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Dear Attendees,

It is a pleasure to welcome you to the MS Research days 2017 in het Van der Valk Hotel in Tiel. A yearly event that all MS scientists within the Netherlands and Belgium look forward to.

Being members of the Scientific Advisory Board of the Dutch MS Research Foundation, we aim at bringing together well-established scientists and young investigators within the MS field to share their recent findings and new concepts. Our mutual goal is to understand how MS originates, its pathological, imaging and clinical aspects, and how we can come to a better monitoring and treatment of MS patients.

This year’s scientific program offers a mix of exciting presentations and speed-dates resulting in a competition to obtain a collaborative start-up research grant. In addition, we are glad to have two renowned keynote speakers: Prof. dr. Robin Franklin (Cambridge University, UK) and Dr. Charlotte Teunissen (VUmc, Amsterdam). Furthermore, there is time allocated to discuss among each other and to enjoy.

Mutual interactions between MS scientists and MS patients are an important aspect of this year’s MS Research days. Learning from patients and their care-takers what their daily fights with MS are about, enforces our drive as MS scientists to conquer the disease. With novel insights, technology and joint scientific forces, we should be able to ‘meet’ the needs of MS patients.

We wish you a motivating and inspiring meeting!

On behalf of the Scientific Advisory Board of the Dutch MS Research Foundation,

Dr. Wia Baron  
UMCG, Groningen

Dr. Anne-Marie van Dam  
VUmc, Amsterdam
# Short program

## Tuesday, 14 November 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Scientific program¹</th>
<th>Location</th>
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<tbody>
<tr>
<td>8:30 – 9:15</td>
<td>Registration</td>
<td>Breakout</td>
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<tr>
<td>9:15 – 9:45</td>
<td>Welcome &amp; Opening</td>
<td>Bloesem</td>
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<tr>
<td>9:45 – 10:45</td>
<td>Keynote lecture 1</td>
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<tr>
<td>10:45 – 11:00</td>
<td>Break</td>
<td>Breakout</td>
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<tr>
<td>11:00 – 12:15</td>
<td>Scientific presentations 1</td>
<td>Bloesem</td>
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<tr>
<td>12:15 – 13:00</td>
<td>Speed dates</td>
<td>Tuin- &amp; Waterkers</td>
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<tr>
<td>13:00 – 13:45</td>
<td>Lunch</td>
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<tr>
<td>13:45 – 15:45</td>
<td>Scientific presentations 2</td>
<td>Bloesem</td>
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<tr>
<td>15:45 – 16:15</td>
<td>Break</td>
<td>Breakout</td>
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<tr>
<td>16:15 – 18:00</td>
<td>Scientific presentations 3</td>
<td>Bloesem</td>
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<tr>
<td>18:00 – 18:30</td>
<td>Drinks</td>
<td>Breakout</td>
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<tr>
<td>18:30 – 21:30</td>
<td>Diner</td>
<td>Breakout</td>
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## Wednesday, 15 November 2017

<table>
<thead>
<tr>
<th>Time</th>
<th>Scientific program¹</th>
<th>Location</th>
<th>Program MS patients (Maas &amp; Waal)</th>
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<tr>
<td>8:30 – 9:30</td>
<td>Welcome and Coffee</td>
<td>Breakout</td>
<td>Reception</td>
</tr>
<tr>
<td>9:30 – 10:30</td>
<td>Keynote lecture 2</td>
<td>Bloesem</td>
<td>Welcome by D. Roos</td>
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<tr>
<td>10:30 – 11:30</td>
<td>Scientific presentations 4</td>
<td>Bloesem</td>
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<tr>
<td>11:30 – 11:50</td>
<td>Break</td>
<td>Breakout</td>
<td>Presentations</td>
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<tr>
<td>11:50 – 12:20</td>
<td>Thesis award &amp; presentation</td>
<td>Bloesem</td>
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<tr>
<td>12:20 – 12:30</td>
<td>Awards</td>
<td>Lunch</td>
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<tr>
<td>12:30 – 13:15</td>
<td>Lunch</td>
<td>Breakout</td>
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<tr>
<th>Time</th>
<th>Joined program for MS patients and MS professionals²</th>
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<tr>
<td>13:15 – 14:15</td>
<td>Sessions A-E Maas &amp; Waal</td>
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<td>Session F Bloesem</td>
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<td>Session G Tuin- &amp; Waterkers</td>
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<tr>
<td>14:15 – 14:30</td>
<td>Short break Maas &amp; Waal</td>
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<tr>
<td>14:30 – 14:50</td>
<td>Presentations nominees speed date sessions Maas &amp; Waal</td>
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<tr>
<td>14:50 – 15:45</td>
<td>Presentations Maas &amp; Waal</td>
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<tr>
<td>15:45 – 16:15</td>
<td>Entertainment</td>
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<td>16:15</td>
<td>Closure</td>
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¹ In English, ² In Dutch
TUESDAY, 14 NOVEMBER 2017

<table>
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<tr>
<th>Time</th>
<th>Scientific program (in English)</th>
<th>Location</th>
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<tr>
<td>8:30–9:15</td>
<td>Registration</td>
<td>Breakout</td>
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<td>9:15–9:45</td>
<td>Welcome &amp; Opening</td>
<td>Bloesem</td>
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</table>
| 9:45–10:45| Keynote lecture  
Prof. dr. R. Franklin  
Ageing and myelin regeneration in the CNS (page 13) |            |
| 10:45–11:00| Break                                                                                          | Breakout  |
| 11:00–12:15| Scientific presentations 1  
Moderators: Dr. A.M.W. van Dam, Dr. B. Broux  
• Distinct cholesterol efflux in grey and white matter astrocytes:  
  Implications for remyelination (page 14)  
  I. Werkman  
• Fronto-limbic dysconnection in patients with MS and depression (page 15)  
  Q. van der Geest  
• MS associated cytotoxic CD4+ T cells escape regulatory T cell mediated suppression (page 16)  
  C. Hoeks  
• Fatty acid metabolism in control of the phenotype of foamy macrophages (page 17)  
  E. Grajchen  
• Regional heterogeneity in oligodendrocyte progenitor cells (page 18)  
  D.H. Lentferink | Bloesem    |
| 12:15–13:00| Speed dates (page 11)                                                                          | Tuin- & Waterkers |
| 13:00–13:45| Lunch  
Prepare proposal speed-dates                                                              | Breakout  |
| 13:45–15:45| Scientific presentations 2  
Moderators: Dr. M.M. van Luijn, Dr. M.M. Schoonheim  
• Down-regulation of mTOR signaling pathway in MS demyelinating lesions of immunopattern III (page 19)  
  R. Rahmanzedeh  
• IFN-γ- and TLR9-induced CXCR3+T-bet+ memory B cells are key drivers of MS (page 20)  
  J. van Langelaar  
• White matter OPCs are more susceptible to IFNγ mediated inhibition of OPC proliferation and differentiation than grey matter OPCs (page 21)  
  J.M. Jongsma  
• Information processing speed and its relation with structural and functional brain changes in multiple sclerosis (page 22)  
  K. Meijer  
• Introducing NeuroKeys: Day-to-day smartphone-based individual disease monitoring, a validation study (page 23)  
  K.H. Lam  
• Immunoregulation during pregnancy in MS: study of NK cell function (page 24)  
  A.R. Lupu  
• The formation of advanced glycation endproducts in the central nervous system of MS patients and its animal model EAE (page 25)  
  S. Wetzels  
• Differential expression of microglial P2Y12R and TMEM119 in white- and grey matter lesions of MS patients (page 26)  
  T.A. van Wageningen | Bloesem    |
<p>| 15:45–16:15| Break                                                                                          | Breakout  |</p>
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<tr>
<th>Time</th>
<th>Activity</th>
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<tr>
<td>16:15–18:00</td>
<td><strong>Scientific presentations 3</strong>&lt;br&gt;<em>Moderators: Dr. W. Baron, Dr. G. Kooij</em>&lt;br&gt;- Dietary modification reduces MS-like disease in adult marmoset monkeys <em>(page 28)</em>&lt;br&gt;- CLIP upregulation on B cells associates with rapid MS onset and is governed by risk allele CLEC16A <em>(page 29)</em>&lt;br&gt;- Cognitive impairment in MS is associated with slowing of resting state oscillatory activity on magnetoencephalography <em>(page 30)</em>&lt;br&gt;- Dimethyl fumarate reduces the frequency and function of inflammatory immune cells in RRMS patients <em>(page 31)</em>&lt;br&gt;- MRI predictors of cognitive decline in MS <em>(page 32)</em>&lt;br&gt;- The Phosphodiesterase 4 (PDE4) inhibitor roflumilast improves remyelination in a mouse model for MS <em>(page 33)</em>&lt;br&gt;- TNFR2 as a target for MS treatment <em>(page 34)</em>&lt;br&gt;Y.S. Kap&lt;br&gt;L. Rijvers&lt;br&gt;E.M. Strijbis&lt;br&gt;G. Montes Diaz&lt;br&gt;A.J.C. Eijlers&lt;br&gt;M. Schepers&lt;br&gt;V. Pegoretti</td>
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<td>18:00–18:30</td>
<td>Drinks&lt;br&gt;<em>Prepare proposal speed-dates</em></td>
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<td>18:30–21:30</td>
<td>Diner (buffet)</td>
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<tr>
<td>Time</td>
<td>Scientific program (in English)</td>
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<td>8:30–9:30</td>
<td>Welcome and Coffee</td>
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<td>Prepare proposal speed-dates</td>
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<td>9:30–10:30</td>
<td>Keynote lecture</td>
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<td>Dr. C.E. Teunissen</td>
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<td>The biology speaks through</td>
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<td>fluid biomarkers</td>
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<td>10:30–11:30</td>
<td>Scientific presentations 4</td>
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<td>prof.dr. N. Hellings,</td>
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<td>dr. H. Vrenken</td>
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<td></td>
<td>• Influx of extracellular</td>
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<td>calcium drives axonal</td>
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<td>degeneration in an animal</td>
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<td>model of MS (page 36)</td>
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<td>M.E. Witte</td>
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<td>• IL-4-activated microglia/</td>
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<td>macro-phages overcome</td>
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<td>fibronectin aggregate-</td>
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<td>mediated inhibition of</td>
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<td>remyelination (page 37)</td>
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<td>P. Wang</td>
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<td>• Oncostatin M and the</td>
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<td>inflamed blood brain barrier:</td>
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<td>good, bad or both? (page 38)</td>
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<td>E. Houben</td>
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<td>• Microscopic cellular</td>
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<td>correlates of macroscopic</td>
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<td>MRI-measured structural</td>
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<td>network properties in MS</td>
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<td>(page 39)</td>
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<td>S. Kiljan</td>
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<td>11:30–11:50</td>
<td>Break</td>
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<td>11:50–12:20</td>
<td>Thesis award &amp; presentation</td>
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<td>12:20–12:30</td>
<td>Awards</td>
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<td>Announcement nominees speed-</td>
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<td>dates</td>
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<td>12:30–13:15</td>
<td>Lunch</td>
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# Complete program

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Organizer</th>
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</table>
| 13:15–14:15   | **Sessions A-E**<sup>3</sup>  
Meet-the-Scientist                                                                 | Maas & Waal     |
| 13:15–14:15   | **Session F**  
Research & Development drugs                                                                 | Bloesem         |
| 14:15–14:30   | Short break                                                                 | Maas & Waal     |
| 14:30–14:50   | **Presentations nominees**  
speed date sessions                                                                 | Maas & Waal     |
| 14:50–15:45   | **Presentations**  
• VUmc MS Center Amsterdam  
• Fundraising  
• Announcement Best Proposal                                                                 | Maas & Waal     |
| 15:45–16:15   | **Entertainment**  
Ik Hou van Holland  
MS Research edition                                                                 |                 |
| 16:15         | **Closure**                                                                 |                 |

<sup>1</sup> Nominees speed-dates have time to prepare their pitch between 12:30–14:15 hours  
<sup>2</sup> Researchers are encouraged to attend parallel sessions F or G.  
<sup>3</sup> Round-table conversations with MS-professional and patients.
Location

CONGRESS VENUE
HOTEL Van der Valk Tiel
Laan van Westroijen 10
4003 AZ Tiel, the Netherlands
Tel +31 344 62 20 20

DIRECTIONS

By car
From Amsterdam / Utrecht
- A2 Den Bosch follow
- Junction Deil exit A15 direction Nijmegen
- A15 exit Tiel / Maurik (exit 33)
- Top turn right
- Left at traffic lights
From Rotterdam
- Follow A15 towards Nijmegen
- Exit Tiel / Maurik (exit 33)
- Top turn right
- Left at traffic lights
From Apeldoorn / Zwolle
- A50 direction Den Bosch follow
- Exit Tiel / Rotterdam (A15)
- Exit Tiel / Maurik (exit 33)
- Top turn left
- At 2nd traffic lights turn left
From Arnhem / Nijmegen
- Follow A15 towards Rotterdam
- Exit Tiel / Maurik (exit 33)
- Top turn left
- At 2nd traffic lights turn left

The hotel offers free parking.

By public transport
Each hour several trains depart from Utrecht, ‘s Hertogenbosch, Nijmegen and Arnhem to Tiel station. It’s a 15 minutes walk from the trainstation to the hotel. You can also make use of the regional bus services, bus stop ‘Laan van Westroijen’.

For more information consult www.ns.nl (train) or 9292.nl (all public transport).
REGISTRATION
The registration desk is located near the entrance of the Bloesem room. Registered participants will receive the short program and a name batch. The program book, including abstracts, is only available electronically.

PRESENTATIONS
Presenters are kindly requested to timely hand in their PowerPoint presentation at the Bloesem room. Technical support is present on Tuesday November 14th from 8:15 till 9:15 am and on Wednesday November 15th from 8:30 till 9:30 am.

WIFI
Free wifi is available in the meeting room area.

FOOD & DRINKS
Coffee and tea are available in the breakout room during the breaks as indicated in the program. The breakout room is available for networking and gathering throughout the Research days. Lunch (Tuesday and Wednesday) and diner (Tuesday) are included and served in the breakout room.

CHAIR
Prof.dr. Dick Hoekstra  
Member Audit Committee of the Dutch MS Research Foundation

JURY THESIS AWARD
Prof.dr. Carlie de Vries (AMC, Amsterdam) - Chair  
Vice-president Scientific Advisory Board of the Dutch MS Research Foundation  
Prof.dr. Niels Hellings (BIOMED, Hasselt University)  
Prof.dr. Jon Laman (UMCG, Groningen)
Instructions for MS speed date session

MS SPEED DATES
The speed dates are meant for MS researchers and clinicians (PhD students and post-docs) to stimulate the collaborations between the different institutes and within the different disciplines. Every participant has three speed dates with a participant from another institute and will write a short research proposal with the best match speed date partner. Only PhD students and post-docs that are present on both Tuesday and Wednesday can participate.

PROGRAM
TUESDAY 12:15 - SPEED DATES
After a short introduction, every participant has 3 speed dates of each 7 minutes. Together with your speed date partner you will discuss which research you are doing and whether you can come up with a joint research proposal.

TUESDAY UNTIL WEDNESDAY 9:30 - WRITE RESEARCH PROPOSAL
With the speed date partner you have the best match, you will write a short research proposal. For this you use the MS-speed date form. Remember to hand in your proposal by 9.30 h on Wednesday morning in the box at the registration desk (one copy).

WEDNESDAY 12:20 - ANNOUNCEMENT NOMINEES
Nominated 3 speed date couples will be announced

WEDNESDAY 14:30 - PRESENTATIONS NOMINEES
The 3 nominated speed date couples will have 5 minutes per couple to present their research proposal (elevator pitch, no slides). The presentation must be understandable for a broad audience (MS patients, their companions, and professionals of different disciplines). The preferred language of speak is Dutch, but couples are allowed to pitch their proposal in English.

WEDNESDAY 14:50 - JURY CONSULTATION
The nominees and jury leave the general meeting room. The jury will ask the nominees some detailed questions about the scientific content of their proposal. The nominees will return to the general meeting room. The jury will select the best proposal.

WEDNESDAY 15.40 - AWARD CEREMONY
The speed date couple with the best research proposal and presentation will be rewarded with a chance to apply for a € 17,000,- research grant!
Instructions for MS speed date session

GUIDELINES
• Only one research proposal per person;
• A multidisciplinary approach is preferred;
• Feasibility should be taken into account.

JURY
Prof. dr. Jack van Horssen (VUmc, Amsterdam) – Chair
Prof. dr. Bart Eggen (UMCG, Groningen)
Dr. Jeffrey Bajramovic (BPRC, Rijswijk)
**Keynote lectures**

**KEYNOTE 1: PROF. DR. ROBIN J.M. FRANKLIN**  
(Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, UK)

*Ageing and myelin regeneration in the CNS*

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents the most compelling example of adult multipotent stem cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in multiple sclerosis (MS), and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination in MS, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. There is now compelling evidence that ageing is the major contributor to the declining efficiency of remyelination and that this is largely due to a failure of stem cell differentiation. This talk will cover some of our recent studies on how ageing effects many aspects of CNS remyelination, including the divergent properties of CNS progenitors of different developmental origin and how changes in the mechanical properties of the ageing brain change the properties of CNS progenitors.

**KEYNOTE 2: DR. CHARLOTTE E. TEUNISSEN**  
(VU Medical Center, Amsterdam)

*The biology speaks through fluid biomarkers*

Body fluid biomarkers have played an important role in MS, as especially the presence of oligoclonal bands has been the cornerstone of MS diagnostics. Nowadays, this role has partly been taken over by imaging metrics. Nevertheless, due to its proximity to the pathological tissue, molecular changes in CSF reveal specific information that can be used for clinical practice, for example the presence of JCV, or the presence of subclinical inflammation. Besides emerging novel CSF biomarkers, novel technological developments have revolutionised blood based analysis of neurospecific proteins, such as neurofilament light proteins. I will therefore defend the prediction that we are entering an era where subclinical biological changes, e.g. due to effective treatment, will be sensitively monitored through biomarkers.
DISTINCT CHOLESTEROL EFFLUX IN GREY AND WHITE MATTER ASTROCYTES: IMPLICATIONS FOR REMYELINATION

Author  Inge Werkman¹, Jo Mailleux², Jerome Hendriks, Wia Baron
Affiliation  ¹Department of Cell Biology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands, ²Department of Immunology, Hasselt University, Hasselt, Belgium

Background
Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system which eventually results in axonal loss mainly due to failure of remyelination. Strikingly, remyelination is far more efficient in grey matter (GM) lesions as compared to white matter (WM) lesions. Oligodendrocytes require high amounts of cholesterol for the formation of myelin, which is among others supplied by astrocytes. Therefore, we hypothesize that GM astrocytes deliver sufficient cholesterol to oligodendrocytes, which may facilitate remyelination in GM MS lesions.

Methods
The localisation of the transcription factor LXRβ, which regulates cholesterol efflux, in GM and WM was determined by immunohistochemistry. Also, the effect of demyelination-related astrocyte activation on cholesterol efflux was examined, using the TLR3 agonist Poly I:C, the TLR4 agonist LPS and a mixture of the pro-inflammatory cytokines TNFα, IFNγ and IL-1β. The expression of the cholesterol transporters ABCA1 and ABCG1 was determined by Western blot. In vitro myelination was studied in the presence of GM or WM astrocytes.

Results
Our data reveal that in control WM and WM MS lesions LXRβ is localized to cytoplasm, which indicates that ABC cholesterol transporters are inactive, while in GM astrocytes LXRβ is localized to the nucleus, which indicates cholesterol transport is active. In vitro, GM astrocytes secreted more cholesterol than WM astrocytes. Interestingly, treatment with pro-inflammatory cytokines decreased cholesterol efflux from both GM and WM astrocytes. The cytokine-induced decrease in cholesterol efflux was mediated via down-regulation of ABCA1, but not ABCG1. Myelination was significantly increased on a feeding layer of GM astrocytes as compared to a WM astrocyte layer. Similar results were obtained with astrocyte-conditioned medium.

Conclusion
Our findings indicate that cholesterol export is enhanced in GM astrocytes, which may facilitate remyelination. The pro-inflammatory environment as evident in demyelinated MS lesions, reduces cholesterol secretion in both GM and WM astrocytes.
Background
Depression occurs frequently in multiple sclerosis (MS) and its clinical manifestation is very similar to that of major depressive disorder (MDD). In MDD, on MRI fronto-limbic disconnection has been observed. To better understand depression in MS, we investigated structural and functional changes of the fronto-limbic system in MS patients with moderate-to-severe depression and non-depressed (nD) MS patients.

Methods
Patients were selected from two different cohorts: 1) 22 moderately-to-severe depressed (D) MS patients (scoring ≥20 on the Beck Depression Inventory; disease duration 8.2±7.7 years,); 2) 21 nDMS patients (scoring <8 on the Hospital Anxiety and Depression Scale – Depression; disease duration 15.3±8.3 years), and 12 healthy controls (HCs). All subjects underwent MRI (1.5T). Brain volumes (white matter (WM), grey matter, amygdala, hippocampus, thalamus), WM lesion load, fractional anisotropy (FA) of fronto-limbic tracts and resting-state functional connectivity (FC) between limbic and frontal areas were measured. A depression rank score was calculated for patients.

Results
Compared to nDMS patients, DMS patients had lower WM volume (P<0.001), and decreased FA of the uncinate fasciculus (P<0.01), despite a significantly shorter disease duration. FC between the amygdala and frontal regions was decreased in DMS compared to nDMS patients (P<0.01). Lower FA of the uncinate fasciculus, and decreased FC of the amygdala could explain 47% of variance in the depression rank score.

Conclusion
On MRI, more pronounced (MS) damage, i.e. structural and functional changes in temporo-frontal (limbic) regions (uncinate fasciculus and amygdala), were found to be specific for depressed compared to non-depressed MS patients, supporting our hypothesis for fronto-limbic dysconnection in MS.
MULTIPLE SCLEROSIS ASSOCIATED CYTOTOXIC CD4+ T CELLS ESCAPE REGULATORY T CELL MEDIATED SUPPRESSION

Author Cindy Hoeks*, Marjan Vanheusden*, Liesbet Peeters, Piet Stinissen, Bieke Broux, Niels Hellings

*Authors contributed equally

Affiliation Hasselt University, Biomedical Research Institute and Transnationale Universiteit Limburg, Diepenbeek, Belgium

A terminally differentiated subset of CD4+ T lymphocytes, characterized by loss of the costimulatory molecule CD28 and gain of cytotoxic activity, arises during aging and chronic inflammation. An age-inappropriate expansion of these cells has been found in autoimmune diseases like rheumatoid arthritis and multiple sclerosis (MS). Our group has recently published that these CD4+ cytotoxic T lymphocytes (CTL) contribute to the pathology of autoimmune diseases, as we were able to show that expansion of CD4+ CTL exacerbates experimental autoimmune encephalomyelitis.

Here we show that CD4+CD28null T cells are phenotypically different from CD4+CD28+ T cells, and that CD4+CD28null T cells evade Treg-mediated suppression in vitro. CD4+CD28null T cells display enhanced levels of pro-inflammatory molecules such as granzyme B, IFN-gamma, IL-1beta, IL-6, IL-22, and GM-CSF and decreased levels of IL-10R and GITR. Blocking of CD4+CD28null derived granzyme B or IFN-gamma completely restored the suppressive capacity of Tregs towards CD4+CD28null T cells. In contrast, blocking of IL-10R or GITR-L did not affect Treg-mediated suppression of CD4+CD28+ T cells. We further showed that Tregs upregulate IFN-gamma when exposed to the secretome of CD4+CD28null T cells. Blocking Treg-derived IFN-gamma partly reinstated Treg mediated suppression of CD4+CD28null T cells. These results suggest that CD4+CD28null T cells can evade Treg suppression through two distinct mechanisms: 1) by becoming less susceptible to Treg activity and 2) by directly altering the functionality of Tregs. Elucidating these pathways may contribute to the development of novel therapeutic interventions specifically targeting age-inappropriate expansion of CD4+ CTL in autoimmune diseases like MS.
FATTY ACID METABOLISM IN CONTROL OF THE PHENOTYPE OF FOAMY MACROPHAGES

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The accumulation of foam cells in the central nervous system (CNS) is a pathological hallmark of multiple sclerosis (MS) and other neurodegenerative disorders. To date, the functional properties of these foam cells as well as the molecular pathways involved in directing their phenotype remain poorly understood. Here, we provide evidence for a complex interplay between fatty acid metabolism and the phenotype of foamy phagocytes in demyelinating disorders.

Several studies demonstrated beneficial effects of myelin uptake on the macrophage phenotype and the importance of lipid signaling pathways in directing this protective phenotype. However, our recent data indicate that sustained accumulation of myelin within phagocytes eventually blunts their protective features and promotes an inflammatory transcriptional profile. We further show that the formation of unsaturated fatty acids through stearoyl-CoA desaturase-1 (SCD1) underlies the observed phenotype shift of macrophages after prolonged myelin uptake. Degradation of the lipid efflux transporter ABCA1 and subsequent accumulation of free cholesterol likely explains the inflammatory impact of SCD1. In line with these findings, we demonstrate that SCD1 deficiency reduces neuroinflammation and promotes CNS repair in well-established ex vivo and in vivo models. Finally, foamy macrophages located in the center of MS lesions show increased lipid load (free and esterified cholesterol), elevated SCD1 expression, and a reduced ABCA1 surface expression, as compared to foamy macrophages in the lesion rim. These observations suggest that foamy phagocytes in MS lesions also accumulate myelin-derived lipids and undergo a temporal phenotype shift. Hence, therapeutic interventions focused at preventing lipid overload in myelin-containing phagocytes offers a promising new therapeutic strategy for the treatment of MS and other neurodegenerative disorders.
REGIONAL HETEROGENEITY IN OLIGODENDROCYTE PROGENITOR CELLS

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Background
In the chronic demyelinating disease multiple sclerosis (MS) remyelination ultimately fails despite the presence of oligodendrocyte progenitor cells (OPCs) in most lesions. Since remyelination is crucial for functional neurological recovery and axonal survival, a therapeutic treatment aimed at restoring remyelination is a prerequisite for halting disease progression. Although MS is often considered a disease of the white matter, it has become increasingly apparent that demyelination in the grey matter is also a prominent feature. Interestingly, spontaneous remyelination appears more pronounced in grey than in white matter lesions. Here, we investigated whether this difference in remyelination capacity may be attributed to inherent molecular differences between grey and white matter OPCs per se.

Methods
OPCs were isolated from cortex and corpus callosum of postnatal day 7 rats via anti-A2B5 magnetic bead isolation. The transcriptomic profile of these cells was compared via 3’-RNAseq. Additionally, functional assays were performed on OPCs obtained from mixed glial cultures derived either from cortical or non-cortical parts of newborn rats.

Results
Our preliminary results show differential expression of genes which may be involved in (re)myelination in A2B5+ OPCs isolated from cortex and corpus callosum. Functional assays on cortical and non-cortical OPCs show an increased proliferation and differentiation of cortical OPCs. Interestingly, cortical OPCs have increased mRNA levels of hes5, a transcription factor that represses myelin gene expression, while non-cortical OPCs are more enriched in genes involved in oligodendrocyte differentiation and myelin formation (ikbkap, lpar1 and nkx6-2).

Conclusion
Regional OPCs display their own intrinsic identity. Cortical OPCs appear to be more immature and may therefore be better equipped for remyelination. Exploring the molecular background of the observed difference in remyelination capacity of regional OPCs will lead to a better understanding of the process of remyelination, and may open therapeutic avenues to enhance remyelination in MS lesions.
SCIENTIFIC PRESENTATIONS 2
Tuesday 13:45-15:45
Moderators: Dr. Marvin van Luijn, Dr. Menno Schoonheim

DOWN-REGULATION OF MTOR SIGNALING PATHWAY IN MS DEMYELINATING LESIONS OF IMMUNOPATTERN III

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Background
Although the pathogenesis of multiple sclerosis was mostly attributed to myelinswallowing macrophages activated by autoimmune T-cells, recent neuropathological studies unraveled that other non-inflammatory processes may be involved. In keeping, it has been shown that in a subpopulation of MS patients (with lesions of immunopattern III), a primary oligodendrocytopathy may antedate other inflammatory events. It has been shown that the primary oligodendrocytopathy may mimic a dying back pathology likely due to impairment in energy system of oligodendrocytes. In our study we investigated the possible alterations of mTOR signaling pathway, which has been shown to play a pivotal role in energy production and myelinating ability of oligodendrocytes. Study design and procedure: The study were performed on archival materials of 20 biopsies-autopsies. with histologically proven active MS lesion of type 3. In summary, paraffin-embedded 5-mm sections were stained with hematoxylin-eosin, Luxol fast blue myelin stain, periodic acid±Schiff (PAS) reaction. In addition, we used CNPase to identify oligodendrocytes and antibodies against proteins of mTOR signaling pathway; PI3K, AKT, TSC, Rheb, mTOR.

Results
The preliminary data show that proteins involved in mTOR pathway (PI3K, AKT, TSC, Rheb, mTOR) are down-regulated in type 3 MS lesions.

Discussion
Studies suggest that the mTOR signaling pathway was evolved to be the master regulator of cellular metabolism and energy homeostasis in cells. Owing to high level of myelin synthesis, oligodendrocytes have been suggested to have very high metabolic rate. The unique features of MS lesions of immunopattern III show that a primary oligodendrocytopathy may underlie the formation of such lesions. Our results show that this primary event may be due to down regulation of mTOR signaling pathway that leads to a disturbance in energy system of oligodendrocytes.
IFN-γ- AND TLR9-INDUCED CXCR3+T-BET+ MEMORY B CELLS ARE KEY DRIVERS OF MS

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A recent breakthrough in MS research is the approval of B-cell depletion as treatment. However, this treatment slows down but does not prevent progression. In MS patients, peripheral B-cell tolerance checkpoints are defective and meningeal ectopic B-cell follicles are found, indicating that yet undefined B-cell subpopulations infiltrate the CNS to trigger local pathology. In this study, we found that CXCR3 and not CXCR4 or CXCR5 is upregulated on accumulating IgG+B cells in the blood of MS patients treated with natalizumab (pre- versus 1 year, 2 and 3 years post-Tx). This accumulation was not seen for naive mature, IgM+ and IgA+ memory B cells, which implicates that CXCR3+IgG+B cells preferentially recruit to the CNS during the MS course. Correspondingly, CXCR3+IgG+B cells were selectively reduced in RRMS versus matched control blood, and were enriched in ex vivo, paired single-cell suspensions from MS CSF and brain tissue compared to blood (NBB). CXCR3 expression is under the direct control of T-bet, which is IFN-γ- and TLR9-inducible and promotes ectopic B-cell follicle formation in mice. To elucidate how the differentiation and function of T-bet+B cells are regulated, we stimulated B cells from RRMS and matched control blood with IFN-γ using a 3T3-CD40L/IL-21 culture system. Intracellular T-bet expression was significantly increased in IFN-γ-induced CXCR3+ B cells in MS. The sensitivity of B cells to IFN-γ stimulation was dependent on the presence of a recently identified genetic MS risk variant, the IFN-γ receptor β-chain (IFNGR2). Supplementation of TLR9 ligand CpG to in vitro cultures of naive mature, but not memory B cells further increased IFN-γ-induced T-bet expression, which was required for switching to IgG1. These findings implicate synergistic upregulation of T-bet in B cells, by IFN-γ and TLR9, underlies their CXCR3-mediated migration into the CNS of MS patients resulting in enhanced recognition of local antigens and pathogenic antibody production.
WHITE MATTER OPCs ARE MORE SUSCEPTIBLE TO IFNγ-MEDIATED INHIBITION OF OPC PROLIFERATION AND DIFFERENTIATION THAN GREY MATTER OPCs

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Background
Multiple sclerosis (MS) is a chronic inflammatory disease, which is characterized by demyelinated lesions. At later stages, repair in the form of remyelination often fails, which leads to axonal degeneration and neurological disability. For the regeneration of myelin, oligodendrocyte progenitor cells (OPCs) have to migrate, proliferate and differentiate into remyelinating oligodendrocytes. Previously, we have shown that differences between grey matter (GM) and white matter (WM) OPCs may account for the increased ability of GM OPCs to remyelinate denuded axons. Here, we aimed to examine and compare the effect of exposure of the MS relevant pro-inflammatory cytokines TNFα and IFNγ on GM and WM OPC behavior.

Methods
OPCs derived from cortical (GM) and non-cortical (mostly WM) forebrain areas were exposed to TNFα, IFNγ or a combination of these. The effect of cytokine treatment on OPC migration (transwell assay), proliferation (Ki67), survival (MTT/LDH), morphology (A2B5) was examined. Also, the differentiation potential (MBP) and myelin membrane formation of cytokine-exposed OPCs that were allowed to mature in the absence of cytokines was assessed.

Results
IFNγ, but not TNFα treatment, decreased OPC proliferation, which was more pronounced in WM OPCs as compared to GM OPCs. In addition, upon exposure to IFNγ both GM and WM OPCs develop very long primary processes which were also less arborized. Cytokine treatment did not affect GM and WM OPC migration and survival. The differentiation of WM OPCs that were briefly exposed to IFNγ was delayed, while combined IFNγ and TNFα treatment delayed differentiation of both GM and WM OPCs.

Conclusion
WM OPCs are more susceptible to IFNγ-mediated inhibition of OPC proliferation and differentiation as compared to GM OPCs, which may contribute to the difference in remyelination efficiency between GM and WM MS lesions. Furthermore, a transient exposure of OPCs to pro-inflammatory cytokines has long term effects OPC maturation.
Information processing speed (IPS) deficits are amongst the first cognitive symptoms in multiple sclerosis (MS) and are highly debilitating. Although both structural and functional alterations have been associated with IPS impairment, it is unclear whether IPS deficits can be explained by predominantly structural damage, predominantly functional changes or both. Therefore, we examined the impact of mild and severe structural and functional brain changes on IPS.

Methods
IPS was measured using the symbol digit modalities test (SDMT) in 330 MS patients and 96 healthy controls. The severity of structural MRI-damage was measured using diffusion tensor imaging (DTI), atrophy and lesion load, while the severity of functional damage was determined by the average level of increased and decreased resting-state functional connectivity. The most important predictors of IPS were identified using a regression model in MS. Subsequently, patients were divided into groups with mild or severe functional and/or structural damage, between which IPS was compared.

Results
Deep grey matter volume, WM integrity and increased functional connectivity were predictors for IPS (all p<0.05). Patients with mild functional and mild structural changes had the best IPS (z-score=-0.40). IPS was mildly impaired for patients with predominantly functional changes (z-score=-0.84) and for patients with predominantly structural changes (z-score=-1.49). Those with both severe functional and severe structural changes were worst off (z-score=-1.95).

Conclusion
Structural damage was associated with worse IPS compared to functional changes. However, severe functional damage did occur in the presence of only limited structural damage, which was associated with a decline in IPS compared to HCs. This disparity suggests that IPS does not fully rely on one or the other, but is reflected by a weighted combination of structural and functional brain changes.
INTRODUCING NEUROKEYS: DAY-TO-DAY SMARTPHONE-BASED INDIVIDUAL DISEASE MONITORING, A VALIDATION STUDY

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Background
Multiple sclerosis (MS) is a disease with a high variety of symptoms and unpredictable disease course. Disease monitoring requires clinical visits, therefore assessing symptoms showing day-to-day fluctuations is challenging. Fatigue is one such symptom, affecting ≥75% of MS patients and impacting quality of life. Due to its subjective, multidimensional and multifactorial nature, current measures of fatigue face unmet needs. To address this, NeuroKeys mobile application has been developed. NeuroKeys is an intelligent keyboard that collects keystroke dynamics and internal smartphone sensor data, previously suggested to be related to internal states like fatigue and stress. NeuroKeys is specially designed for unobtrusive and continuous monitoring, providing the patient and treating physician with (prospective) insight in the day-to-day fluctuations of fatigue.

Methods
A single centre, prospective observational cohort study is scheduled to start in October 2017 to explore feasibility and validity of NeuroKeys in a population of one-hundred MS patients (50 relapsing-remitting and 50 progressive MS) and one-hundred controls. Clinical visits, consisting of conventional measures in MS, take place at baseline and every three months thereafter for a total of 12 months. In the one year follow-up duration patients will use NeuroKeys and wearables.

Analyses
Linear and logistic regression models will be utilized to investigate associations between the mobile application and established measures of fatigue, disease activity and progression. In addition, in-house datascientists of Neurokeys will build individual predictive models utilizing Machine Learning techniques in supervised and unsupervised learning. Of special interest are Neural Networks, Nearest Neighbour approaches and hierarchical models to deal with timeseries data.
Natural killer (NK) cells are able to modulate immune response through cytokine production and/or target cell lysis based on “missing self” recognition. Some evidence suggests that impaired regulatory function of NK cells may be involved in the pathobiology of multiple sclerosis (MS) while NK cell induced self-tolerance could help to control neuroinflammation and reduce MS disease activity. NK cells have also been found to play a key role during pregnancy to protect the semi-allogeneic fetus from immune rejection. Intriguingly, epidemiological evidence clearly demonstrates that pregnancy reduces the number of relapses in MS patients, especially in the third trimester. Recent scientific data also support the NK cell contribution to the protective role of pregnancy in MS, but the mechanisms regulating NK cell subtypes in pregnant women with MS are not fully understood.

To explore the functional competence of NK cell subsets during pregnancy (3rd trimester) and postpartum period (PP, 3 months) in MS patients, we have measured gene expression (qPCR), phenotype and function of NK cells using the surface marker CD107a and intracellular IFNγ expression following a 5 hours PBMCs:K562 cells co-culture. Our data showed no significant differences in expression of CD107a and IFNγ in PP compared with the 3rd trimester. However, the evolution of CD107a and IFNγ expression from 1st trimester to PP was similar for both PBMCs:K562 sample and positive control (PMA/Ionomycin stimulated PBMCs) showing that the capacity of NK cells to respond to tumor promoting and/or infectious stimuli (PMA / Ionomycin) was preserved. The relative expression of perforin in NK cells was higher in PP vs 3rd trimester. However, the increase in perforin expression was not associated with a corresponding increase in CD107a expression (needed for NK cells to release perforin containing cytotoxic granules). All obtained results suggest that NK cells would be less responsive to pro-cytotoxic phenotype stimuli, as a protective mechanism in order to support normal pregnancy. This could in part explain the beneficial role of pregnancy in MS.

This work was supported by Stichting MS Research in the frame of an MSIF Du Pré Grant.
In multiple sclerosis (MS), the pro-inflammatory activation of innate immune cells induces a metabolic shift towards glycolysis. Moreover, it is known that astrocytes mainly rely on glycolysis to provide neurons with energy. Glycolysis can lead to the formation of reactive dicarbonyl byproducts (e.g. MGO and GO) that are potent glycating agents involved in the formation of advanced glycation endproducts (AGEs). AGEs are shown to induce pro-inflammatory effects. Glyoxalase 1 (Glo1) is the rate-limiting enzyme that neutralizes MGO and GO, thereby preventing the formation of AGEs. In this study, we hypothesized that AGEs are formed in the central nervous system (CNS) during neuroinflammatory responses, potentially contributing to the disease pathology. By using UPLC-MS/MS, we determined AGEs (CML, CEL, MG-H1), MGO and GO in the plasma, spinal cord, and brain of mice subjected to experimental autoimmune encephalomyelitis (EAE), an animal model for MS. We demonstrate that AGEs and MGO are significantly increased in the spinal cord and brain of EAE mice compared to controls. In line, Glo1 activity was decreased in the CNS of EAE mice. To confirm the presence of AGEs in human active MS lesions, fluorescence immunohistochemistry (IHC) was used to stain for AGEs combined with various cell markers such as Iba, GFAP, and neurofilament to localize AGEs in macrophages, astrocytes, and neurons respectively. IHC revealed that AGEs are accumulating predominantly in GFAP+ astrocytes in active MS lesions. Collectively, our data demonstrate that AGEs are generated during neuroinflammatory responses and accumulate in astrocytes in MS lesions.
DIFFERENTIAL EXPRESSION OF MICROGLIAL P2Y12R AND TMEM119 IN WHITE- AND GREY MATTER LESIONS OF MULTIPLE SCLEROSIS PATIENTS

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Background
In Multiple Sclerosis (MS), demyelination in white-matter lesions (WML’s) is accompanied by an influx of leukocytes, including macrophages, and activation of local microglial cells. Demyelination in grey-matter lesions (GML’s) occurs in a relative absence of infiltrating leukocytes and limited activation of microglia. This overt difference in inflammatory status of WML’s and GML’s has questioned the role of macrophages and microglia in MS pathology. In order to elucidate the role of endogenous microglia in MS, cell-type specific markers are of utmost importance.

Methods
We utilized immunohistochemistry on post-mortem material of MS patients containing inflammatory active and chronic-active WML’s; and less inflammatory subpial GML’s to study the expression of the recently proposed microglia-specific protein TMEM119 and the purinergic receptor P2Y12, as well as the expression of HLA-DR and Iba-1. In addition, to determine if TMEM119 and P2RY12 were regulated by inflammatory stimuli we measured mRNA levels of TMEM119 and P2RY12 in post-mortem isolated human microglial cells treated with IL-4 or IFNγ+LPS compared to control.

Results
In normal appearing white- and grey-matter, TMEM119 and P2RY12 positive (+) cells were present in ramified microglia. In the core of active WML’s, when HLA-DR+ immunoreactivity increased, there was a loss of P2RY12 and TMEM119 immunoreactivity. Still, amoeboid-shaped and morphologically reactive microglial TMEM119+ and P2RY12+ cells co-localizing with HLA-DR were found at the edge of active WML’s. Similarly, TMEM119+ and P2RY12+ cells co-localizing with HLA-DR were observed in the rim of chronic-active WML’s. In the core of chronic-active WML’s, TMEM119+ cells remained absent, but reactive P2RY12+ cells re-appeared showing no co-localization with HLA-DR. In less inflammatory subpial GML’s, no loss of TMEM119 and P2RY12 immunoreactivity was observed compared to non-affected GM. Moreover, TMEM119+ and P2YR12+ cells as well as Iba-1+ cells showed a ramified phenotype in these lesions. At the mRNA level, P2RY12 was reduced after IFNγ+LPS treatment and increased after IL-4 treatment. TMEM119 mRNA level was decreased by both treatments.
Conclusion
In WML's, expression of microglial TMEM119 and P2RY12 is probably affected by inflammatory factors, and thus remains difficult to visualize endogenous microglia. However, in GM, TMEM119 and P2RY12 are present in ramified microglia irrespective of demyelination and both proteins can be used as genuine microglial markers.
The interplay between diet, gut microbiota and Epstein-Barr virus (EBV) infection with the immune system is thought to play a determining role in the development of multiple sclerosis (MS). Experimental autoimmune encephalomyelitis (EAE) in common marmosets (Callithrix jacchus), a Neotropical primate, is a translationally relevant model of MS. We noticed that after the introduction of a new dietary supplement in our marmoset colony, the frequency of marmosets in which clinically evident EAE could be induced had decreased from 100 to 40%. This finding prompted the here reported controlled study in marmoset twins where the effects of the new and classic dietary supplement on factors contributing to EAE susceptibility were compared. One sibling of eight adult dizygotic bone-marrow chimeric twin pairs raised on the new diet were fed the classic diet starting eight weeks before EAE induction with rhMOG/IFA; the other sibling was maintained on the new diet. In the monkeys reverted to the classic diet a 100% (8 of 8) EAE incidence was observed, whereas in the monkeys maintained on the new diet the EAE incidence was 75% (6 of 8). Although six animals of the new diet group developed clinically evident EAE, their spinal cord demyelination was significantly lower than in the classic diet group and this was associated with reduced pro-inflammatory T cells in lymphoid organs. Typing of the marmoset gut microbiota using 16S V4 rRNA sequencing demonstrated a unique, Bifidobacteria-dominated composition. Starting at 3 weeks post-EAE induction, we observed a diet-related divergence of gut microbiota composition between twin siblings. In addition, the new diet was associated with reduced expression of CalHV3, an EBV-related herpesvirus with a crucial pathogenic role in the model. In conclusion, we report a marked effect of dietary modification on the susceptibility of adult, outbred, conventionally-housed marmosets to MS-like disease.
CLIP UPREGULATION ON B CELLS ASSOCIATES WITH RAPID MS ONSET AND IS GOVERNED BY RISK ALLELE CLEC16A

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C-type lectin CLEC16A is located next to CIITA (the master regulator of HLA class II genes) at a risk locus for many autoimmune diseases, including MS. Previously, our group revealed that CLEC16A is upregulated in MS and triggers the biogenesis of HLA-II peptide-loading compartments (MIIC) in myeloid antigen-presenting cells. Since T-cell activation by B cells critically contributes to early MS, we assessed whether key members of the HLA class II pathway in B cells are dysregulated during MS onset, and how CLEC16A is involved in this process.

FACS analysis of surface HLA-DR, CD74 and CLIP expression was performed for B cells from CIS patients who developed CDMS within 1 year, or remained CIS for at least 5 years (n=33), as well as for human EBV+ and EBV- B-LCL (n=19). Surface levels were related to the expression of 15 MS risk genes, including CLEC16A. CLEC16A was silenced using different shRNA constructs to define its role in HLA-DR, CD74 and CLIP surface expression (FACS) and MIIC biogenesis (confocal imaging) in B cells.

CLIP-containing HLA-DR molecules were more abundant, while CD74 was less present on blood B cells from CIS patients who rapidly develop CDMS. CLIP/HLA-DR surface ratios were elevated, and only correlated to CLEC16A (r=0.78) in EBV+ versus EBV- B-LCL. CLEC16A knockdown in EBV+ B-LCL significantly reduced CLIP/HLA-DR ratios and upregulated CD74 on the plasma membrane. Moreover, CLEC16A-silenced B cells showed extensive scattering of MIIC throughout the cytoplasm, indicating disrupted transport of CLIP-containing HLA-DR molecules between perinuclear regions and the plasma membrane.

These data demonstrate that the HLA class II pathway in B cells is dysregulated in early MS and is functionally controlled by MS risk allele CLEC16A. The abundance of CLIP-loaded HLA-DR molecules on B cells is mechanistically linked to their ability to escape from peripheral tolerance checkpoints during MS onset.
COGNITIVE IMPAIRMENT IN MULTIPLE SCLEROSIS IS ASSOCIATED WITH SLOWING OF RESTING STATE OSCILLATORY ACTIVITY ON MAGNETOEENCEPHALOGRAPHY

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Background
Neurophysiological measures of brain function, such as magnetoencephalography (MEG), are widely used in clinical neurology and have strong relations with cognitive impairment and dementia, but are presently still underdeveloped in multiple sclerosis (MS). This study aims to assess the value of clinically applicable quantitative MEG measures of neuronal activity in evaluating and predicting cognitive impairment in MS.

Methods
Eyes-closed resting-state MEG measurements of 83 patients with long-standing, clinically definite MS and 34 healthy controls (HC) were analyzed and related to neuropsychological evaluations, based on an expanded brief repeatable battery of neuropsychological tests (BRB-N). “Cognitive impairment” (CI) was defined as Z-scores (using HC as reference) of ≤-2 on two or more domains; Z-scores > -1.5 on all domains as “cognitively preserved” (CP) and all scores in between as “mildly cognitively impaired” (MCI). Time series were estimated using a beamforming approach, for 78 cortical regions of interest (ROIs) based on an automated anatomical labelling atlas (AAL). For each subject five artifact-free epochs (~13 seconds, sample frequency 1250Hz) were selected; peak frequencies and relative power were calculated for each of six frequency bands and each ROI. Subsequently, global relative power and peak frequency were averaged over all 78 ROIs. Associations with cognitive impairment were performed using linear modelling correcting for age, gender and educational level where appropriate.

Results
Of all 83 patients, 37 were labeled as CP, 18 as MCI, and 28 as CI. The CI-MS group had a significantly lower peak frequency (β=0.266;P=0.049) than HCs, indicating higher relative alpha1-power and overall slowing of neuronal activity. Increased global relative alpha1-power was associated with impaired overall cognitive performance (β=0.304;P=0.005) but specifically with attention (β=0.408;P<.001), working memory (β=0.388;P<.001 and verbal memory (β=0.257;P=0.020). Increased global relative theta-power was associated with worse performance on verbal memory tasks (β=0.333;P=0.003).

Conclusion
These findings indicate a clinically relevant global slowing of neuronal activity in MS patients, affecting cognition, and hold promise for the application of resting-state MEG in a clinical setting as a biomarker for cognitive disturbances. Future studies are warranted to assess the prognostic value of these neurophysiological changes in MS.
Dimethyl fumarate (DMF) is an oral therapy for multiple sclerosis (MS) with a yet unknown working mechanism. The aim of this study was to perform an extensive immunophenotypic analysis of the innate and adaptive immune system of MS patients under DMF treatment using 3 different study designs: a 12 month follow-up study (n = 12), a cross-sectional study (untreated n = 25, DMF treated n = 25) and an in vitro study. The distribution of innate, T and B cell subtypes and T helper cytokine expression, including interferon (IFN)-γ, granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-17, was determined by flow cytometry. DMF was added to in vitro B cell cultures to analyze B cell apoptosis (annexin V) and expression of costimulatory molecules (CD40, CD80, CD86), antigen presentation major histocompatibility complex (MHC)II and B cell activating factor receptor (BAFFR).

After 12 months of DMF treatment, monocyte and natural killer cell frequencies were increased while pro-inflammatory T and B cell subsets, including memory and effector memory CD4+ and CD8+ T cells and double negative, class-switched and non class-switched memory B cells decreased. A decreased percentage of IFN-γ+, GM-CSF+ and IL-17+ T helper cells was detected. Further, naïve CD4+ and CD8+ T cells, transitional and naïve B cells increased. The cross-sectional study indicated that DMF was fully effective after 6 months of therapy. In vitro experiments demonstrated DMF induced B cell apoptosis in a direct and concentration dependent manner. DMF tended to increase the frequency of regulatory B cells. Additionally, DMF decreased B cell expression of CD40, MHCII and BAFFR.

DMF treatment induced a persistent redistribution of the adaptive and innate immune system favoring anti-inflammatory responses in MS patients. One of the working mechanisms of DMF was to directly induce B cell apoptosis and reduce expression of functional markers on B cells.
MRI PREDICTORS OF COGNITIVE DECLINE IN MS

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Background
Different cross-sectional MRI correlates of cognitive impairment have been discovered, but due to a lack of longitudinal data, it is still unclear which measures best predict future cognitive decline. The objective of this study was to evaluate which baseline structural MRI measures best predict cognitive decline during a 5-year follow-up period.

Methods
A total of 234 clinically definite MS patients and 60 healthy controls (HC) were seen twice, with a 5-year interval (mean=4.94 year, SD=0.94). Structural MRI measures (3T) at baseline included cortical, deep grey and white matter volumes, lesion volume as well as white matter integrity. The Practice Adjusted Reliable Change Index (RCI) was computed to assess cognitive changes during follow-up and divided by interval duration to obtain a yearly cognitive decline rate. Patients were classified as having cognitive decline when the cognitive domain decline rate exceeded Z=-0.25/yr on at least two cognitive domains.

Results
The MS patient group demonstrated an average yearly cognitive decline rate (RCI) of Z=-0.05/yr, with a three times faster rate in progressive compared to relapsing patients (-0.10/yr vs -0.03/yr, p<0.01). Patients that demonstrated cognitive decline during follow-up already showed more severe structural damage at baseline, including lower white matter integrity, higher lesion volumes, lower cortical and deep grey matter volumes, as well as a higher proportion of progressive patients (42% vs 14%). Cognitive decline in MS patients was independently predicted (adjusted R² = 0.53, p<0.01) by lower age, lower regional cortical grey matter volumes, higher average cognitive performance at baseline, progressive disease phenotype and lower level of education.

Conclusions
These results demonstrate that baseline MRI measures help to predict future cognitive decline, with cortical grey matter volumes being the strongest predictors. Future studies are now needed to elucidate the mechanisms of how these structural abnormalities lead to disturbances in brain function and cognitive impairment.
THE PHOSPHODIESTERASE 4 (PDE4) INHIBITOR ROFLUMILAST IMPROVES REMYELINATION IN A MOUSE MODEL FOR MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic autoimmune disease (CNS) that results in central nervous system demyelination and cognitive impairment. Pathway analysis shows that cyclic adenosine monophosphate (cAMP) directs oligodendrocyte precursor cell (OPC) differentiation into mature myelinating oligodendrocytes, a prerequisite for remyelination. Phosphodiesterase 4 (PDE4) inhibitors suppress the breakdown of cAMP, thereby augmenting cAMP levels and facilitating OPC differentiation. We hypothesize that the PDE4 inhibitor roflumilast promotes in vivo remyelination in an animal model for remyelination.

The impact of roflumilast (5µM) on ex vivo remyelination was studied in lysolecithin-demyelinated mouse brain slices. In vivo, cuprizone-induced demyelinated 10-week-old male C57bl6 mice (6 weeks, 0.3% cuprizone w/w) were treated for seven days with subcutaneous roflumilast (1mg/kg or 3mg/kg) or vehicle injections upon withdrawal of the cuprizone. Immunohistochemistry for myelin basic protein (MBP) and transmission electron microscopy (TEM) were applied to study (re)myelination. Cognitive performance (e.g. spatial memory in the object location task (OLT)) was monitored during de- and remyelination processes.

Demyelinated brain slices showed an increase in remyelination upon roflumilast (5µM) treatment. In vivo, roflumilast (3mg/kg) treatment induced a significant increase in corpus callosum and dentate gyrus remyelination and an restored spatial memory. TEM analyses confirmed an increase in myelin sheath thickness on ultrastructural level in the corpus callosum of roflumilast treated mice. The improved remyelination and spatial memory performance following roflumilast treatment at a dosage 100-fold the regular cognition enhancing dose, prompt us to conclude that roflumilast boosts the endogenous repair mechanisms in cuprizone fed mice and thereby improves cognitive performances.
TNFR2 AS A TARGET FOR MS TREATMENT

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Background
The pathology of several autoimmune diseases has been associated with impaired regulation of Tumor Necrosis Factor (TNF) alpha. TNF is a major cytokine involved in the induction and maintenance of inflammation and it determines its opposing actions via two receptors: TNFR1 and TNFR2. In both in vitro and in vivo studies, TNFR1 directly and indirectly induces neurotoxicity under chronic condition while TNFR2 showed neuroprotection. Anti-TNF therapies are successfully used to treat diseases such as rheumatoid arthritis, colitis and psoriasis. However, clinical studies with a non-selective inhibitor of TNF (Lenercept) in patients with multiple sclerosis (MS) had to be halted due to exacerbation of clinical symptoms. Although the opposing effects of TNF receptors were obscure at that time, TNF and TNFR levels were found elevated in cerebrospinal fluid (CSF) and serum and correlating with the severity of MS. Altogether, these findings suggest an ambiguous role of TNF in MS, worth to be investigated. Interestingly, a TNFR1 antagonist was highly protective in an experimental autoimmune encephalomyelitis (EAE) mouse model.

Methods
Here, we hypothesize that an agonist of TNFR2 could be equally effective or even superior to blocking TNFR1 in various in vitro and in vivo models of MS and demyelination, including MOG induced EAE and cuprizone mediated demyelination. Furthermore, beneficial effects of TNFR2 agonist treatment and remyelination process will be monitored by using a small animal positron emission tomography (PET). In order to functionally evaluate compounds that are specific for human TNFRs, we have previously generated knock-in mice expressing TNFRs with a humanized extracellular domain (huTNFR-ki). These novel mouse lines will be used to test TNFR2 agonists in in vivo and ex vivo models for MS.

Results
There is evidence to believe that activating TNFR2 has beneficial functions such as neuroprotection, immune regulation and tissue regeneration. Recently, we could show neuroprotection against NMDA mediated Nucleus basalis lesion in huTNFR2ki mice by using TNFR2 agonist. Further, preliminary data of the PET results on the cuprizone model will be shown which will reveal the potential effect of TNFR2 agonist on remyelination in this mouse model.
Conclusion
In conclusion, unravel the difference between TNFRs and their downstream cascades might lead to new insights in MS pathology with major implications for its effective treatment.
In multiple sclerosis (MS) infiltration of immune cells into the central nervous system (CNS) leads to axonal degeneration, which in turn contributes significantly to irreversible neurological disability. Previously, we identified a mechanism of axonal degeneration in both MS and experimental autoimmune encephalomyelitis (EAE). This mechanism, which begins with focal axonal swelling followed by axonal transection and fragmentation, was termed focal axonal degeneration (FAD). Here we set out to identify the molecular mechanism that drive FAD. First, we investigated intra-axonal calcium levels in EAE mice in vivo, by using two-photon microscopy of the spinal cord of transgenic mice expressing a FRET-based ratiometric calcium sensor in neurons. This revealed that in EAE lesions a significant proportion of axons have elevated levels of intracellular calcium, whereas calcium levels were tightly controlled in healthy animals. Importantly, high calcium levels were also found in axons with normal morphology, suggesting that a rise in intra-axonal calcium precedes and perhaps initiates FAD. Indeed, time-lapse experiments indicate that intra-axonal calcium levels predict both swelling and fragmentation of axons in a neuroinflammatory environment. Furthermore, removal of extracellular calcium with EGTA lead to a decrease in intra-axonal calcium levels and rescued axons from fragmentation. Next, we identified ROS and NO as potential culprits of increased intra-axonal calcium levels and axonal degeneration in both MS and EAE. It has been previously hypothesized that ROS/NO induce a rise in axonal calcium by reversing the axolemmal Na/Ca2+ - exchanger. Local application of Bepridil, which blocks the Na/Ca2+ - exchanger, did not affect calcium levels or FAD in the spinal cord of EAE mice, suggesting that reversal of the Na/Ca2+ - exchanger is not essential for axonal degeneration in EAE. We are currently exploring other pathways involved in the ROS/RNS-induced rise in intra-axonal calcium.
**Background**

Remyelination is common in early stages of the chronic demyelinating disease multiple sclerosis (MS), but fails -despite the presence of oligodendrocyte progenitor cells (OPCs)- at later stages, resulting in secondary axonal degeneration, and disease progression. Among other factors, the persistent presence of fibronectin aggregates (aFn) in MS lesions frustrates OPC differentiation and induces proinflammatory and anti-inflammatory features in macrophages, thereby impeding remyelination. The aim of this study was to determine the potential of IL-4, as the inducer of pro-regenerative microglia and macrophages, to overcome aFn-mediated inhibition of remyelination.

**Methods**

The ability of aFn to modulate alternatively (IL-4)-activated microglia and bone marrow-derived macrophages were examined by qPCR, Western blot (iNOS and arginase), immunocytochemistry (iNOS) and ELISA (TNFα). The effect of microglia/macrophage conditioned medium on OPC differentiation was determined by immunocytochemistry for the myelin protein MBP. The influence of IL-4 on remyelination was assessed in demyelinated aFn-containing organotypic forebrain slice cultures.

**Results**

aFn coatings induced iNOS expression and tend to induce the release of TNFα by IL-4-activated microglia and macrophages. Conditioned medium of microglia and macrophages that were grown on aFn, inhibited OPC differentiation, which is not observed upon addition of conditioned medium of IL-4-treated aFn-exposed microglia and macrophages. Similarly, exposure to IL-4 counteracted aFn-mediated inhibition of remyelination in organotypic forebrain slice cultures. The underlying mechanism appeared an IL-4-induced release of proMMP7 that active form initiates the degradation of aFn. In addition, IL-4 treatment exerted a direct effect on OPC myeline membrane formation by increasing the number of MBP sheath-positive cells.

**Conclusion**

Fibronectin aggregates promote pro-inflammatory features in alternatively (IL-4)-activated microglia and macrophages. In addition, microglia and macrophages grown on aFn secrete factors that impair OPC differentiation. However, IL-4-activated microglia and macrophages overcome aFn-mediated inhibition of remyelination. Hence, skewing microglia and macrophages towards the alternatively-activated phenotype may be a first step to overcome fibronectin aggregate-mediated inhibition of remyelination in MS lesions.
Multiple sclerosis (MS) is a chronic disabling disease of the central nervous system (CNS), characterized by focal areas of inflammation in which myelin, oligodendrocytes (OLGs) and neurons are damaged. In healthy brain, leukocyte infiltration into the CNS is limited by the blood-brain barrier (BBB), consisting of tightly sealed and highly specialized endothelial cells (ECs) surrounded by a continuous basement membrane and astrocytic end feet. In MS however, this tightly regulated immune surveillance is hampered, leading to infiltration of myelin-specific T-cells into the CNS parenchyma.

Oncostatin M (OSM), a member of the interleukin (IL)-6 cytokine family, is produced in lesions of MS patients and we demonstrated in previous research that OSM protects against demyelination in the cuprizone mouse model and enhances neurite outgrowth during spinal cord injury. High expression of the OSM receptor (OSMR) was found on brain microvascular endothelial cells (BMECs). Therefore, we hypothesize that OSM also has a protective role in CNS damage at the level of the BBB. In vitro experiments reveal a decrease (p<0.01) in vascular cell adhesion protein 1 (VCAM-1) expression on BMECs after OSM (25 ng/ml) treatment under inflammatory conditions. The same trend is visible for intercellular adhesion molecule -1 (ICAM-1). For mRNA levels of tight (occludin, claudin5, ZO-1) and adherens junctions (catenin alpha 1, VE-cadherin), no effect of OSM is detected, although the effect on the protein level and junction structure needs to be elucidated. To investigate the effect of OSM on the BBB in vivo, the experimental autoimmune encephalomyelitis (EAE) was induced in OSMR KO animals. Here, a reduced disease score (p=0.0351, F(1,42)=4.743) is present in OSMR KO mice. The in vitro data imply a protective effect of OSM on the BBB, while the in vivo data suggest the opposite. Additional in vitro experiments including permeability assays and immunohistochemistry for tight and adhesion junctions, next to tissue analysis of the EAE model are necessary to reveal the true role of OSM on the BBB in MS.
Background
The healthy structural connectome supports an efficient information flow in the brain, featuring an optimal tradeoff between segregation and integration. In multiple sclerosis (MS), a disruption of this structural brain network has been observed (e.g. a decrease in small worldness). However, the cellular substrates of structural network characteristics in MS are elusive up to now. In this study, we investigate the microscopic cellular correlates of macroscopic network measures of integration and segregation within individual MS patients in a postmortem setting.

Methods
Postmortem MRI was performed in eight MS patients, including whole-brain diffusion weighted imaging. Since MS patients have white matter lesions hampering tractography algorithms, a white matter tract atlas was constructed in a group of age and sex matched healthy controls using tractography between 92 AAL atlas regions. Next, diffusion weighted images of the MS subjects were analyzed to construct an individual FA weighted connectome. Measures of segregation (clustering coefficient) and integration (fiber length) of each region in the network were computed per subject. After scanning, brains were dissected into tissue blocks, including the superior frontal gyrus, inferior frontal gyrus, superior temporal gyrus, inferior parietal lobule and the cingulate gyrus. Subsequently, microscopic cellular tissue characteristics (i.e. axonal density, neuronal size and total cell number) were investigated using immunohistochemistry and subsequent quantification through microscopy and image analyses. The association between regional macro-scale network features and micro-scale cellular features was investigated using Spearman rank correlation.

Results
A higher clustering coefficient was correlated with smaller neuronal size (N=33; rho=-0.451; p=0.008) and lower axonal density (N=32; rho=-0.403; p=0.022). Longer
average fiber length of a region was correlated with larger neuronal size (N=33; rho=0.458; p=0.007) and higher axonal density (N=32; rho=0.409; p=0.020).

**Conclusion**
These findings indicate that segregative and integrative features of macro-scale brain organization have distinct micro-scale cellular characteristics in MS.
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