+$ B cells. Each immune cell level at the approximately time of a relapse (blue circle) or at the time of a new emerging demyelinating CNS activity on magnetic resonance imaging (MRI, defined as a new or enlarging T2 lesion; orange circle) is depicted. The lower limit of normal for each cell type is indicated by a horizontal line.

cells accompanied by a low CD4/CD8 ratio) and B cells were decreased at the approximate time of herpes infection (Fig. 4B+C). Interestingly, activated (HLA-DR^+) T cells appeared to be present at an increased level in most of the patients suffering a herpes infection at the approximate time of disease manifestation (Fig. 4C).

Although not as markedly observed as with herpes infections, an association of lymphopenia with the occurrence of upper respiratory tract, urinary tract and other infections may be suggested, as those infections were also reported more frequently during periods of lymphopenia (Supplemental Figs. S6A and S7). Overall, 24 of the 44 patients (54.5%) who developed any infection experienced grade III or IV lymphopenia during the study. Infections developed in 24 of the 33 (72.7%) patients treated with cladribine who experienced grade III or IV lymphopenia and in 20 of 47 (42.6%) patients that did not experience severe lymphopenia during the study. Neutropenia and hypogammaglobulinemia do not represent risk factors for infections in our cohort (Supplementary Figs. S5 and S7).

4. Discussion

In line with immunophenotyping studies using data from pivotal clinical trials (Baker et al., 2017, Comi et al., 2019, Stuve et al., 2019) we observed a selective mainly lymphocyte-depleting, and long-lasting effect of cladribine. This was consistent with the relative levels of enzymatic activity of deoxycytidine kinase (DCK) and 5'-nucleotidases of these adaptive immune cells, determining sensitivity to cladribine toxicity (Ceronie et al., 2018).

All lymphocyte subsets appear vulnerable to cladribine-mediated cytotoxicity, whereas the magnitude and kinetics of depletion between subsets differed considerably: NK cells were the most rapid to reach nadir, followed by B cells, $CD4^+$ and finally $CD8^+$ T cells. Cladribine induced a marked depletion of B cells, a moderate reduction in T cells and smaller reductions in NK cells. These findings are in line with the relatively high B cell expression levels of DCK compared to T cells and NK cells (Baker et al., 2017, Ceronie et al., 2018). Combined with the higher turnover of B cells, this might mediate the B cell-selective

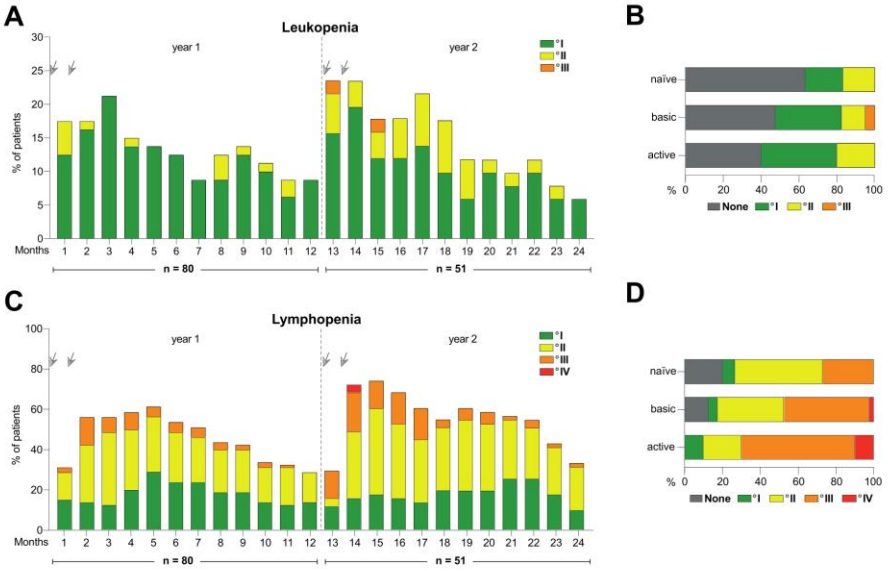

Fig. 3. Safety outcomes of our cohort: Hematological abnormalities. (**A**+**C**) Absolute leukocyte (**A**) and lymphocyte (**C**) levels were determined on a monthly basis in patients treated with cladribine in year 1 (n=80) and year 2 (n=51). Cladribine was administered as monthly courses (gray arrows) at baseline (month 0) and months 1, 12, and 13. Proportions of patients with leukopenia (**A**) or lymphopenia (**C**) severity grade according to CTCAE version 5.0 at the respective time points of analysis are shown. (**B**+**D**) Proportions of patients with leukopenia (**B**) or lymphopenia (**D**) severity grade at their nadir divided in different treatment groups according to the most recent disease modifying therapy (DMT) received (naïve (n=30) platform (n=40) and escalation (n=10)). *Platform* includes the following DMTs: interferon-beta, glatiramer acetate, teriflunomide, and dimethyl fumarate. *Escalation* includes fingolimod, natalizumab and daclizumab.

Table 3
Lymphopenia induced by oral cladribine.

Lymphopenia Grade [a] Patients, No. (%)	Our cohort (n = 80)	Reference cohort [8] (n = 430)
Total	69 (86.3)	386 (89.8)
None (> 1.0 10^3/µl)	11 (13.7)	44 (10.2)
Grade 1 (< 1.0 - 0.8 × 10^3/µl)	7 (8.8)	276 (64.2)
Grade 2 (< 0.8 - 0.5 × 10^3/µl)	31 (38.8)	
Grad 1/2 lymphopenia	38 (47.5)	
Grade 3 (< 0.5 - 0.2 × 10^3/µl)	29 (36.3)	107 (24.9)
Grade 4 (< 0.2 × 10^3/µl)	2 (2.5)	3 (0.7)
Grade 3/4 lymphopenia	31 (38.8)	110 (25.8)
Lymphopenia leading to treatment discontinuation	6 (7.5)	4 (0.9)

Frequency of lymphopenia from baseline to 24 months (our cohort) or 96 weeks (Reference cohort) of follow-up. Data from our cohort is compared to those obtained from the CLARITY trial (named as 'Reference cohort'), presented in the publication from Cook et al., 2011 [9]. This data is from patients with early to more advanced relapsing multiple sclerosis receiving the approved dose of cladribine 3.5mg/kg as monotherapy. Each patient's highest lymphopenia grade is depicted.

[a] Lymphocyte levels were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. No: Number.

depleting effect of cladribine (Baker et al., 2017, Ceronie et al., 2018).

Importantly, sustained depletion of lymphocyte subsets persisted well beyond drug administration, providing further evidence that cladribine may represent a form of IRT.

Generally, cladribine treatment resulted in a profound reduction in relapse rate and MRI activity. In this context, it is reasonable to suggest that the reported lymphocyte depletion underlines the reduction in disease activity. However, considering T cells are a major factor, our results and those of previously published studies (Baker et al., 2017, Comi et al., 2019, Stuve et al., 2019) revealed an only modest depletion of T cells (≤50-60%) by cladribine, probably less than that considered necessary for optimal disease control (von Kutzleben et al., 2017, van Oosten et al., 1997). Similarly, Baker *et al.* indicate that the levels of peripheral T cell depletion do not account for cladribine's efficacy (Baker et al., 2017) and we did not observe a depletion or repopulation pattern of T cells that related to disease activity.

Indeed, the most striking effect of cladribine is the predilected depletion of B cells. Converging lines of evidence suggest that memory B cells may be particularly susceptible to cladribine (Baker et al., 2017, Ceronie et al., 2018). Interestingly, memory B cell depletion represents a consistent feature of highly-active DMTs (Baker et al., 2017, Baker et al., 2017) – notably, at least in conditions other than MS, in a manner that appears to reflect their level of treatment efficacy (Baker et al., 2017) (Baker et al., 2017). However, in our study, $CD19^+$ counts at the approximate time point of disease activity did not show a specific pattern. A possible explanation is that using the total CD19 count masks the contribution of the memory B cell population, which constitutes approximately 30% of the circulating $CD19^+$ pool (Morbach et al., 2010).

Of interest, serum immunoglobulin levels remained largely unaffected. This minimal effect on immunoglobulins likely suggests that cladribine does not directly deplete plasma cells. This is consistent with the finding that plasma cells have a comparatively lower ratio of DCK to 5'-nucleotidases expression compared to the particularly high levels of other B cell subtypes (Ceronie et al., 2018).

Regarding the relevance of immune cell reductions on the safety profile of cladribine, we had a higher incidence of severe lymphopenia in our real-world setting compared to data from the clinical development program (38.8% versus 27.9% of patients) (Cook et al., 2011). Our

Table 4
Infections during treatment with oral cladribine

Infections Patients, No. (%)	Our cohort (n = 80)	Reference cohort [8] (n = 430)
Any infection	44 (55.0)	205 (47.7)
Any infection, Year 1 / week 0-48	38 (47.5)	165 (38.4)
Any infection, Year 2 / week 48-96 [a]	17 (33.3)	141 (32.8)
Mild [b]	32 (40.0)	159 (37.0)
Moderate [b]	20 (25.0)	116 (27.0)
Severe [b]	3 (3.8)	2 (0.5)
Total herpes infections	8 (10.0)	14 (3.3)
Herpes simplex	7 (8.8)	6 (1.4)
Herpes zoster	2 (2.5)	8 (1.9)
Urinary tract infection	7 (8.8)	4 (0.9)
Upper respiratory tract infection	31 (38.8)	54 (12.6)
Other infections [c]:	4 (5.0)	7 (1.6)
Dental infection	3 (3.8)	N/S
Lymphadenitis	3 (3.8)	N/S
Febrile infection not further defined	2 (2.5)	3 (0.7)
Conjunctivitis	2 (2.5)	8 (1.9)
Vaginal infection		

Frequency of infections from baseline to 24 months (our cohort) or 96 weeks (Reference cohort) of follow-up. Data from our cohort is compared to those obtained from the CLARITY trial (named as 'Reference cohort'), presented in the publication from Cook et al., 2011 [9]. This data is from patients with early to more advanced relapsing multiple sclerosis receiving the approved dose of cladribine 3.5mg/kg as monotherapy.
Herpes simplex infections included oral herpes, genital herpes and herpes dermatitis.
[a] Some patients had both, infections in year 1 and year 2, as well as mild, moderate and severe infections so may have been counted more than once. The percentage in year 1 and 2 is calculated based on the total of 80 and 51 patients, respectively.
[b] Infections were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.
[c] Reported in ≥2% of patients in our cohort.
No: Number; N/S: Not specified.

results may also be contrasted to the simulated lymphocyte-based treatment scenarios (using data from the CLARITY trial) (Terranova et al., 2019), which predicted that by allowing a 6-month delay ~99% of patients would be eligible for the second-year treatment.

Although we observed a comparable incidence of overall infections in our real-world cohort (55.0% versus 47.7%), more cases of upper respiratory tract (38.8% versus 12.6%) and herpes infections (10% versus 3.3%) occurred. Interestingly, in our cohort, infections seemed to be temporally linked to the dosing period in years 1 and 2, and there was a trend for patients to acquire infections if they were previously treated with dimethyl fumarate, with the most pronounced association for herpes infections. Moreover, patients with lymphopenia were at greater risk for infections. This association was strongest for herpes infections (94.8% of patients with concomitant lymphopenia). So far, the EU SmPC for cladribine states that anti-herpes prophylaxis should be considered during the time of grade IV lymphopenia. However, 63.1% of patients with herpes infection in our cohort had grade II-III lymphopenia, suggesting that herpes prophylaxis is appropriate even at lower grades and should be guided instead by the critical time interval after cladribine dosing.

Notably, approximately 97% of the infections in our cohort were considered as mild or moderate in severity. This might be partially explained by the semi-selective mechanism of cladribine: the limited activity on cells of the innate immune system and the relatively minor impact on $CD8^+$ T cells and plasma cells – as suspected by the largely unaffected immunoglobulins – might have implications for maintained protection from bacterial and viral infections. Similarly, NK cells are part of the immune surveillance mechanisms for infections. As evidenced by the DCK/5'nucleotidase expression of NK cells, this cell type was more sensitive to cladribine (Ceronie et al., 2018). However, depletion was relatively transient.

Our study has several limitations. First, it is limited by its exploratory nature. Moreover, we only analyzed peripheral blood, and events occurring within the central nervous system or lymphoid tissues were not captured.

It is therefore perhaps not surprising that peripheral blood immune cell levels have not demonstrated biomarker activity to predict disease activation. Moreover, since we focused on the major innate and adaptive immune cells, it is possible that key pathogenic cells were not considered.

To conclude, our immunophenotyping results show that following the two first treatment years, the real-world effects of cladribine are comparable with the effects observed in clinical trials. Cellular effects exceeded the dosing intervals, providing further evidence that cladribine represents a form of selective IRT. Moreover, severe lymphopenia occurred more frequently compared to the clinical development program, as did treatment-associated discontinuation of cladribine. The infectious AE profile further supports the notion that cladribine selectively reduces adaptive immune cells while preserving certain components of the innate immune system. The minor effect of cladribine on immunoglobulin levels may reflect another mechanism of preserved immunosurveillance.

CRediT authorship contribution statement

Conceptualization: Leoni Rolfes, Steffen Pfeuffer, Sven G. Meuth and Refik Pul; Data acquisition: Leoni Rolfes, Steffen Pfeuffer, Mariella Schmidt, Niklas Huntemann, Chuanxin Su, Jelena Skuljec, Jana Hackert, Derya Aslan, Refik Pul; Analysis and interpretation of data: Leoni Rolfes, Steffen Pfeuffer, Marc Pawlitzki; Data visualization and Figures: Leoni Rolfes; Writing - original draft preparation: Leoni Rolfes, Steffen Pfeuffer; Writing - review and editing: Marc Pawlitzki, Tobias Ruck, Christoph Kleinschnitz, Sven G. Meuth and Refik Pul; Critical revision of manuscript for intellectual content: Jelena Skuljec, Chuanxin Su, Derya Aslan, Jana Hackert, Tim Hagenacker, Konstanze Kleinschnitz, Mariella Schmidt, Niklas Huntemann, Tobias Ruck; Supervision: Sven G. Meuth and Refik Pul.

Availability of data and material

Data will be shared with qualified investigators upon request, please contact leoni.rolfes@med.uni-duesseldorf.de.

Funding information

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Declaration of Competing Interest

Leoni Rolfes: received travel reimbursements from Merck Serono and Sanofi Genzyme, research support from Diamed, Merck Serono and Novartis. Her research is funded by the Interdisciplinary Center for Clinical Studies (IZKF) Muenster. **Steffen Pfeuffer**: received travel grants from Sanofi Genzyme and Merck Serono, lecturing honoraria from Sanofi Genzyme, Mylan Healthcare, and Biogen, and research support from Diamed, Merck Serono, and the German Multiple Sclerosis Society Northrhine-Westphalia. **Mariella Schmidt**: None. **Niklas Huntemann**: None. **Chuanxin Su**: none. **Jelena Skuljec**: none. **Derya Aslan**: received honoraria for lecturing from Merck and travel grants from Sanofi. **Jana Hackert**: received travel grants and honoraria for lecturing from Celgene, Merck, Novartis, Roche and Sanofi Genzyme. **Konstanze Kleinschnitz**: none. **Tim Hagenacker**: none. **Marc Pawlitzki:** received research funding from Novartis. His research is founded by the German Multiple Sclerosis Society North Rhine-Westphalia (DMSG) and the program "Innovative Medizinische Forschung "(IMF) of the Medical Faculty of the University of Muenster. **Tobias Ruck**: reports grants from German Ministry of Education, Science, Research and

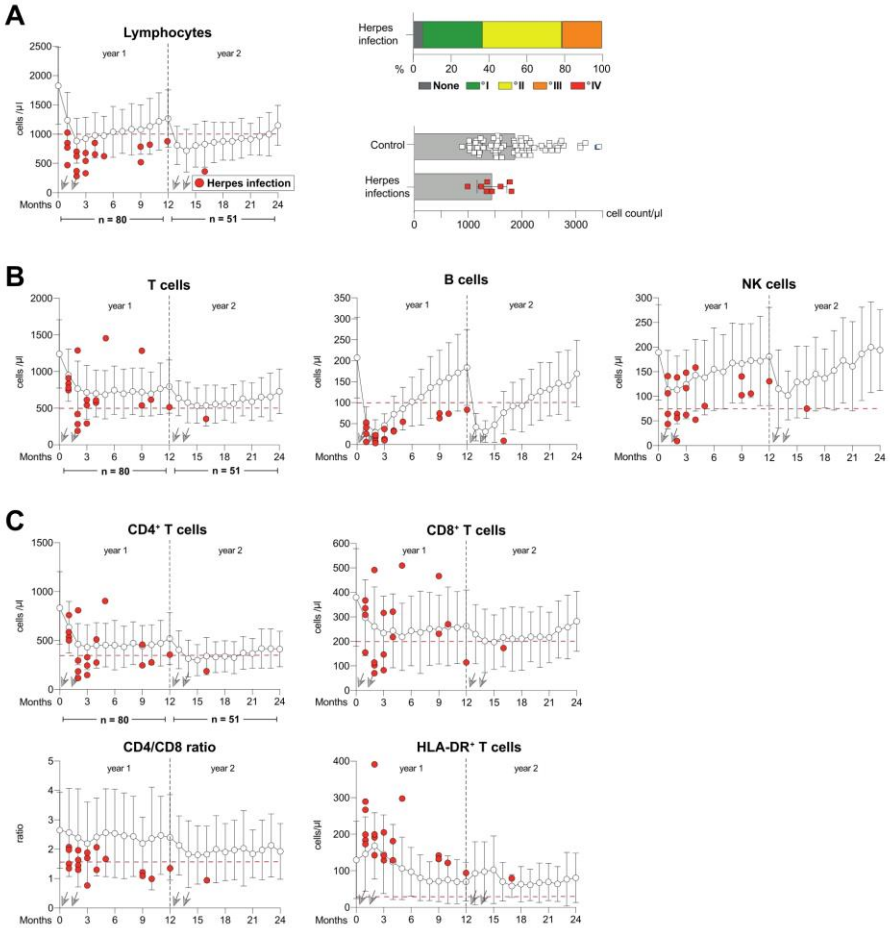

Fig. 4. Safety outcomes of our cohort: Herpes infections are related to lymphopenia. (**A-C**) Cladribine was administered as monthly courses (gray arrows) at baseline (month 0) and months 1, 12, and 13. The results of treatment year 1 (n=80) and year 2 (n=51) represent the mean absolute cell counts ± standard deviation of (**A**) absolute lymphocytes, (**B**) lymphocyte subsets ($CD3^+$ T cells, $CD19^+$ B cells and $CD16^+/CD56^+$ natural killer (NK) cells), and (**C**) T cell subsets, including $CD4^+$ T-helper and $CD8^+$ cytotoxic T cells, $HLA\text{-}DR^+$ activated T cells and the CD4/CD8 ratio. Absolute lymphocyte (subset) counts at disease manifestation in patients with herpes infections (in total 19 cases in 8 patients – 17 of herpes simplex and 2 of herpes zoster) are shown (red circles). The lower limit of normal for each cell type is indicated by a horizontal line. (**A**) The lymphopenia severity grade according to CTCAE version 5.0 at the respective time points of herpes infection (n=8; upper panel) are depicted. The baseline lymphocyte counts of those patients who developed a herpes infection are compared to those without herpetic infectious event (n=72; lower panel).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2022.103931.

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4.12 Immune Response to Seasonal Influenza Vaccination in Multiple Sclerosis Patients Receiving Cladribine

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Immune Response to Seasonal Influenza Vaccination in Multiple Sclerosis Patients Receiving Cladribine

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Abstract: Cladribine has been approved for the treatment of multiple sclerosis (MS) and its administration results in a long-lasting depletion of lymphocytes. As lymphopenia is known to hamper immune responses to vaccination, we evaluated the immunogenicity of the influenza vaccine in patients undergoing cladribine treatment at different stages vs. controls. The antibody response in 90 cladribine-treated MS patients was prospectively compared with 10 control subjects receiving platform immunotherapy (NCT05019248). Serum samples were collected before and six months after vaccination. Response to vaccination was determined by the hemagglutination-inhibition test. Post-vaccination seroprotection rates against influenza A were comparable in cladribine-treated patients and controls (H1N1: 94.4% vs. 100%; H3N2: 92.2% vs. 90.0%). Influenza B response was lower in the cladribine cohort (61.1% vs. 80%). The increase in geometric mean titers was lower in the cladribine group vs. controls (H1N1: +98.5 vs. +188.1; H3N2: +225.3 vs. +300.0; influenza B: +40.0 vs. +78.4); however, titers increased in both groups for all strains. Seroprotection was achieved irrespective of vaccination timing and lymphocyte subset counts at the time of vaccination in the cladribine cohort. To conclude, cladribine-treated MS patients can mount an adequate immune response to influenza independently of treatment duration and time interval to the last cladribine administration.

Keywords: multiple sclerosis; cladribine; immunization; influenza; vaccination

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1. Introduction

In the last two decades, the approval of new and effective disease-modifying treatments (DMT) led to a significant change in the therapeutic strategy of multiple sclerosis (MS). New so-called immune reconstitution therapies (IRTs) have the potential to induce long-term or even permanent drug-free remission in people with MS [1]. These therapies deplete components of the immune system, intending to allow its renewal [2]. In this context, cladribine represents the first short-course oral IRT approved for the treatment of active relapsing MS after having been positively evaluated in placebo-controlled randomized clinical trials [3,4]. Cladribine is a synthetic purine analogon that induces lymphocyte depletion due to an accumulation of intracellular chloro-deoxyadenosine triphosphate. Immunophenotyping studies showed that cladribine only modestly affects T cells, whereas it vastly reduces B-cell counts [5], particularly class-switched and unswitched memory B cells [6,7].

Given those immunocompromising effects, the impact of this drug on immunization responses cannot be ruled out. Therefore, it is recommended to complete vaccination requirements as per local prescribing information before initiation of cladribine to optimize vaccine effectiveness. However, protection from some pathogens, such as influenza viruses, requires annually repeated vaccinations.

The immunogenicity of the influenza vaccine in MS patients depends on the immunomodulatory treatment they received. A sufficient vaccination response to influenza has been shown in patients receiving interferon (IFN)-β, glatiramer acetate, dimethyl fumarate, and teriflunomide therapy [8–13]. In contrast, a reduced likelihood of seroprotection following influenza vaccination was observed in individuals on fingolimod, natalizumab, or the B-cell-depleting therapy ocrelizumab [9,11,14].

With respect to cladribine, a study of 12 patients demonstrated seroprotection after influenza vaccination in all participants, regardless of the time passed since the last treatment administration or total lymphocyte count [15]. However, the study was retrospective in design, included a limited number of patients, and a control group was not established. Consequently, larger and prospective studies are required to evaluate whether cladribine treatment influences the likelihood of response to influenza immunization.

In this study, we analyzed the immunogenicity of the seasonal influenza vaccine 2020/2021 in patients with relapsing MS receiving immunomodulatory cladribine therapy, with baseline sampling and follow-up after 6 months in both the study population and controls (MS patients treated with platform DMTs).

2. Materials and Methods

2.1. Study Design

CIRMS (Cladribine on Immune Responses in Multiple Sclerosis) was designed as a large prospective observational study to assess response rates to the seasonal influenza vaccine in participants with relapsing MS treated with cladribine (ClinicalTrials.gov identifier: NCT05019248). The vaccine-specific antibody responses to the H1N1, H3N2, and B strain 2020/2021 influenza vaccine viruses were measured prior to immunization and six months postvaccination. All included participants had an indication for a seasonal influenza vaccination according to the German national recommendations by the Standing Committee on Vaccination.

Initially, the study was designed to incorporate a larger cohort of 200 patients in order to allow a non-inferiority analysis of the primary endpoint as well as confirmation of various secondary endpoints and immunologic analyses. However, the 2019 coronavirus disease pandemic (COVID-19) and the associated reduction in in-person patient contacts, as well as the limited availability of seasonal influenza vaccine, resulted in slow recruitment, so the originally planned patient count was not achieved. Therefore, we present here results from a smaller study population that were sufficient for a descriptive analysis of the primary outcome and several secondary outcomes (cladribine group: $n = 90$; control group: $n = 10$).

2.2. Study Population

All adult patients diagnosed with relapsing MS according to 2017 revised McDonald criteria [16] who underwent treatment with cladribine at the University Hospitals Essen and Duesseldorf, Germany, and who chose to receive a seasonal influenza vaccine on a routine basis were offered to participate in this study. Patients were included from September 2020 to March 2021. Administration of cladribine was performed according to national and international guidelines as well as to the most recent summary of product characteristics (cladribine group; $n = 90$).

The control participants (referred to as "platform DMTs"; $n = 10$) were recruited during the same period and were either treatment naïve ($n = 1$) or received injectable or oral DMTs approved for relapsing MS, namely IFN-β ($n = 3$), glatiramer acetate ($n = 2$), dimethyl fumarate ($n = 2$), or teriflunomide ($n = 2$).

Patients were excluded if they had (i) prior treatment with B-cell-targeted therapies, lymphocyte-trafficking blockers, alemtuzumab, cyclophosphamide, mitoxantrone, azathioprine, mycophenolate mofetil, cyclosporine, methotrexate, total body irradiation, or bone marrow transplantation; (ii) immunosuppressive treatment for diseases other than MS or long-term corticosteroid treatment; (iii) systemic high dose corticosteroid therapy or apheresis procedures 6 weeks prior to vaccination; (iv) contraindications against vaccination.

2.3. Vaccination

We initially planned to recruit only patients vaccinated with a single dose of the tetravalent inactivated unadjuvanted split influenza virus vaccine that contained A/Guangdong-Maonan/SWL1536/2019 (H1N1) pdm09 (H1N1GM19), A/Hong Kong/2671/2019 (H3N2, H3N2HK19), B/Washington/02/2019 (BWAS19), and B/Phuket/3073/2013, as recommended for the northern hemisphere by the World Health Organization for 2020/2021, according to the manufacturer's specification (in detail, the following vaccines were used: Influsplit®, Flucelvax®, Influvac®, and Vaxigrip®). However, due to the COVID-19 pandemic, the demand for influenza vaccination increased, resulting in a shortage of the seasonal vaccine for 2020/21. Consequently, several patients had to be vaccinated with a single dose of the trivalent influenza virus vaccine. Therefore, the evaluation was reduced to the immune response against the following influenza strains: H1N1GM19, H3N2HK19, and BWAS19.

In order to evaluate whether the immune response mounted to antigenic stimulation depends on the duration and timing of cladribine therapy, patients were classified into 5 cohorts: those vaccinated shortly (at least 4 weeks) before initiation of cladribine (−3 to −1 months to baseline, cohort 1), early after first cladribine admission (+1 to +6 months to baseline, cohort 2), at the end of the first-year treatment course (+6 to +11 to baseline, cohort 3), shortly after the second treatment course (+13 to +18 months to baseline, cohort 4), or after completing the second year of treatment (>24 months to baseline and >12 months to last cladribine admission, cohort 5). Controls received influenza immunization during treatment with a platform DMT. By implementing this control group, we took advantage of eliminating bias due to disease-specific dysregulations in peripheral immune responses. Based on previous studies, treatment with platform DMTs does not impact the vaccination responses to influenza [8–13].

2.4. Study Endpoints

The primary endpoint was the proportion of patients with a positive response to the influenza vaccine measured six months after vaccination. A positive response to the vaccine was defined as receiving seroprotection (specific hemagglutination-inhibition (HI) titers $\geq$ 1:40). As secondary endpoints, we assessed geometric mean antibody titers (GMTs) prior to and six months postvaccination, as well as the proportion of patients with seroconversion (i.e., a prevaccination antibody titer $\leq$ 10 and a postvaccination HI titer $\geq$ 40). Finally, adverse events were monitored and collected for all subjects throughout the duration of the study. The severity of adverse events was graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

2.5. Blood Sampling and Processing

Quantitative antibody titer responses to seasonal influenza vaccines were measured by hemagglutination inhibition assays (HAI). The HIA was applied as described before [9,15,17]. Serum samples for analyses were drawn directly before vaccination (i.e., on the day of vaccination) and six months after vaccination. Five prevaccination samples—all in the cladribine group—could not be processed. However, postvaccination samples were obtained and analyzed from all patients in both groups. Samples were stored at −80 °C until use in the blinded analyses.

In brief, a two-fold dilution series of sera was prepared in phosphate-buffered saline (initial dilution 1:20) and incubated with four hemagglutinin units of whole inactivated

H1N1GM19, H3N2HK19, or BWAS19 virus for 1 h. Prior to reading, 1% turkey erythrocytes were added for 1 h. For H1N1GM19 and H3N2HK19 viruses, all tests were performed with positive ferret sera. For BWAS19, sheep serum was used as a positive control. All sera were tested in duplicate. Serum HI titer was expressed as the reciprocal of the highest dilution at which hemagglutination was 50% inhibited [9,17].

The lymphocyte cell counts were assessed via standard hematology laboratory measures. Lymphocyte subsets were assessed in a central laboratory of the University Medicine Essen (CD19+ B cells, CD3+ T cells) using flow cytometry. The total amount of primary immunoglobulin classes (IgG and IgM) was analyzed via latex-enhanced assay by kinetic nephelometry according to manufacturer guidelines [18]. Data were presented as absolute numbers (cells/μL) or unit volumes (immunoglobulins, g/L).

2.6. Statistical Analyses

Baseline epidemiologic characteristics were evaluated using descriptive statistics. Comparisons among patient subgroups were made using the χ^2-test or Fisher's exact test for categorical variables or the Mann–Whitney rank sum test or Kruskal–Wallis test for continuous variables. All analyses of vaccine response were summarized using descriptive statistics, including the number of patients, mean, and 95% confidence intervals. Unless otherwise stated, the calculation of proportions was based on the number of patients in the analysis set of interest.

3. Results

3.1. Patients

In total, 90 cladribine-treated patients and 10 control individuals were included in this study and vaccinated against influenza. Except for five prevaccination samples in the cladribine group, complete blood samples (samples before and 6 months after vaccination, respectively) were available from all patients. Demographic characteristics at the time of vaccination between cladribine-treated patients and the control group were generally well-balanced (Table 1). However, 40% of patients in the cladribine group had not received prior DMT, compared to only 10% in the control group. The other patients were previously treated with different substances including IFN-β, glatiramer acetate, and dimethyl fumarate. Our cladribine patients had a median age of 41 (interquartile range (IQR): 31–52) years and a median disease course of 78.5 (IQR: 36.5–180.3) months since MS onset. The median EDSS score was 2.5 (IQR: 1.5–4.0) indicating a moderate disability burden. A comparison of the individual cladribine cohorts showed a significant difference in the annualized relapse rate at vaccination (Table 1). Here, cohorts 1 and 2 demonstrated higher disease activity. Similarly, patients in cohort 2 were younger at the median than the median of the overall cohort (34 (IQR:29–52) vs. 41 (IQR: 31–52)). This is likely due to two factors: (i) only patients with a pronounced active disease course were newly started on cladribine therapy in 2020 since, due to the onset of the COVID-19 pandemic and the associated uncertainty regarding the risk of infection and potentially worse disease outcomes under escalating DMTs (especially in older patients), indications for adjustments were more hesitant; (ii) insufficient time needed for the stabilization of disease activity under cladribine therapy.

Table 1. Epidemiological parameters at vaccination. Mo: months; BL: baseline; CLD: cladribine; DMT: disease-modifying therapy; yrs: years; No.: numbers; MS: multiple sclerosis; IQR: interquartile range; EDSS: expanded disability status scale; DMF: dimethyl fumarate; TERI: teriflunomide. *: significance level determined using Kruskal–Wallis test; #: significance level determined using Fisher's exact test.

	−3 to −1 mo to BL (*n* = 19)	**+1 to +6 mo to BL (*n* = 15)**	**+6 to +11 mo to BL (*n* = 19)**	**+13 to +17 mo to BL (*n* = 12)**	**>24 mo to BL; >12 mo to CLD (*n* = 25)**	**Platform DMT Control (*n* = 10)**	***p***
Age, yrs, median (IQR)	40 (31–53)	34 (29–52)	41 (35–50)	49 (34–53)	41 (32–51)	53 (31–61)	0.462 *
Male sex, No. (%)	7 (37)	4 (27)	8 (42)	3 (25)	9 (36)	5 (50)	0.797 #
MS duration, mo, median (IQR)	80 (8–160)	13 (7–63)	48 (10–187)	137 (15–231)	68 (25–172)	78 (37–180)	0.075 *
Annualized relapse rate, median (IQR)	1 (1–2)	1 (1–2)	1 (0–1)	1 (0–1)	1 (0–1)	0 (0–0.5)	0.031 *
EDSS, median (IQR)	2.5 (2–4.5)	2 (1–4)	2 (1–3.5)	3.5 (1.5–4.5)	2.5 (2–4)	1.5 (1.5–2)	0.130 *
Number of previous DMT, median (IQR)	1 (0–3)	1 (0–1)	1 (0–2)	1 (0–2)	2 (1–3)	1 (0–1)	0.071 *
Last previous DMT, No. (%)							
Naïve	5 (26)	7 (47)	11 (58)	7 (58)	6 (24)	1 (10)	0.201 #
Platform	5 (26)	2 (13)	2 (11)	1 (8)	8 (32)	5 (50)	
DMF/TERI	7 (37)	6 (40)	4 (21)	3 (25)	8 (32)	4 (40)	
Active	2 (11)	0 (0)	2 (11)	1 (8)	3 (12)	0 (0)	
Vaccine used, No. (%)							
Influsplit®	9 (47)	3 (20)	9 (47)	6 (50)	16 (64)	7 (70)	0.116 #
Flucelvax®	4 (21)	6 (40)	2 (11)	2 (17)	3 (12)	0 (0)	
Influvac®	4 (21)	4 (27)	8 (42)	4 (33)	6 (24)	3 (30)	
Vaxigrip®	2 (11)	2 (13)	0 (0)	0 (0)	0 (0)	0 (0)	
Smoker status, No. (%)	8 (42)	7 (47)	5 (26)	2 (17)	11 (44)	3 (30)	0.464 #

3.2. Response to Influenza Vaccine

Prevaccination GMTs (HI units) for the influenza strains were comparable between the overall cladribine and control group (H1N1GM19: 80.2 (±95% confidence interval: 46.0–141.3) vs. 76.4 (35.5–164.3); H3N2HK19: 172.2 (83.0–374.1) vs. 252.4 (87.1–731.1); BWAS19: 16.8 (11.5–24.7) vs. 15.2 (9.0–25.9), Table 2). Postvaccination, mean increases in the titers were lower in the cladribine group vs. the control group (H1N1GM19: +98.5 vs. +188.1; H3N2HK19: +225.3 vs. +300.0; BWAS19: +40.0 vs. +78.4); however, titers increased in both groups for all strains six months after vaccination. Comparison between the individual cladribine cohorts showed that increases in GMT levels postvaccination were highest when vaccination preceded cladribine initiation (cohort 1). In particular, the mean titer increase in influenza A strains was lower during the first two years of treatment compared to cohort 1 (H1N1GM19: cohort 1 vs. cohort 2 vs. cohort 3 vs. cohort 4: +153 vs. +70.3 vs. +32 vs. +83.9, respectively; H3N2HK19: +279.1 vs. +157.1 vs. +197.8 vs. +133.9, respectively).

The majority of cladribine patients already had seroprotective antibody titers before vaccination (H1N1GM19: 78.8%, H3N2HK19: 84.7%, BWAS19: 18.8%). Postvaccination seroprotective titers were maintained in those patients. In cladribine recipients, seroprotection rates to all strains were higher after vaccination than before vaccination (H1N1GM19: 94.4%; H3N2HK19: 92.2%; BWAS19: 61.1%). Except for the influenza B strain, postvaccination seroprotection rates were comparable with those of the control group (H1N1GM19: 100%; H3N2HK19: 90.0%; BWAS19: 80%). Seroconversion rates were also comparable between cladribine-treated patients and controls (H1N1GM19: +15.6% vs. 20.0%; H3N2HK19: +7.5% vs. 0%; BWAS19: +42.3% vs. 50%, respectively). However, the interpretability of seroconversion was limited by the small number of patients with seronegative prevaccination titers (HI titer $\leq$ 10) in both groups.

Concerning the timing of influenza vaccination in relation to the treatment onset, we further observed only mild variation in seroprotection rates, indicating that an effective humoral response can be mounted independently of the duration of cladribine treatment as well as the time interval to the last cladribine administration (Figure 1).

3.3. Predictors of Response

We also explored whether the serological response was impacted by lymphocyte counts measured at the time of vaccination. Total lymphocyte counts in patients vaccinated shortly before cladribine initiation were mostly within the normal range (Figure 2). In contrast, patients vaccinated within the first six months after a course of cladribine, and especially within the second year of treatment, typically showed grade I or II lymphopenia. None of the patients in our cohort had severe lymphopenia at the time of vaccination. Notably, most patients maintained or achieved seroprotection independently of total lymphocyte count or subset distribution (Figure 2).

Table 2. Geometric mean titer levels pre- and postvaccination.

A.	**Platform DMT Control**		**Overall CLD COHORT**	
strain	**pre (*n* = 10)**	**post (*n* = 10)**	**pre (*n* = 85)**	**post (*n* = 90)**
H1N1GM19	76.4 (35.5–164.3)	264.5 (170.5–410.3)	80.2 (46.0–141.3)	178.7 (113.4–284.6)
H3N2HK19	252.4 (87.1–731.1)	552.4 (209.2–1459.0)	172.2 (83.0–374.1)	397.5 (192.5–849.1)
BWAS19	15.2 (9.0–25.9)	93.6 (42.0–208.3)	16.8 (11.5–24.7)	56.8 (31.3–105.2)

B	**Platform DMT Control**		**Cohort 1: −3 to −1 mo to BL**		**Cohort 2: +1 to +6 mo to BL**		**Cohort 3: +6 to +11 mo to BL**	
strain	**pre (*n* = 10)**	**post (*n* = 10)**	**pre (*n* = 16)**	**post (*n* = 19)**	**pre (*n* = 14)**	**post (*n* = 15)**	**pre (*n* = 19)**	**post (*n* = 19)**
H1N1GM19	76.4 (35.5–164.3)	264.5 (170.5–410.3)	77.1 (45.1–131.9)	230.2 (147.2–360.0)	59.3 (38.4–91.6)	129.6 (63.8–263.3)	78.5 (44.9–137.2)	110.5 (77.8–156.9)
H3N2HK19	252.4 (87.1–731.1)	552.4 (209.2–1459.0)	164.7 (92.2–294.3)	443.8 (265.2–742.6)	144.9 (75.2–279.3)	302.0 (127.6–714.9)	149.2 (67.3–330.6)	347.0 (187.0–644.1)
BWAS19	15.2 (9.0–25.9)	93.6 (42.0–208.3)	17.7 (11.9–27.0)	54.2 (29.7–98.4)	11.6 (9.1–14.8)	38.9 (19.8–76.3)	17.4 (13.2–22.8)	48.6 (28.5–82.7)

C	**Platform DMT Control**		**Cohort 1: −3 to −1 mo to BL**		**Cohort 4: +13 to +17 mo to BL**		**Cohort 5: >24 mo to BL; >12 mo to CLD**	
strain	**pre (*n* = 10)**	**post (*n* = 10)**	**pre (*n* = 16)**	**post (*n* = 19)**	**pre (*n* = 11)**	**post (*n* = 12)**	**pre (*n* = 25)**	**post (*n* = 25)**
H1N1GM19	76.4 (35.5–164.3)	264.5 (170.5–410.3)	77.1 (45.1–131.9)	230.2 (147.2–360.0)	87.3 (47.1–161.6)	171.2 (105.7–277.2)	102.6 (65.2–161.5)	166.4 (115.4–239.9)
H3N2HK19	252.4 (87.1–731.1)	552.4 (209.2–1459.0)	164.7 (92.2–294.3)	443.8 (265.2–742.6)	72.1 (27.4–190.0)	206.0 (78.0–544.1)	249.7 (148.6–419.3)	533.8 (287.9–989.9)
BWAS19	15.2 (9.0–25.9)	93.6 (42.0–208.3)	17.7 (11.9–27.0)	54.2 (29.7–98.4)	18.2 (10.9–30.3)	54.6 (34.2–87.3)	20.2 (15.1–27.1)	51.2 (33.5–78.2)

(**A**): Comparison of platform disease-modifying treatment (DMT) controls and the overall cladribine (CLD) cohort subjected to vaccination. (**B**): Comparison of platform DMT controls and patients subjected to vaccination directly prior to CLD treatment (left columns) to vaccination during year one of CLD treatment (right columns). (**C**): Comparison of platform DMT controls and patients subjected to vaccination directly prior to CLD treatment (left columns) to vaccination during year two of CLD treatment and patients having completed year two in the absence of re-treatment or administration of other DMT (right columns). Data are shown as geometric mean ± 95% confidence interval. BL: baseline; mo: months.

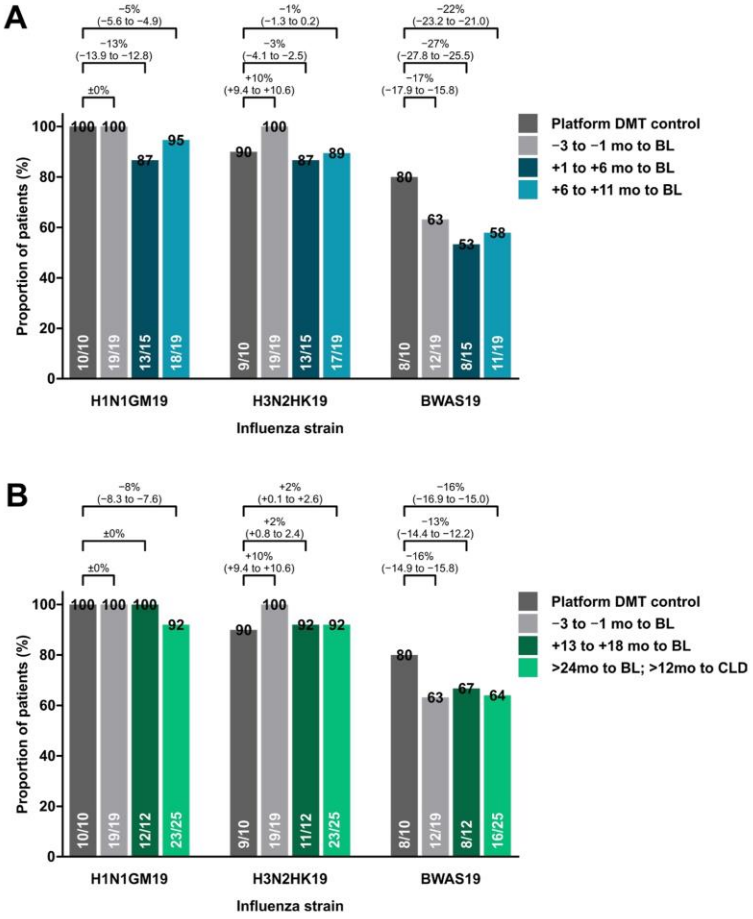

Figure 1. Proportion of patients developing seroprotection to individual influenza strains. (**A**): Proportion of patients with seroprotection among controls and patients having received their vaccination closely to cladribine induction or during year one of treatment. (**B**): Proportion of patients with seroprotection among controls and patients having received their vaccination in year two of cladribine treatment. Data are shown as absolute risk reduction ± 95% confidence interval. DMT: disease-modifying treatment; BL: baseline; CLD: cladribine.

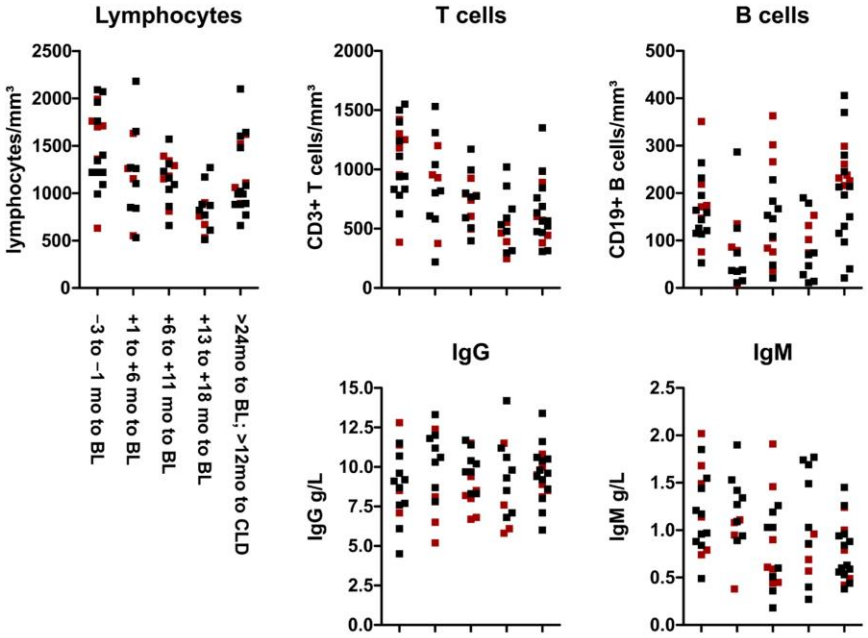

Figure 2. Blood lymphocyte and immunoglobulin levels at vaccination.

Scatter plots indicate patients with seroprotection following vaccination (black boxes) and without sufficient response to at least one strain (red boxes). Error bars show the 95% confidence interval. BL: baseline; CLD: cladribine; IgG: immunoglobulin G; IgM: immunoglobulin M.

3.4. Safety during the Immunization Period

There were no deaths, serious adverse events, or adverse events leading to study discontinuation in either group postimmunization. Measures of cladribine safety during the six months postimmunization were consistent with the phase III safety profile in patients from the clinical development program [19,20], and no new safety signals were identified. Most of the cladribine recipients (51 out of 90, 56.7%) experienced at least one adverse event. Lymphopenia was the most frequently observed event in 34 patients (37.8%).

Except for four cases of grade 3 lymphopenia during the study duration, all adverse events were of mild or moderate intensity. A total of five infectious events occurred in four cladribine patients (4.4%, three cases of upper respiratory tract infection, one case of urinary tract infection, and one case of herpes infection), all infections were rated as mild or moderate. No case of influenza occurred. Two patients (20%) in the control group experienced infectious events, all were mild or moderate in intensity. Similar to the cladribine group, there were no cases of influenza in the control group.

4. Discussion

The data reported here demonstrate that patients with relapsing MS treated with cladribine can mount adequate humoral responses to inactivated influenza vaccine.

The humoral response to influenza vaccines depends, among other factors, on the immunogenicity of the strains included. Here, subjects received locally available trivalent or quadrivalent seasonal influenza vaccines (2020/2021). Due to the COVID-19 pandemic and the associated risk of severe complications of co-circulation of influenza and SARS-

CoV-2 viruses, the World Health Organization pointed to the 2020–2021 anti-influenza campaign as being of particular relevance. Consequently, vaccines were rare and many patients in our cohort received the trivalent vaccine. Hence, analysis of the fourth strain (influenza B, Phuket) was omitted.

While an increase in GMTs against all influenza strains was lower in cladribine-treated MS patients than in the control group, titers increased in both groups for all strains 6 months after vaccination. Of note, lower GMTs compared with healthy controls or patients on platform DMTs are not specific to cladribine therapy but have also been described for other highly active agents such as fingolimod, natalizumab, and B-cell depleting therapies [9,21,22]. Furthermore, seroprotection rates increased in cladribine recipients for all strains tested, and except for the influenza B strain, seroprotection rates after vaccination were comparable to those in the control group. Thus, the cladribine patients were able not only to maintain their pre-existing specific humoral immunity to influenza but also to mount an immune response anew under therapy. However, the interpretation of per-protocol seroconversion that included an HI titer < 10 (i.e., seronegativity) was challenged by the small number of patients who met this criterion.

Currently, there are few studies on the safety and efficacy of vaccines in MS patients treated with cladribine [15,23,24]. The data available to date relate solely to COVID-19 immunization and few cases of vaccination against seasonal influenza (n = 12) and herpes zoster (n = 31) during the 2-year prospective phase IV study MAGNIFY [15]. Interestingly, our observations on influenza immunization support previous findings on this vaccine, as well as on the other vaccines mentioned above. All studies noted that patients receiving cladribine were shown to develop specific humoral immunity and the vaccination was considered safe.

In this study, we chose to have a control group under therapy with a platform DMT. By implementing this control group, we take advantage of eliminating bias due to disease-specific dysregulations in peripheral immune responses. Based on previous studies, treatment with basic DMTs does not impact the vaccination responses to influenza [8–13]. Nevertheless, a compression to healthy individuals might be interesting and can be the subject of further studies. Data from the literature that allow a comparison with our cohort (e.g., same viral strains, same demographic structure, same time to the outcome, same outcome parameters) do not currently exist. However, it can be said from the literature that in the 2020/21 season the immunological response to H1N1 and H2N3 was higher than to the influenza B strains, even in healthy populations [25].

Whereas the anti-inflammatory activity of cladribine has been attributed to the depletion of memory B cells [26], seroconversion and protection after vaccination upon vaccination in cladribine-treated MS patients are likely the result of immature/naïve B-cell repopulation, occurring after the development of a 1 to 3% B-cell repopulation [27]. In our previous study, we showed that cladribine-treated patients maintained 1% B cell levels and CD19+ B cells recovered to at least 10–20 CD19+ B cells/µL rapidly after cladribine dosing [5]. Therefore, the selective kinetics of lymphocyte repopulation induced by cladribine, including incomplete reduction and subsequent prompt recovery of immature/naïve B cells [26], may explain why vaccine responses in cladribine-treated MS patients resembled those treated with platform DMTs. In contrast, humoral responses to the influenza vaccine are blunted in patients treated with other active DMTs with different mechanisms of action [9,11,14,28].

Under this assumption, it is not surprising that vaccination elicited an effective immune response in all cohorts and that no clear relationship between absolute CD19+ B-cell counts at the time of vaccination and seroprotection rates could be demonstrated, given the depletion and repopulation dynamics mentioned before (with B-cell counts > 1 to 3%).

In addition, it should be noted that sex differences in response to vaccines and especially with regard to the influenza vaccination have been described previously in the literature [29–31]. In our cohort, however, we did not find any differences in immune response or adverse events regarding male and female participants. However, the previ-

ously reported sex-specific immunologic differences once again highlight the importance of having a balance of women and men in subgroup analyses of vaccine trials.

Moreover, the effect of cladribine treatment on vaccine response in patients with active secondary progressive MS (which is generally covered by the cladribine label in Germany) was not evaluated in this study. While the MS disease course itself would not be expected to affect vaccine responses, age-related decline in immunity is known to impair antibody responses to vaccines in older adults [32]. Since patients with secondary progressive MS tend to be older, they might experience a reduced vaccine response to cladribine.

In addition, the durability of responses to influenza vaccine during treatment is unknown and should be the subject of future studies. Based on our study design with a long follow-up time point (6 months) and the analyzed sub-cohorts of cladribine treatment, it can be expected that the antibody response upon influenza vaccination lasts at least for one influenza season, as demonstrated for patients under platform DMTs [21].

Our study, of course, has some general limitations. Although this analysis was performed prospectively, preconceived patient numbers were not reached, so an indicated non-inferiority approach was statistically not possible. Further, we divided the patient group into smaller groups, also reducing the power of the study. Moreover, our control group was considerably smaller than the cladribine group. In addition, the vaccination response depends on various aspects, such as the immunogenicity of the influenza vaccine, including vaccine factors, adjuvants, individual factors, and repeated vaccination. In our study, patients were immunized with different vaccines. Although all vaccines used contained the same virus strains (tri- or tetravalent), they did so in different doses and with different adjuvants. This further increases the degree of variability but, as mentioned above, was not feasible otherwise due to the vaccine shortage in the acute pandemic. Moreover, the proportion of individuals with seroprotection was already high at baseline. This is likely attributable to the fact that since the 2009 swine flu, the pandemic H1N1 virus strain has been circulating worldwide (with the usual variability) and is, therefore, considered one of the reference strains for seasonal influenza vaccines (so-called pdm09-like virus strains). The same applies to H3N2, where a similar strain has already been integrated into the northern hemisphere vaccines in 2017/2018 and 2016/2017. On the other hand, the influenza B strain BWAS19—in line with our results—was not included in earlier vaccines. Moreover, our patient cohort is likely to have undergone a selection bias resulting from recruiting exclusively at tertiary centers. Nonetheless, this is the first large and prospectively conducted cohort study to assess vaccination response to influenza in this patient cohort.

5. Conclusions

In summary, seasonal influenza vaccination is effective and safe in patients who received treatment with cladribine in our cohort, regardless of timing after treatment administration or total lymphocyte count. In keeping with the paradigm of "de-risking immunotherapy" [33], it is recommended to complete vaccination requirements before the initiation of cladribine. However, the annual influenza epidemic season requires a regular refresher of vaccination. In this context, we here demonstrate that vaccination, even after cladribine initiation, generates a substantial humoral vaccine response in most MS patients that is comparable to those treated with platform therapies.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and the study was approved by local ethical boards (Institutional review board of the Heinrich-Heine-University Duesseldorf, Germany (2021-1474-a); Institutional review board of the University Duisburg-Essen (22-10594-BO)).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. All authors were given unrestricted access to data published herein during manu-script preparation.

Data Availability Statement: Anonymized data will be shared with qualified investigators upon reasonable request.

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4.13 Skin Reactions in Patients with Multiple Sclerosis Receiving Cladribine Treatment

ARTICLE OPEN ACCESS CLASS OF EVIDENCE

Skin Reactions in Patients With Multiple Sclerosis Receiving Cladribine Treatment

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Abstract

Objective
To report 77 patients with multiple sclerosis (MS) who developed skin-related adverse events (AEs) following treatment with cladribine.

Methods
We evaluated our prospective bicentric cladribine cohort. Cladribine-treated patients with a skin AE were identified.

Results
Two hundred thirty-nine cladribine-treated patients with MS were evaluated. Seventy-seven patients (32%) showed at least 1 skin AE at median 1 month after cladribine initiation (range: 1–12). Within first 3 months following last cladribine exposition, hair thinning (n = 28, 12%), skin rash (n = 20; 8%), mucositis (n = 13, 5%), and pruritus (n = 6, 3%) were observed. Furthermore, 35 patients (15%) developed herpes virus infections (time since last cladribine exposition: median 83 [range: 10–305]). In 15 patients, herpes zoster infection was severe (CTCAE grade ≥ 3) and required hospitalization. Delayed skin AEs (≥3 months after a cladribine treatment cycle) involved 1 case of leukocytoclastic vasculitis and 2 cases of alopecia areata. Finally, 2 patients presented with in total 3 isolated precancerous lesions (1 leukoplakia simplex and 2 actinic keratosis) and 1 patient developed a squamous cell carcinoma.

Conclusion
Skin AEs are common in patients with MS treated with cladribine. Until risk management plans have been adjusted to include these phenomena, clinicians should perform a thorough clinical follow-up and in suspicious cases seek early interdisciplinary support. In light of the observed delayed skin reactions, we further emphasize the necessity of careful clinical surveillance of cladribine-treated patients for yet undescribed secondary autoimmune events.

Classification of Evidence
This study provides Class IV evidence that skin-related AEs are frequent in patients with MS following cladribine in a real-world setting.

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Glossary

AE = adverse event; **DMT** = disease-modifying therapy; **MS** = multiple sclerosis; **RRMS** = relapsing-remitting MS.

Skin-related adverse events (AEs) have been described in several disease-modifying therapies (DMTs) approved for treatment of relapsing-remitting multiple sclerosis (RRMS).[1] However, little is known about the incidence and clinical presentation of such AEs in patients treated with cladribine. Dermatomal herpes infections and rare cases of skin rash are the only described (transient) skin-related AEs of cladribine so far.[2,3] Moreover, there has long been uncertainty whether cladribine treatment increases the risk of malignancy.[2,4]

Here, we present our prospective, bicentric cohort of 239 cladribine patients, 77 (32%) of whom developed at least 1 skin-related AE of varying severity.

Methods

We evaluated our prospective, bicentric cohort of patients with RRMS who received oral cladribine according to the latest summary of product characteristics. Following treatment induction, patients were evaluated at least every third month. Patients with skin-related AEs were identified, and baseline characteristics (e.g., age, sex, concomitant diagnosis and therapies, smoker status, and family history) as well as data regarding the AE (date of AE, concomitant clinical events, supportive medications, and lymphocyte count) were evaluated.

Standard Protocol Approvals, Registrations, and Patient Consents

Ethical approval was obtained from local authorities (2020-459-f-S), and patients gave informed consent.

Data Availability

Data will be shared with qualified investigators on request; please contact leoni.rolfes@ukmuenster.de.

Results

Two hundred thirty-nine cladribine-treated patients with RRMS were longitudinally evaluated (table 1). Seventy-seven patients (32%) developed 1 or more (median number 1.5 [range: 1–6]) skin AEs. Generally, acute skin reactions (<3 months following a cladribine treatment course) represented the most common phenomenon, with 54 patients (22.6%) reporting at least 1 event (table 2). In detail, 28 patients (11.7%) described diffuse hair thinning, and 20 patients (8.4%) experienced a skin rash, including diffuse erythema (n = 13, 5.4%), nummular eczema (n = 2, 0.8%), and papulopustular acneiform eruption (acneiform rash, n = 5, 2.1%, figure, A–C). Of interest, 6 patients who developed a rash at cladribine initiation experienced it at reexposure during the second course. In 2 cases (0.8%), skin rash was accompanied by pruritus, whereas in 4 patients (1.7%), pruritus represented as an isolated symptom (figure D). Furthermore, 13 patients (5.4%) demonstrated with transient mucositis, including 2 cases of dental abscess, even leading to teeth loss in 1 patient and surgical abscess splitting in the other. Most cases of acute symptoms resolved without specific treatment (except 7 cases of symptomatic treatment with local steroids and/or antihistamines due to skin rash and pruritus). However, 6 cases (2.5%) of hair thinning and 3 (1.3%) of acneiform rash persisted during the follow-up (median 21 [range: 6–26] months).

Moreover, 35 patients (14.6%) developed a herpetic skin infection (herpes zoster: n = 16, 6.7% [figure E]; herpes simplex: n = 19, 7.9%; figure F and table 2). Of note, 88% of herpes zoster infections (n = 15, including 1 zoster ophthalmicus, 2 disseminated infections (figure E), and 1 trigeminal nerve affection) were rated as severe (CTCAE grade ≥ 3) and needed hospitalization. Because an integrated analysis of safety data from the cladribine development program suggested a correlation between lymphopenia and the incidence of herpes zoster,[2] we evaluated lymphocyte counts at infection onset. Indeed, 88% (n = 15) of herpes zoster and 74% of herpes simplex infections manifested during lymphopenia (median lymphocyte count herpes zoster: 570 [range: 220–1,120] cells/μL; herpes simplex: 860 [range: 420–1,150] cells/μL).

We further observed 7 cases (2.9%) of delayed skin reactions (onset ≥3 months following cladribine treatment, table 2). Those included 3 cases of acneiform rash (median 144 [range: 130–174] days following cladribine therapy), 1 case of delayed hair thinning (day 206 of cladribine therapy), and—of particular interest—1 case of leukocytoclastic vasculitis and 2 cases of alopecia areata (22/20/13 months after cladribine initiation), probably representing secondary autoimmune complications of cladribine treatment in MS. In detail, a 42-year-old woman received the diagnosis of leukocytoclastic vasculitis, based on the clinical presentation (palpable purpura on both lower legs, figure G), findings in the direct immunofluorescence and good response to local as well as systemic steroid treatment. She showed no symptoms of systemic vasculitis, and further laboratory tests were inconspicuous. During 3 months of follow-up, purpuras improved in size and palpability while the patient continues to take steroids in an oral tapering regime.

Moreover, 2 female patients (27 and 38 years old) reported smooth, circular areas of complete hair loss. In both cases, consultation of a dermatologist resulted in the diagnosis of alopecia areata based on the classical clinical presentation (figure H). Laboratory tests including thyroid hormones and

Table 1 Baseline Characteristics of the Cladribine Cohort (Total N = 239 Patients)

Cladribine cohort	
Patients, no. (%)	239
Age, y, median (IQR)	40 (33–46)
Male patients, no. (%)	91 (38)
Time since RMS onset, y, median (IQR)	4.2 (0.9–9.0)
EDSS baseline, median (IQR)	2.0 (1.5–3.0)
ARR; baseline, median (IQR)	1 (0–2)
Total number of previous DMTs, median (IQR)	1 (0–2)
Last previous DMT, no. (%)	
Treatment naive	72 (30)
Basic treatment	117 (49)
Escalation treatment	50 (21)
Reason for switch to CLD, no. (%)	
Treatment initiation	72 (30)
Disease progression	118 (50)
Adverse events	48 (20)
Washout duration, d, median (IQR)	49.0 (17–88)
Follow-up duration, mo, median (IQR)	23.7 (16.2–29.5)

Abbreviation: IQR, interquartile range.
Disease duration was defined as the time between diagnosis and last follow-up date. Basic includes the following DMT: interferon-beta, glatiramer acetate, teriflunomide, and dimethyl fumarate. Escalation includes fingolimod, natalizumab, and daclizumab. The Expanded Disability Status Scale and the annualized relapse rate refer to the time before cladribine initiation.

thyroid antibodies revealed no alternative underlying course. Alopecia persisted during follow-up periods of 6 and 19 months, respectively.

Finally, single cases of skin carcinomas have been described in cladribine-treated patients with MS.[5-7] Here, we observed in total 3 precancerous lesions (leukoplakia simplex and 2 cases of actinic keratosis) and 1 case of squamous cell carcinoma in 2 cladribine-treated patients with MS. In detail, a 41-year-old woman presented with oral leukoplakia that was first noted 67 days following cladribine exposition (figure I). Biopsy confirmed the diagnosis of leukoplakia simplex. No specific therapy was initiated and restitution occurred during a 1-year follow-up. However, at day 153 of cladribine treatment, the patient developed actinic keratosis (figure J). The lesion was excised, followed by topical therapy with diclofenac sodium.

Moreover, a 69-year-old female patient with MS required escalation treatment due to persisting relapse activity. Ultraviolet damaged skin and a medical history of actinic keratosis were evident, however stable for years. Of note, 68 days after the first cladribine treatment cycle, she presented with several firm and indolent lesions with central hyperkeratosis (figure K). Biopsies revealed the diagnosis of 4 squamous cell carcinomas and 4 actinic keratoses. The lesions were excised, and no further specific treatment was initiated. She is regularly screened (every 6 months) for reemerging disease activity.

Discussion

Skin-related AEs, especially skin rash, have been described before in cladribine treatment for oncologic purpose[8]; however, are less-considered AEs of cladribine treatment in MS[3] and are not mentioned in the risk management plans of MS so far.

In this prospective study, we demonstrate that skin-related AEs are frequent, with 77 of 239 cladribine-treated patients (32%) experiencing at least 1 treatment-emerged AE. Acute to subacute AEs thereby represent the most common phenomenon (22.6%), with hair loss being the dominant manifestation. Non-hematologic toxicity might be an underlying mechanism (especially in regard to alopecia, mucositis, and acneiform rash), probably also being an explanation for other side effects reported by controlled clinical trials (e.g., nausea, diarrhea, and fatigue).[2,4,9] Moreover, skin rash or pruritus might further represent immune-mediated skin phenomena. Previously, it has been postulated that cladribine-induced immune dysregulation might not be the direct cause of skin rash in hemato-oncologic patients, rather than predisposes patients to be hypersensitive to other drugs.[8] However, none of our patients experienced skin rash associated with concomitant therapy. Generally, most cases of acute reactions resolved without specific treatment while cladribine was continued. Only in 1 patient, the AE (squamous cell carcinoma) led to treatment discontinuation.

Moreover, our cohort indicates a high number of skin infections (herpes zoster: n = 16, 6.7%; herpes simplex: n = 19, 7.9%), contrasting the herpes zoster/simplex infection rate of 2.0%/3.0% in the cladribine 3.5 mg/kg group of the CLARITY trial and its extension.[4,9] Importantly, there was a high percentage of severe disease courses (88% of herpes zoster infections) that required inpatient therapy.

Of interest, 2 patients showed hair loss due to alopecia areata, and 1 presented with a leukocytoclastic vasculitis. Because there are spontaneous manifestations described, we cannot unequally demonstrate a causal relationship between those phenomena and cladribine in these reported cases. However, the autoimmune pathophysiology against the background of long-lasting changes of cladribine in the immune system, the identification of other cases of alopecia areata associated with DMTs,[1] and the typical time course of secondary autoimmunity beginning from 13 months after initiation of immune reconstitution[1] might argue in favor of a lymphopenia-associated secondary autoimmune disease. Indeed, both patients (1 with alopecia areata and 1 with leukocytoclastic vasculitis) from whom we had blood counts at the time of autoimmunity showed lymphopenia (850 and 910 cells/μL). Of interest, both patients developed autoimmunity from a

Table 2 Manifestation of Cladribine-Related Skin Reactions (Total N = 239 Patients)

Skin reaction	Patients, no. (%)	Time since last treatment cycle, d, median (range)
Patients with at least 1 skin reaction	77 (32.2)	36 (2–272)
Acute skin reactions	54 (22.6)	18 (2–75)
Hair thinning	28 (11.7)	16 (3–65)
Skin rash		28 (2–65)
Diffuse erythema	13 (5.4)	
Acneiform rash	5 (2.1)	
Nummular eczema	2 (0.8)	
Mucositis	13 (5.4)	34.5 (2–75)
Pruritus	6 (2.5)	11 (2–54)
Skin infections	35 (14.6)	83 (10–305)
Herpes zoster	16 (6.7)	90 (10–237)
Herpes simplex	19 (7.9)	83 (16–305)
Delayed skin reactions	7 (2.9)	159 (7–272)
Acneiform rash	3 (1.3)	144 (130–174)
Hair thinning	1 (0.4)	206
Alopecia areata	2 (0.8)	122 (7–237)
Leukocytoclastic vasculitis	1 (0.4)	272
Precancerous/cancerous lesions	2 (0.8)	67 (35–153)
Leukoplakia simplex	1 (0.4)	67
Actinic keratosis	2 (0.8)	106 (59–153)
Squamous cell carcinoma	1 (0.4)	120

Skin reactions of the 76 identified cases are classified into acute events (≤3 months after a cladribine treatment cycle), skin infections, delayed skin reactions (onset >3 months following cladribine treatment), and precancerous lesions. Number of patients with a respective event is outlined; percentage is given in relation to the entire cohort of 239 cladribine-treated patients. Duration since last treatment cycle refers to the time period between the last cladribine intake (irrespective of whether it is the first or second cycle) and the first manifestation of the respective skin reaction.

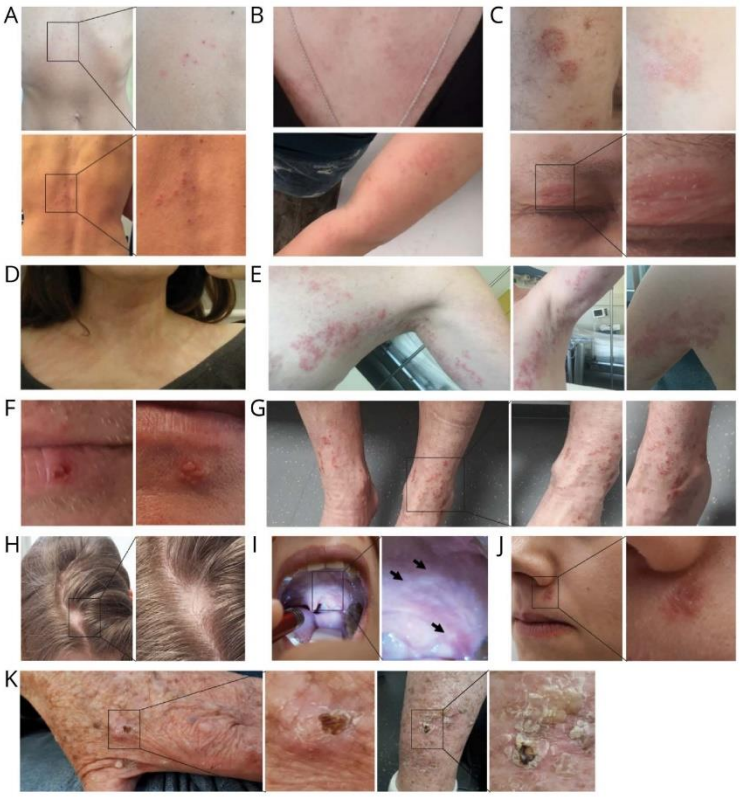
moderate state of immunosuppression with a $CD19^+$ B cell of 51 cells/μL and 100 cells/μL, respectively. T-cell counts were normal (724 and 670 $CD3^+$ cells/μL; 411 and 334 T helper cells/μL; 253 and 150 cytotoxic T cells/μL). Of note, a significant increase in the relative proportion of T cells expressing the activation marker HLA-DR was observed in the patients with alopecia areata (increase from 4.8% to 16.8% at the onset of autoimmunity). Because the last cladribine treatment course particularly narrowed the difference between activated ($CD3^+$ $HLA\text{-}DR^+$) and nonactivated ($CD3^+HLA\text{-}DR^-$) T cells, it can be speculated that this may have caused an imbalance to the disadvantage of regulatory T cells. Indeed, there are several lines of evidence suggesting that alopecia areata is a primary T cell–mediated autoimmune condition. In contrast, the pathomechanism of leukocytoclastic vasculitis is still unclear. T helper cells appear to be involved in disease induction,[10] but in this particular case showed no dynamics during disease onset.

Moreover, it is currently unknown whether the reported cases showed expression of risk alleles for the respective autoimmune phenomenon; however, this should be investigated in future cases to further assess individual risk profiles.[11]

In addition, and in view of cladribine's mechanism of action, the risk of malignancy was thoroughly evaluated in clinical studies. Although CLARITY and its extension outlined a higher incidence of overall malignancies in the verum group,[9] independent reviews showed a similar rate of malignancy with cladribine compared with other DMTs or in comparison to healthy population.[2] Here, we report 3 precancerous skin lesions and 1 case of malignant squamous cell carcinoma in 2 cladribine-treated individuals (0.8%), not exceeding the expected overall rate of neoplasms as outlined by CLARITY.[9] Of note, benign (seborrheic keratosis, skin papilloma, and melanocytic nevus) and malignant (squamous cell carcinoma, basal cell carcinoma, and malignant melanoma) isolated skin carcinomas have been previously described in the context of cladribine treatment.[5-7]

In conclusion, we report 77 cases of skin AEs, associated with cladribine treatment. Taking into account the high frequency,

Figure Cladribine-Related Skin Reactions



(A) Acneiform eruption consisted of follicle-based papules without comedones and lesions typically occurred on the face, scalp, chest, and back, sparing the extremities. (B) Representative images of 1 patient, experiencing diffuse skin rash. (C) Nummular eczema typically occurred as itchy coin-sized round- to ovoid-shaped red plaques in 2 cladribine-treated individuals. (D) An example of a female patient with severe and unbearable itching. (E) Disseminated herpes zoster infection of left Th1 to Th4 dermatomes. (F) Representative images of herpes simplex infection following cladribine treatment. (G) Typical presentation of a leukocytoclastic vasculitis with palpable purpura, accompanied by pain and burning at both legs, in a 42-year-old female cladribine-treated patient with MS. (H) A case of alopecia areata with typical hair loss in one round spot on the scalp. (I) Oral leukoplakia simplex in a 41-year-old cladribine-treated patient. Typical white changes of the buccal mucosa are depicted (black arrows). (J) Actinic keratosis manifested as a rough, dry patch of skin, of approximately 1.5 cm in diameter in a 41-year-old cladribine-treated patient. (K) A 69-year-old female patient with MS developed squamous cell carcinomas on sun-exposed skin (hand and legs), appearing as hyperkeratotic plaque with central ulceration and/or crusted surface.

as well as the in part autoimmune origin, it should be carefully evaluated whether patients prone to skin irritation should receive treatment with cladribine. Moreover, we emphasize the necessity of careful clinical surveillance of cladribine-treated patients to warrant early diagnosis and prompt treatment. In this context, we consider that a complete clinical examination of the skin at regular intervals should be mandatory for all patients. In particular, our cases of alopecia areata and leukocytoclastic vasculitis further demonstrate that clinicians must to be aware of and screen yet undescribed (secondary) autoimmune phenomena. Large register studies are necessary to establish the true incidence and the clinical impact of skin AEs in cladribine-treated patients with MS in the future.

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Jana Hackert, MD	University Hospital Essen, Essen, Germany	Acquisition of data
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Tobias Ruck, MD	University Hospital Münster, Münster, Germany	Critical revision of the manuscript for intellectual content
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Refik Pul, MD	University Hospital Essen, Essen, Germany	Study concept and design; acquisition and interpretation of data; and drafted the manuscript

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5. ABKÜRZUNGSVERZEICHNIS

BTK	Bruton-Tyrosin-Kinase
CD	*cluster of differentiation*
CDW	*confirmed disability worsening*
DMT	*disease-modifying therapy*
EDSS	*expanded disability status scale*
IRT	Immunrekonstitutionstherapie
JCV	JC-Virus
MRT	Magnetresonanztomografie
MS	Multiple Sklerose
NEDA	*no evidence of disease activity*
PML	Progressive multifokale Leukoenzephalopathie
PMS	Progrediente Multiple Sklerose
PPMS	primär chronisch-progrediente Multiple Sklerose
RMS	relapsing (schubförmige) Multiple Sklerose
S1PRM	Sphingosin-1-phosphat-Rezeptormodulator
SPMS	sekundär chronisch-progrediente Multiple Sklerose
ZNS	zentrales Nervensystem

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8. ERKLÄRUNG

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Gießen, im August 2024

Dr. med. Steffen Pfeuffer