

# **Biomarker Challenges and Opportunities**

## **Neuroinflammation in Multiple Sclerosis comes in more than a single flavor...**

Biomarkers of Neuroinflammation Workshop  
National Academy of Sciences, March 20-21, 2017

**Amit Bar-Or, MD, FRCPC**

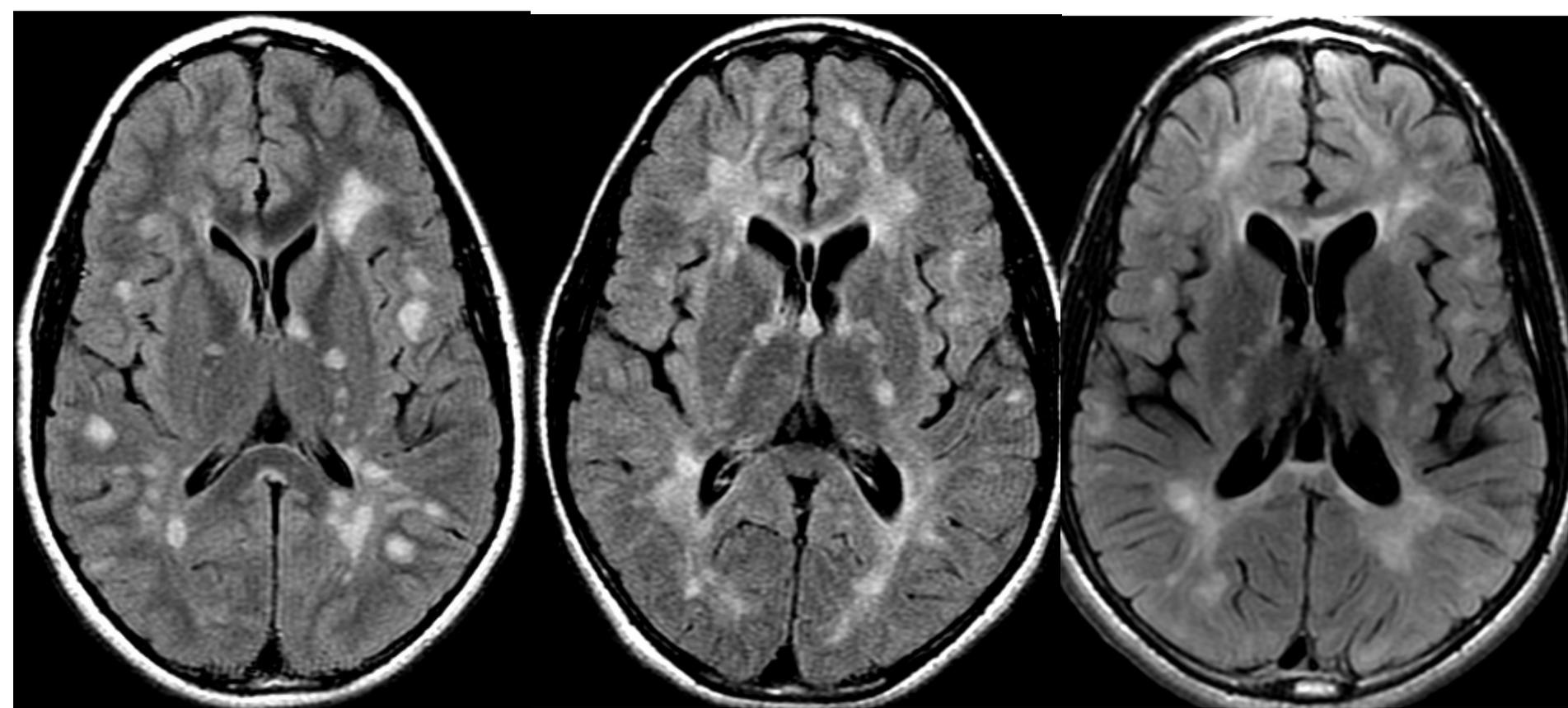
Melissa and Paul Anderson President's Professor  
Director, Center for Neuroinflammation and Neurotherapeutics  
Chief, Multiple Sclerosis Division, Department of Neurology  
Perelman School of Medicine, University of Pennsylvania

## Disclosures

Dr. Bar-Or Has participated as a speaker in meetings sponsored by and received consulting fees and/or grant support from:

Biogen Idec, Diogenix, Roche/Genentech, Sanofi-Genzyme, GlaxoSmithKline, Medimmune, Novartis, Ono Pharma, Teva Neuroscience, Celgene/Receptos Inc, and Merck/EMD Serono.

## MS: Major cause of neurological disability in young adults



**Dynamic, multifocal and diffuse, destructive process**  
**Non-random global distribution**  
**Female predilection (after puberty)**  
**Highly variable and unpredictable individual course**

# Therapeutic Era: Multiple Options Available

IFN $\beta$

Copolymer glatiramer acetate

S1P functional antagonist (fingolimod)

Selective cell subset modulation/losses (BG12/fumarate)

Pyrimidine synthesis inhibitor (teriflunomide: )

Anti-VLA-4 (natalizumab)

Anti-CD25 (daclizumab)

Anti-CD52 (alemtuzumab)

[Anti-CD20: (ocrelizumab)]

## How to Monitor? Choose? Sequence?

# **Where We Stand in the Clinic (any biomarkers used in making decisions?)**

## **Diagnosis of CNS inflammatory diseases**

**MRI: multi-focal T2 lesions (>> OCB, IgG Index)  
anti-AQP4 Ab (anti-MOG Ab?)**

## **Treatment initiation/considering change:**

**MRI 'Gd+' and  $\Delta$  in T2; (atrophy)**

**IFN $\beta$  Neutralizing Ab**

**Anti-JC Abs**

**(Lymphopenia) ; B cell counts...)**

# Multiple Candidate biomarkers for MS

CSF (serum) neurofilament LC (NFL)

Chitinase 3 like 1

Particular miRNAs

B cell chemoattractant CXCL13, Osteopontin

Monocyte macrophage marker CD163

Glial activation marker YKL-40

Lateral olfactory tract usher substance (LOTUS), an  
endogenous Nogo receptor-1 antagonist

Haematopoietic precursor stem cells (HPSC)

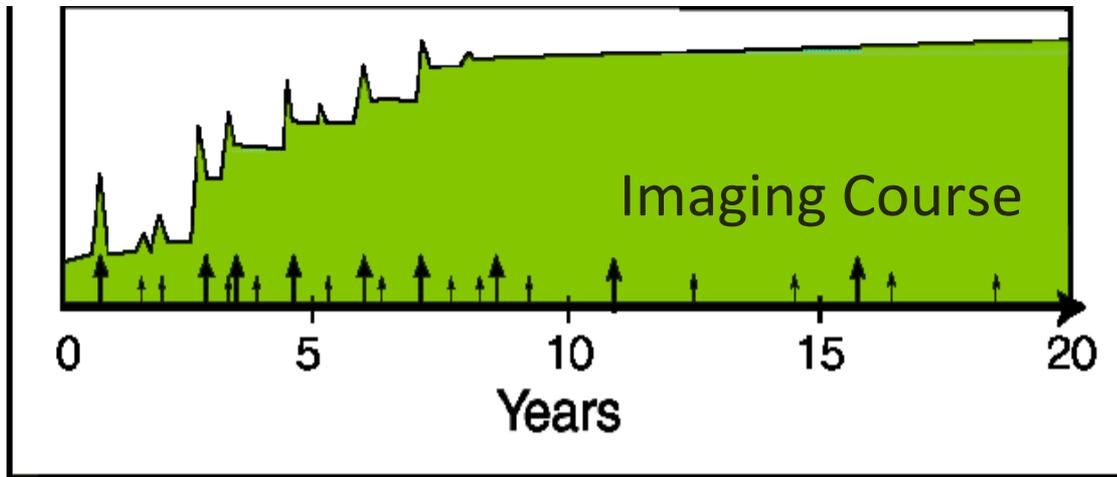
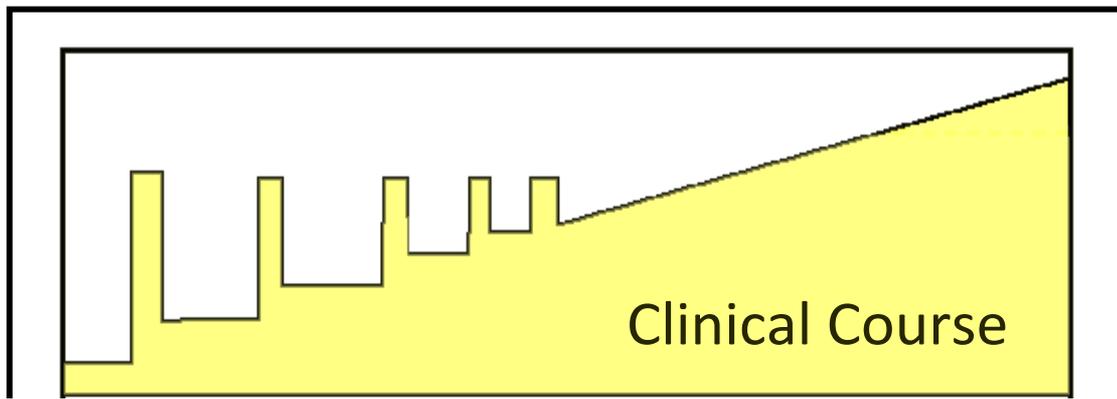
myelin reactive T cells

Kir4.1 antibodies

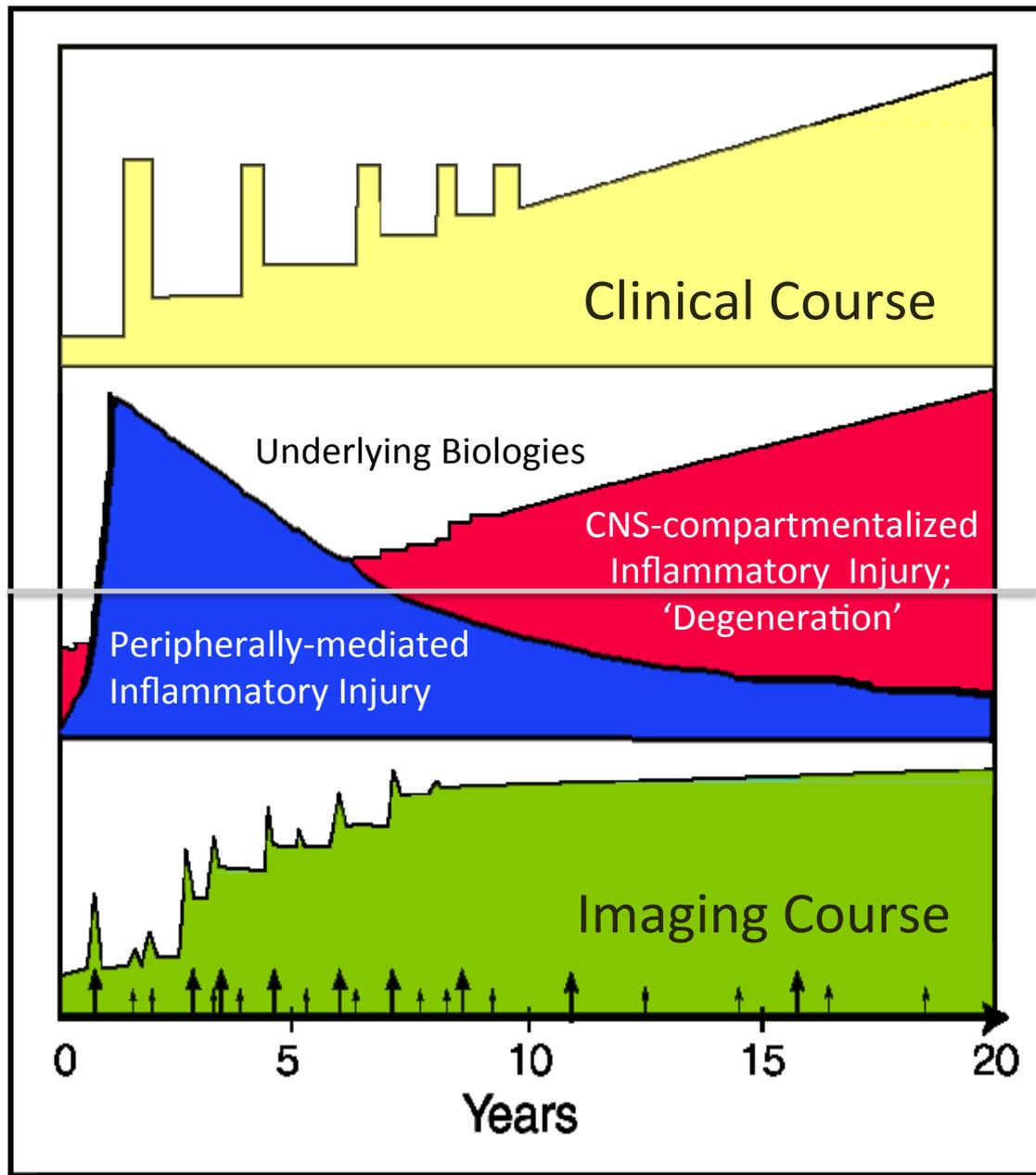
Microbiome associated lipopeptides

...

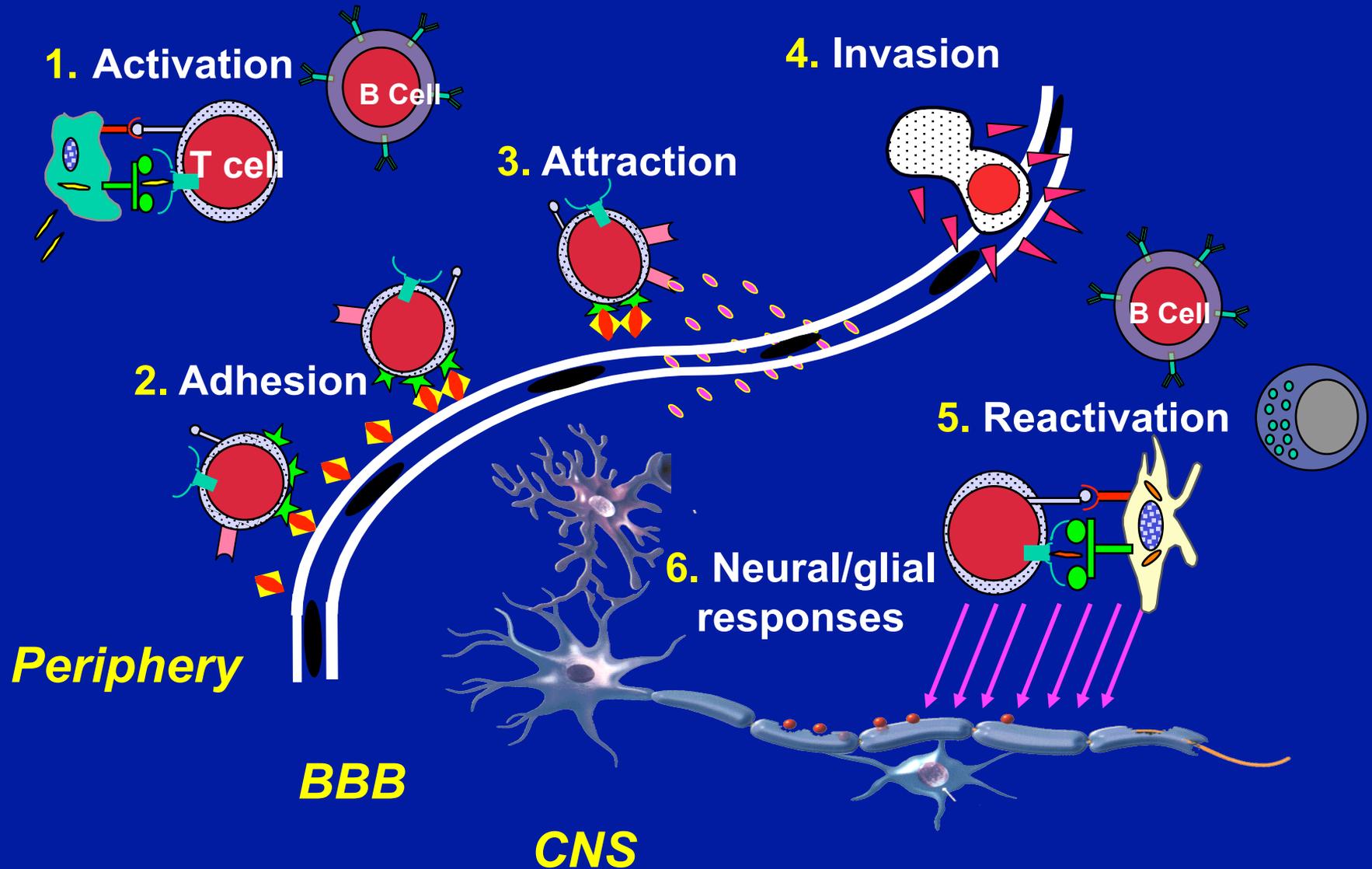
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Clinical  
Threshold



# Simplified Model of MS Immunopathogenesis



# Rituximab in Relapsing- Remitting Multiple Sclerosis: A 72-Week, Open-Label, Phase I Trial

Amit Bar-Or, MD,<sup>1</sup> Peter A. J. Calabresi, MD,<sup>2</sup>  
Douglas Arnold, MD,<sup>1,3</sup> Clyde Markowitz, MD,<sup>4</sup>  
Stuart Shafer, MD,<sup>5</sup> Lloyd H. Kasper, MD<sup>6</sup>  
Emmanuelle Waubant, MD<sup>7</sup> Suzanne Gazda, MD,<sup>8</sup>  
Robert J. Fox, MD,<sup>9</sup> Michael Panzara, MD,<sup>10</sup>  
Neena Sarkar, PhD,<sup>11</sup> Sunil Agarwal, MD,<sup>11</sup> and  
Craig H. Smith, MD<sup>11</sup>

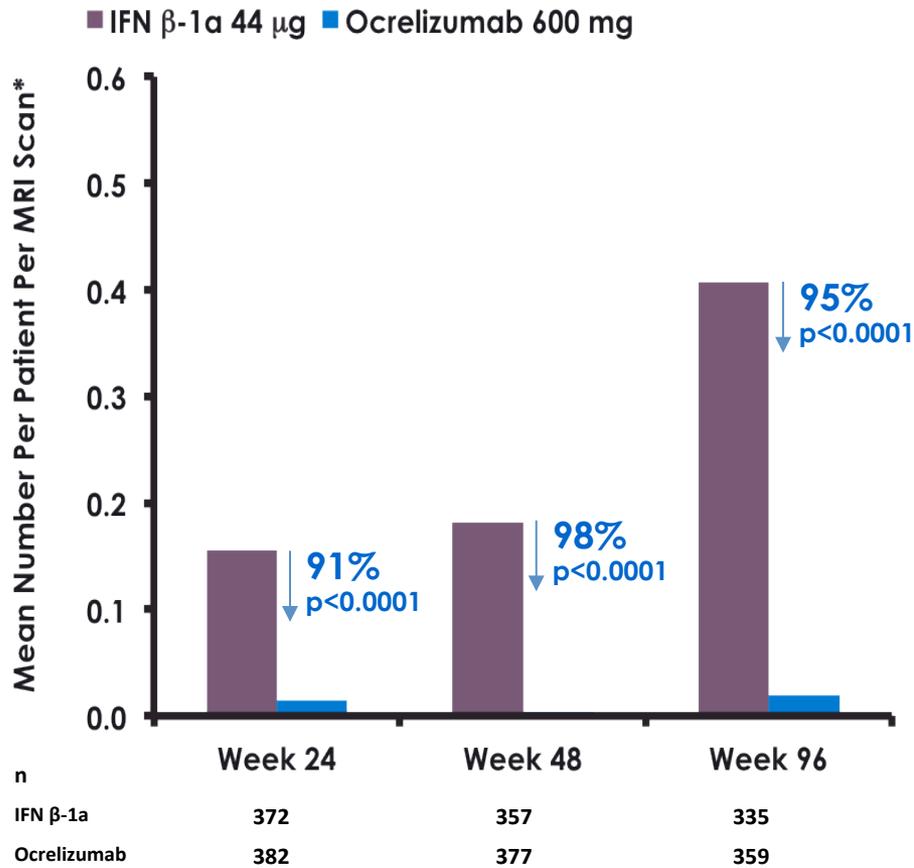
ORIGINAL ARTICLE

# Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis

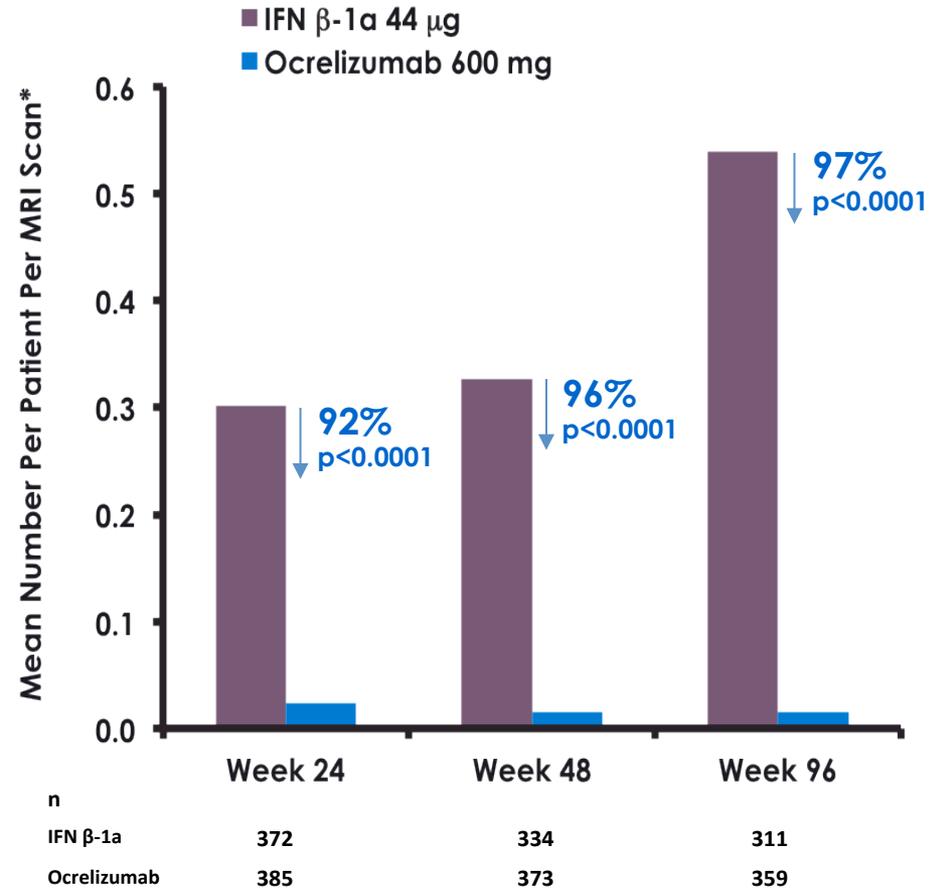
S.L. Hauser, A. Bar-Or, G. Comi, G. Giovannoni, H.-P. Hartung, B. Hemmer,  
F. Lublin, X. Montalban, K.W. Rammohan, K. Selmaj, A. Traboulsee,  
J.S. Wolinsky, D.L. Arnold, G. Klingelschmitt, D. Masterman, P. Fontoura,  
S. Belachew, P. Chin, N. Mairon, H. Garren, and L. Kappos,  
for the OPERA I and OPERA II Clinical Investigators\*

# Phase 3 $\alpha$ CD20 (ocrelizumab) B cell depletion in RRMS

## OPERA I



## OPERA II

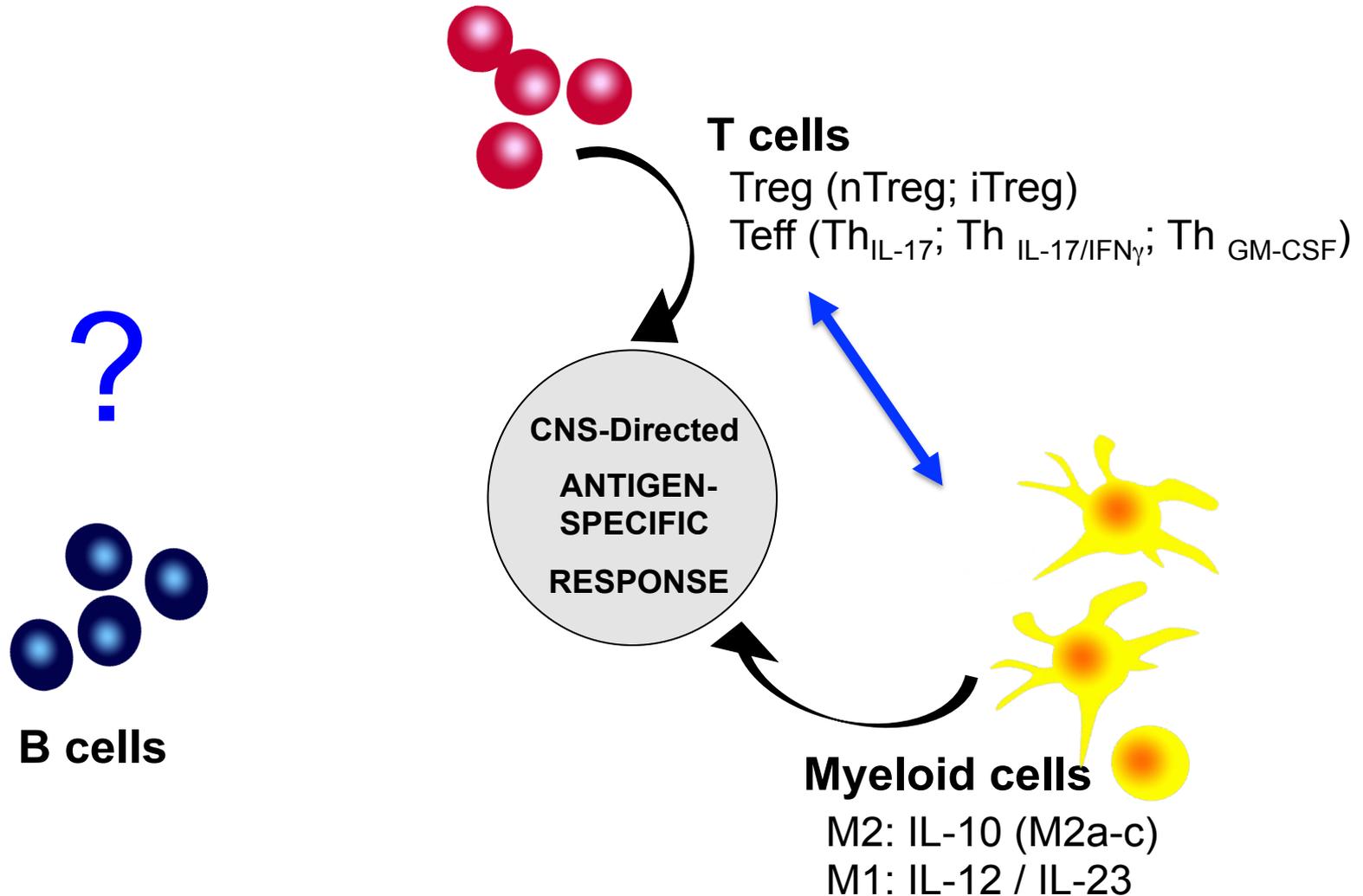


Hauser et al  
NEJM, Jan 2017

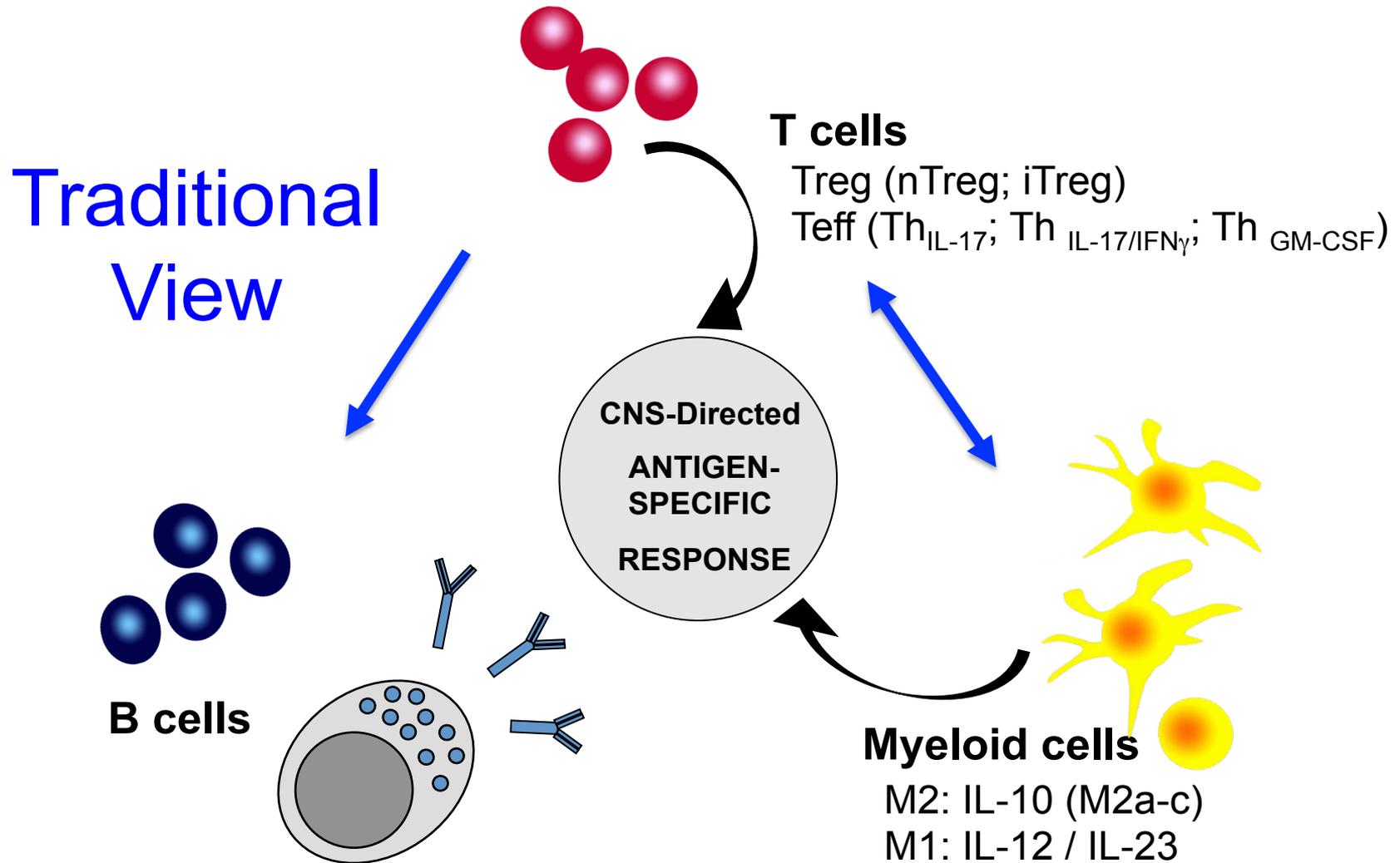
ITT

\*Adjusted by baseline T1 Gd lesion (present or not), baseline EDSS (<4.0 vs  $\geq$ 4.0) and geographical region (US vs ROW).  
EDSS, Expanded Disability Status Scale; Gd<sup>+</sup>, gadolinium enhancing; IFN, interferon; MRI, magnetic resonance imaging; ROW, rest of the world.

# Autoimmunity and Multiple Sclerosis



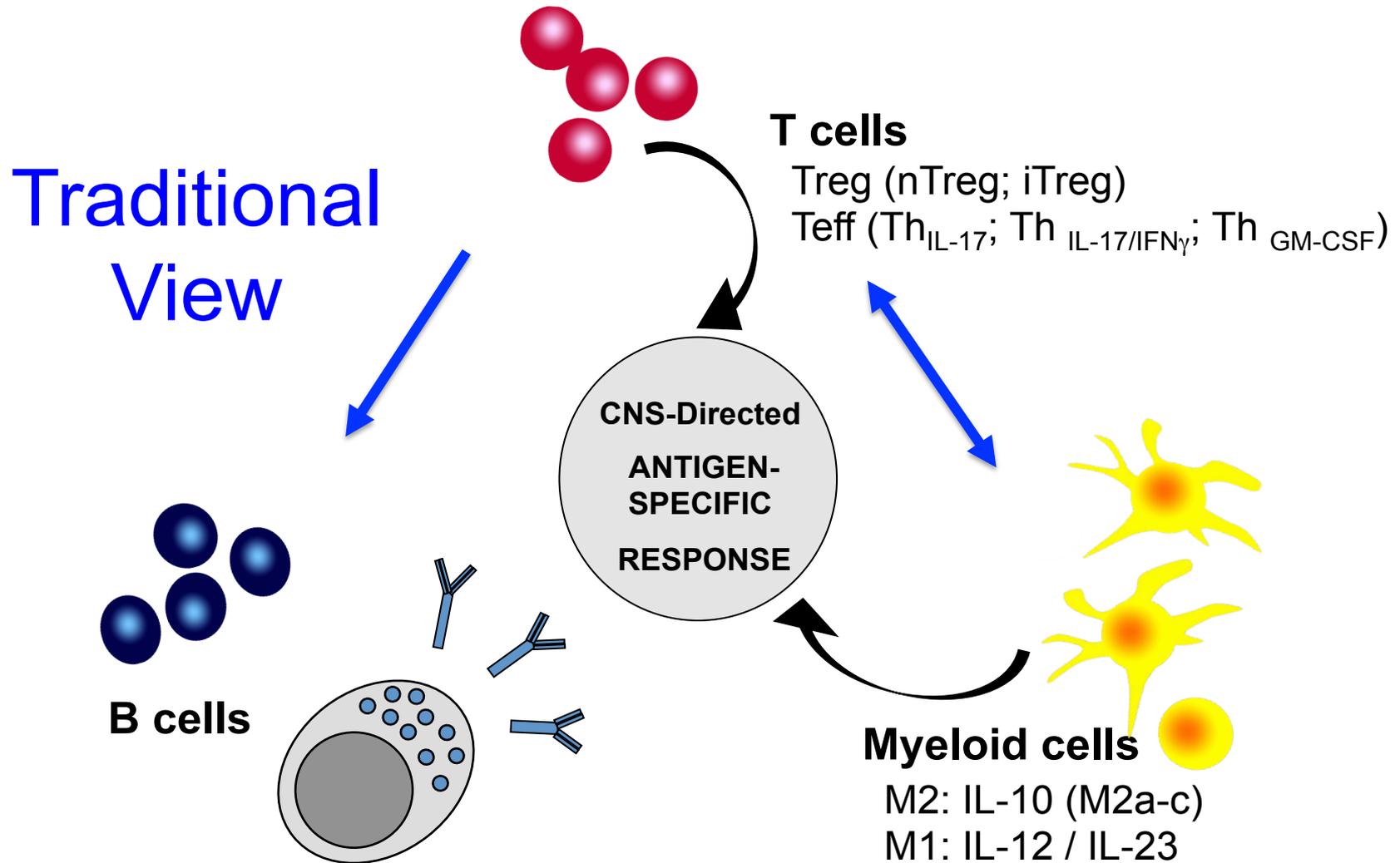
# Autoimmunity and Multiple Sclerosis



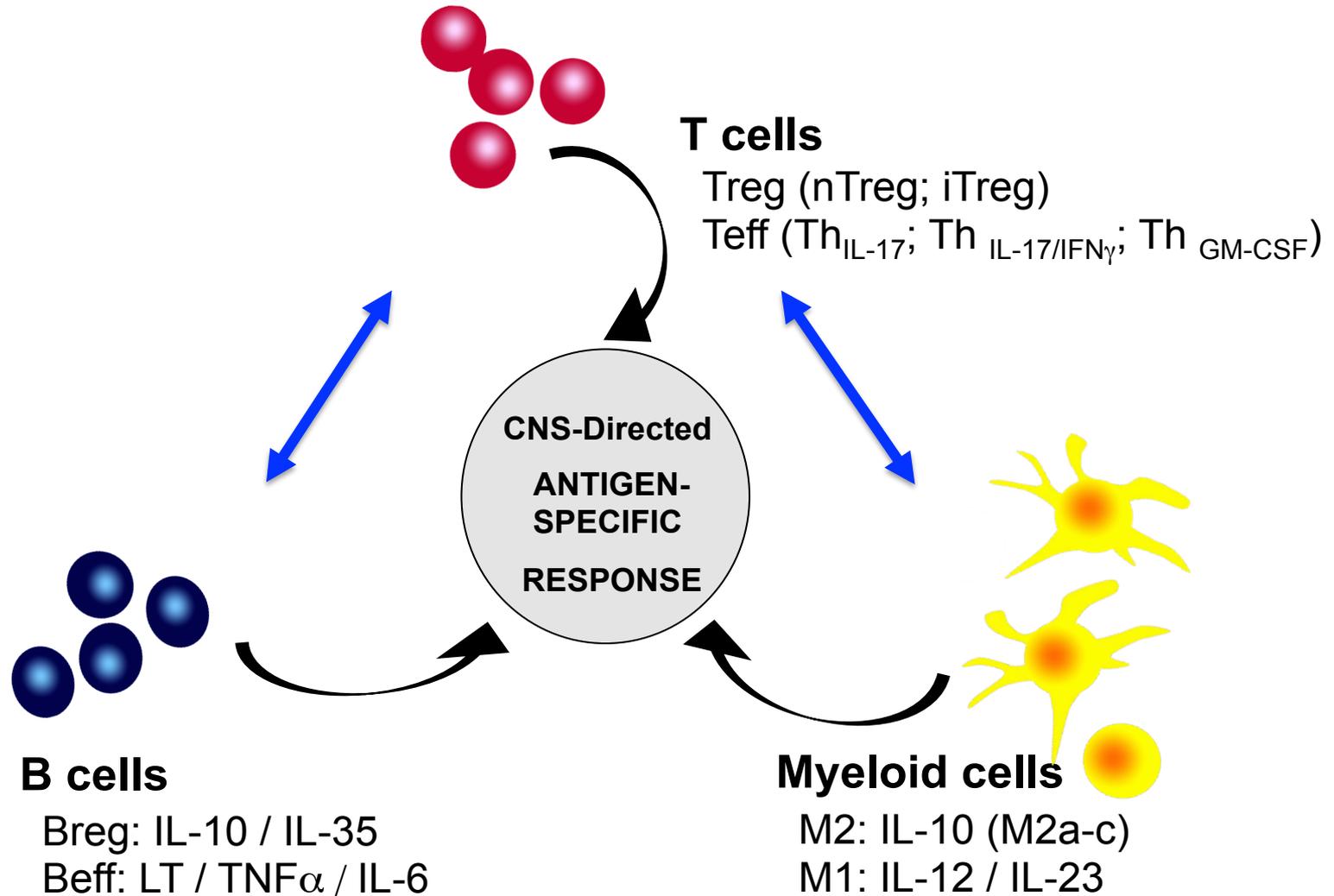
# Abnormal B-Cell Cytokine Responses A Trigger of T-Cell-Mediated Disease in MS?

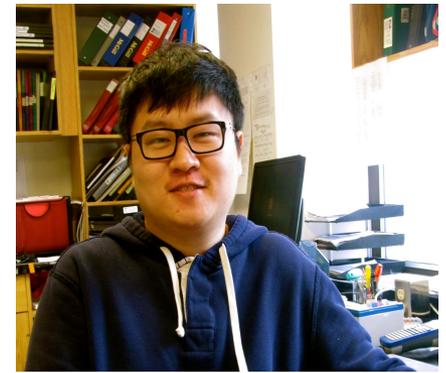
Amit Bar-Or, MD, FRCP,<sup>1,2</sup> Lama Fawaz, PhD,<sup>1</sup> Boli Fan, MSc,<sup>1,2</sup>  
Peter J. Darlington, PhD,<sup>1</sup> Aja Rieger, BSc,<sup>1</sup> Christine Ghorayeb, MSc,<sup>1</sup>  
Peter A. Calabresi, MD,<sup>3</sup> Emmanuelle Waubant, MD, PhD,<sup>4</sup>  
Stephen L. Hauser, MD,<sup>4</sup> Jiameng Zhang, PhD,<sup>5</sup> and  
Craig H. Smith, MD<sup>5</sup>

# Autoimmunity and Multiple Sclerosis



# Autoimmunity and Multiple Sclerosis

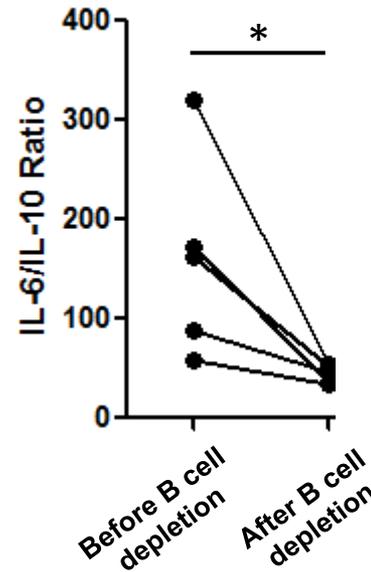
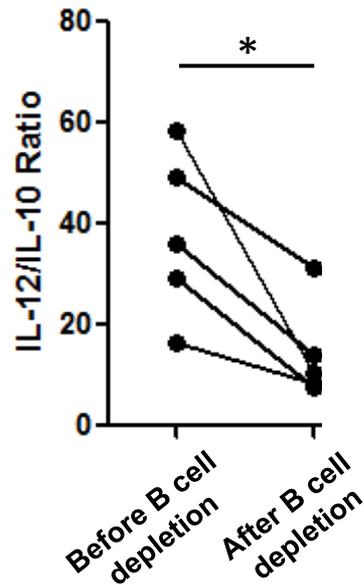




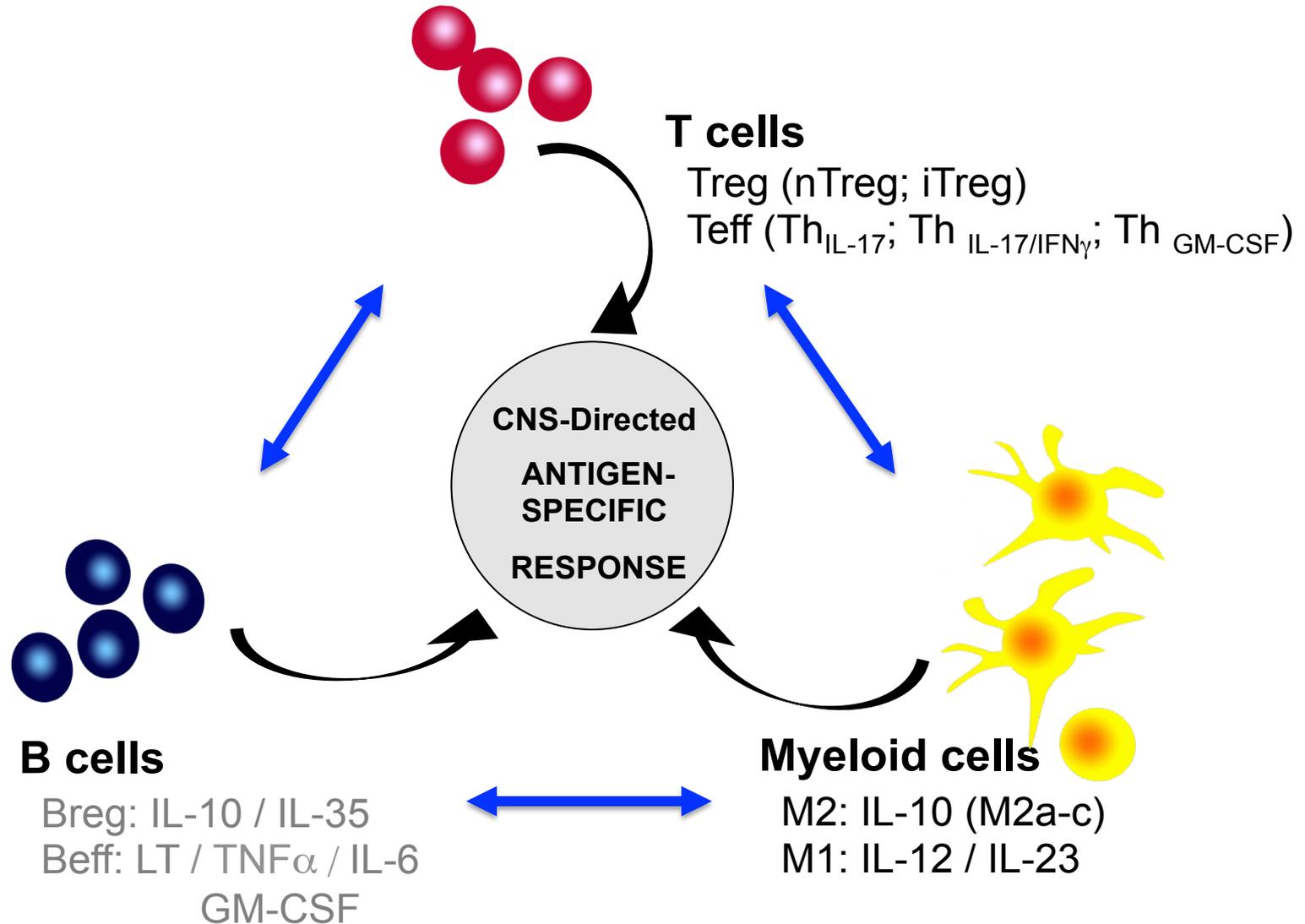
MULTIPLE SCLEROSIS

# Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy

Rui Li,<sup>1</sup> Ayman Rezk,<sup>1</sup> Yusei Miyazaki,<sup>1</sup> Ellen Hilgenberg,<sup>2</sup> Hanane Touil,<sup>1</sup> Ping Shen,<sup>2</sup> Craig S. Moore,<sup>1</sup> Laure Michel,<sup>3</sup> Faisal Althekeair,<sup>1</sup> Sathy Rajasekharan,<sup>1</sup> Jennifer L. Gommerman,<sup>4</sup> Alexandre Prat,<sup>3</sup> Simon Fillatreau,<sup>2</sup> Amit Bar-Or,<sup>1,5\*</sup> on behalf of the Canadian B cells in MS Team



# Autoimmunity and Multiple Sclerosis



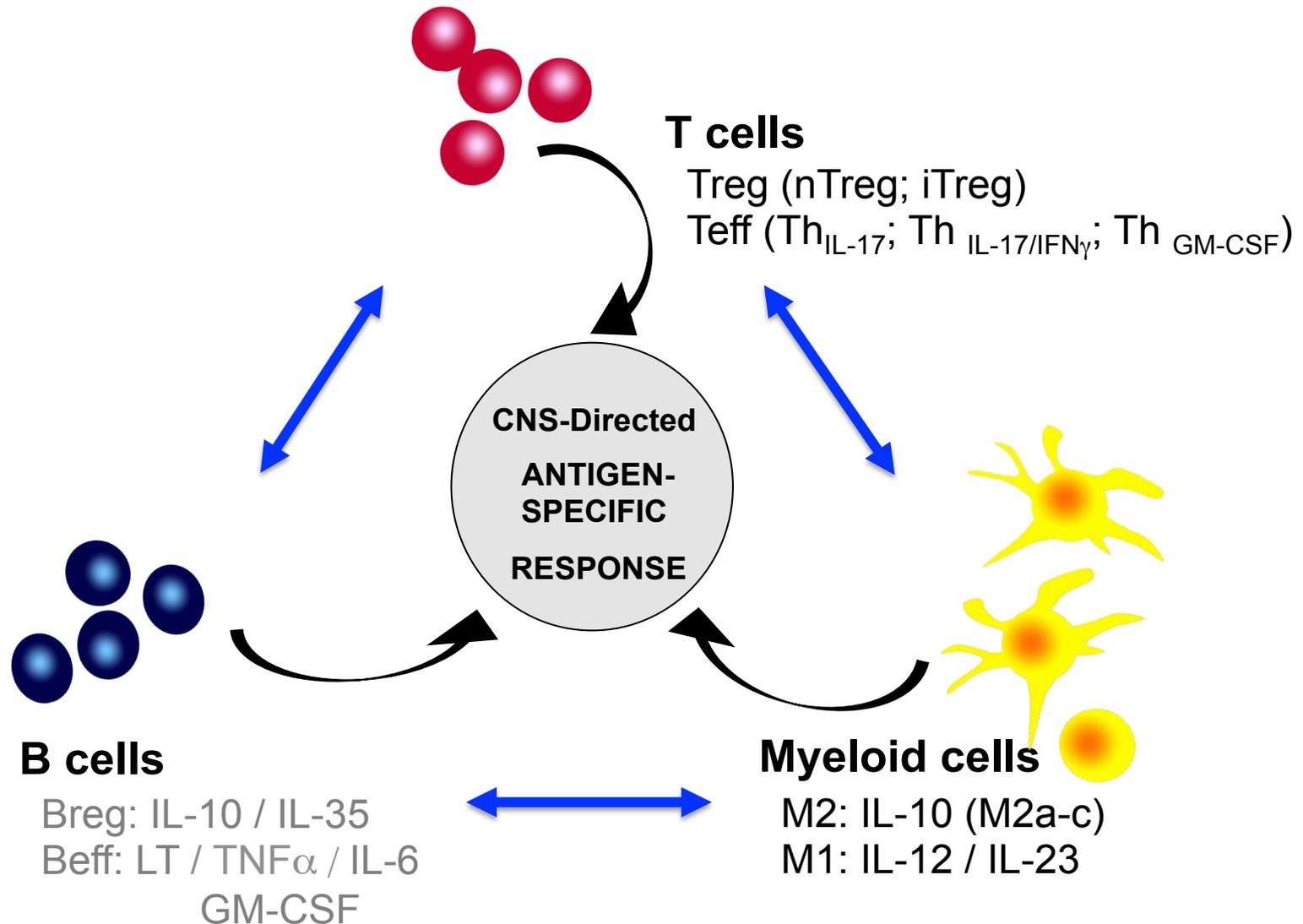
Mechanisms underlying aberrant B cell cytokine pursued for biomarker potential:

(1) miR 132 regulates B cell LT and TNF $\alpha$  secretion

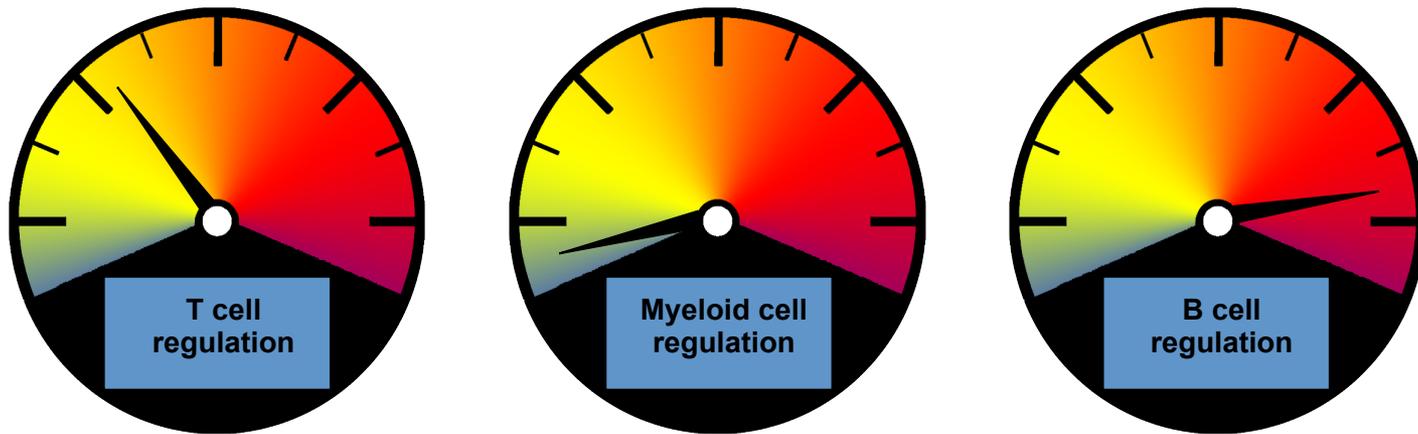
(2) STAT5/6 signaling reciprocally regulates B cell pro-inflammatory and anti-inflammatory cytokines

# Autoimmunity and Multiple Sclerosis

## Co-operativity Captures disease 'state'



# Importance of concurrent measure of relevant biologies



Functional Immune profile to capture person's disease 'state'

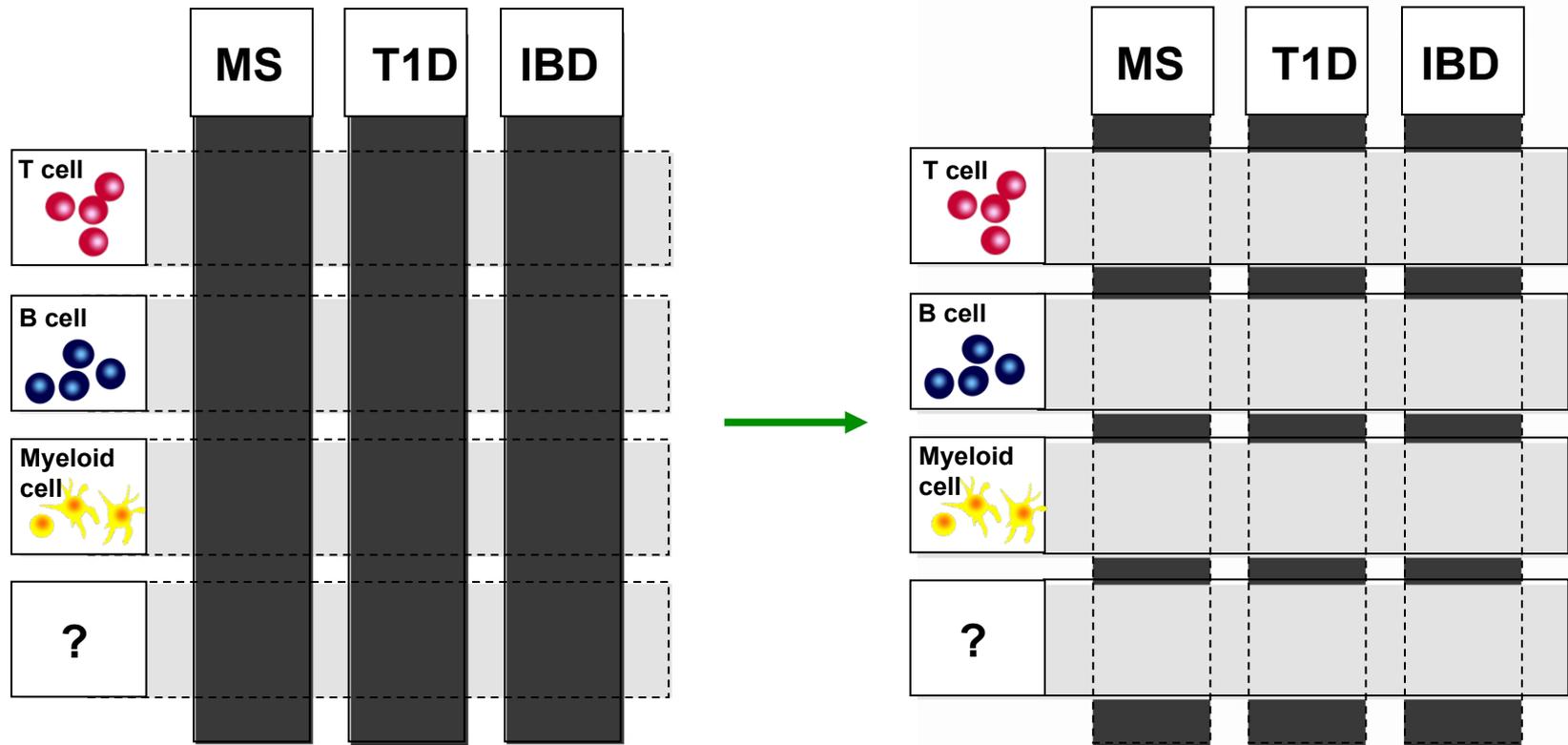
Guide individualized care (Precision Medicine):

Treatment Selection (both efficacy and safety)

On-treatment Monitoring; Stop and Switch

# Evolving beyond disease 'Silos':

(Example of autoimmune diseases; AID)

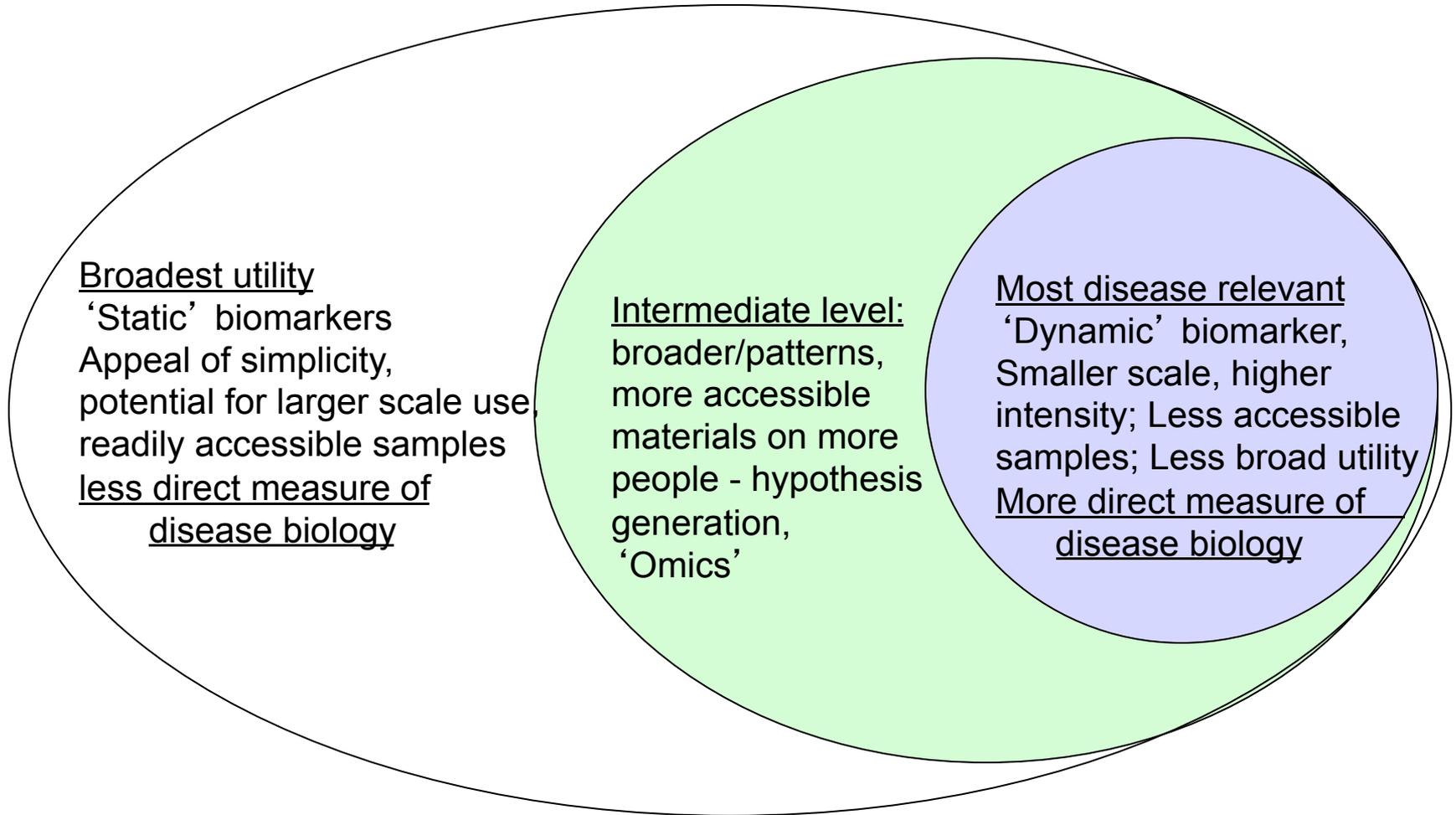


Relevance of dynamic (viable) cell-based measures to define functional Immune profile capturing person's disease 'state'

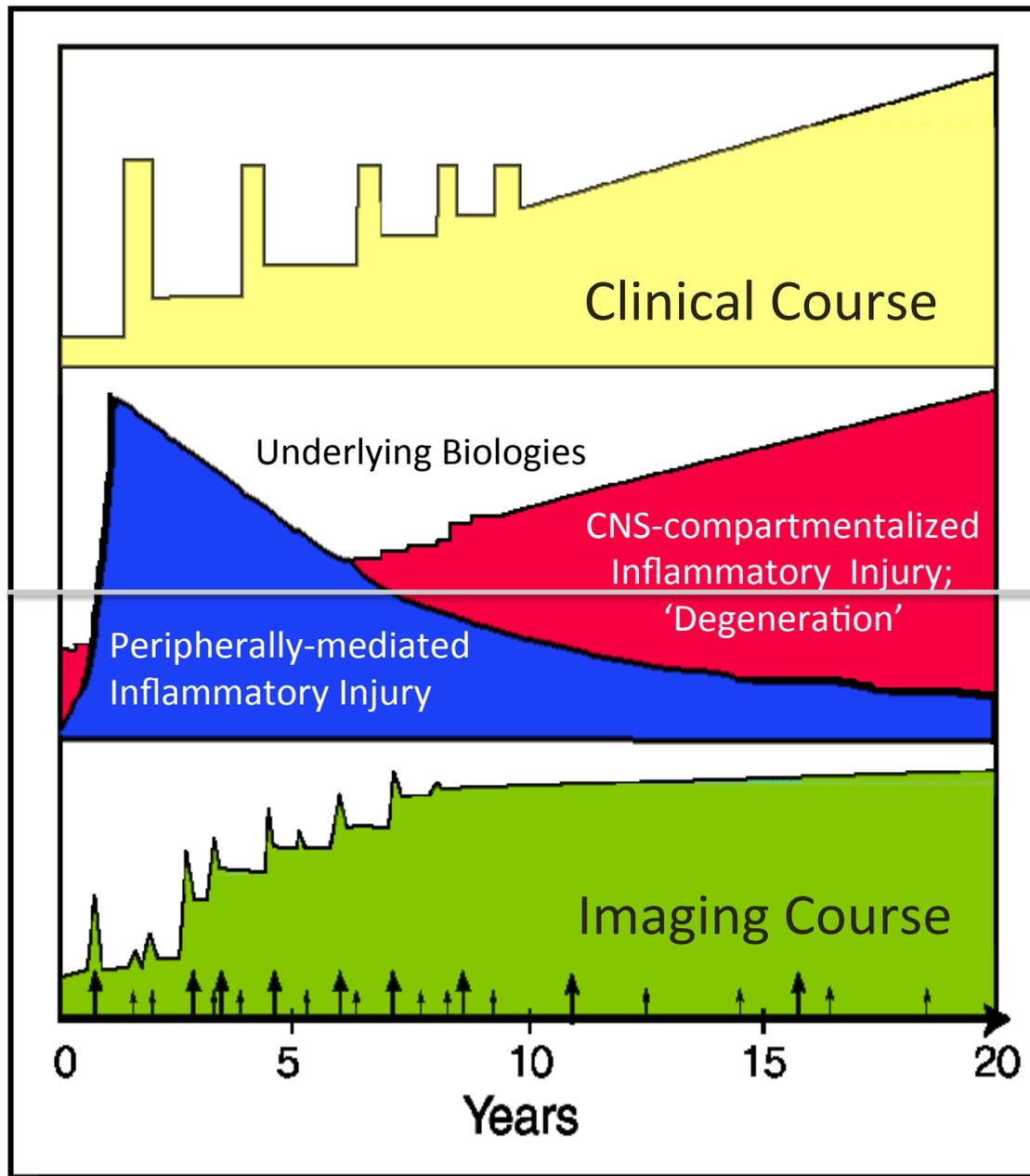
# Pros and cons to either 'Static' or 'Dynamic' Biomarkers

Ideal: develop in parallel, iteratively, with one guiding the other

Opportunity: 'Onion Peel' model of biomarker development



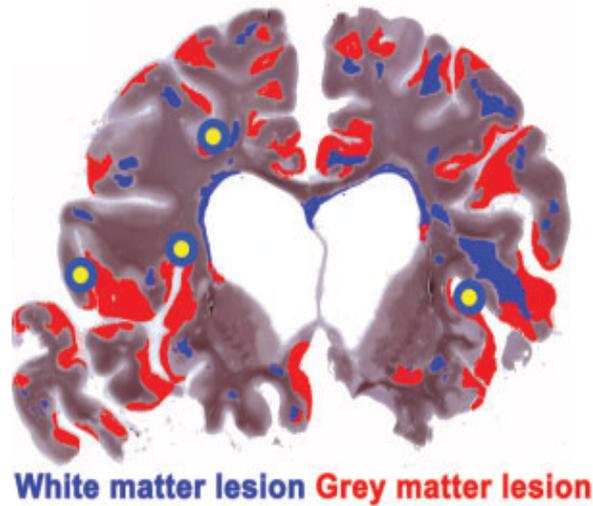
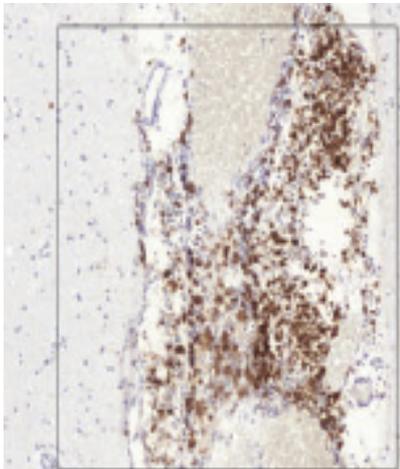
Clinical  
Threshold



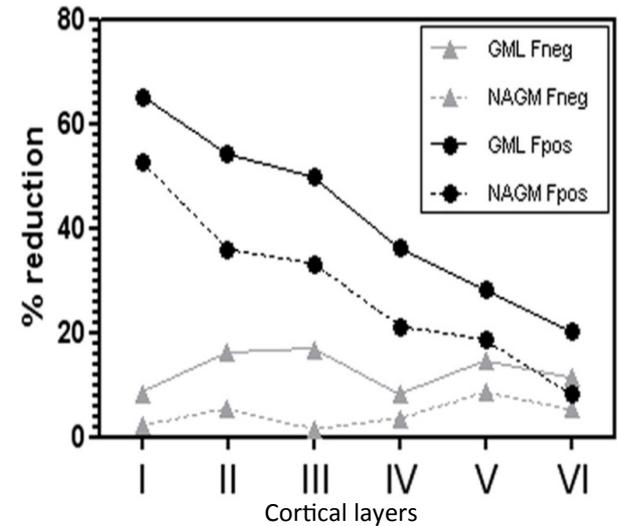
# White matter AND Grey matter pathology in MS



Meningeal inflammation



Percent reduction in NeuN+ neurons



Microglial activation: similar graded pattern from surface, inwards

Attractive hypothesis: Secreted factor(s) released from meningeal immune cells contribute to the subpial cortical injury in MS

However: Not all autopsy studies have found relationship between meningeal inflammation and subpial cortical injury ...

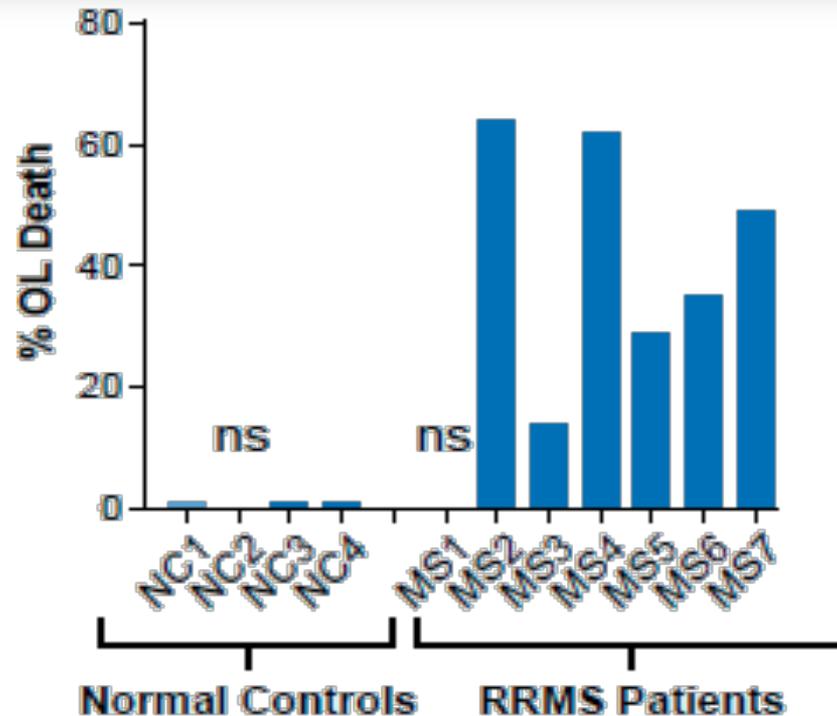


ELSEVIER



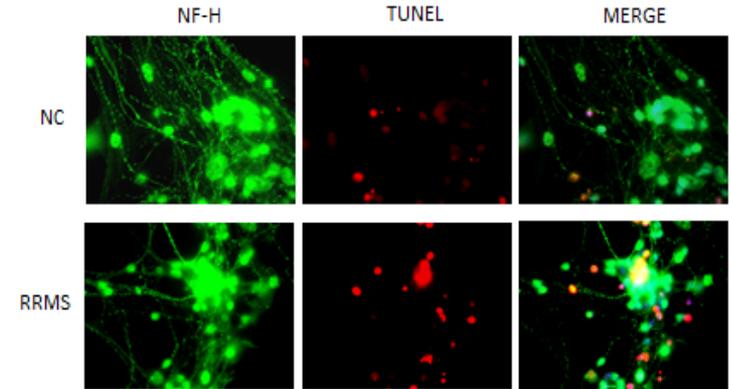
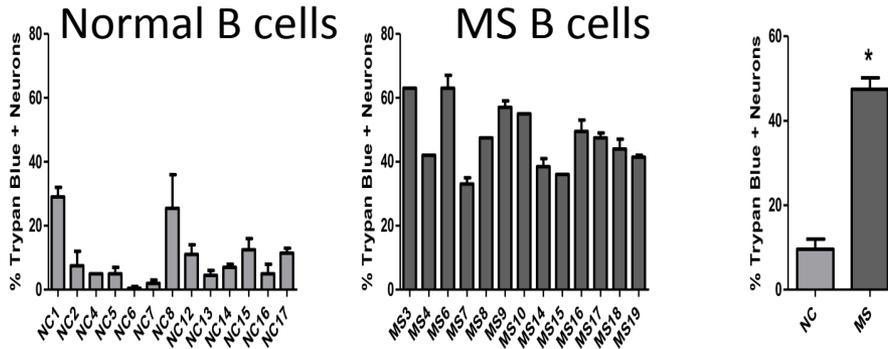
## Secretory products of multiple sclerosis B cells are cytotoxic to oligodendroglia *in vitro*

Robert P. Lisak<sup>a,b,\*</sup>, Joyce A. Benjamins<sup>a,b</sup>, Liljana Nedelkoska<sup>a</sup>, Jennifer L. Barger<sup>a</sup>, Samia Ragheb<sup>a,b</sup>, Boli Fan<sup>c,e</sup>, Nadia Ouamara<sup>c,e</sup>, Trina A. Johnson<sup>c,e</sup>, Sathyanath Rajasekharan<sup>c,e</sup>, Amit Bar-Or<sup>c,d,e,\*\*</sup>

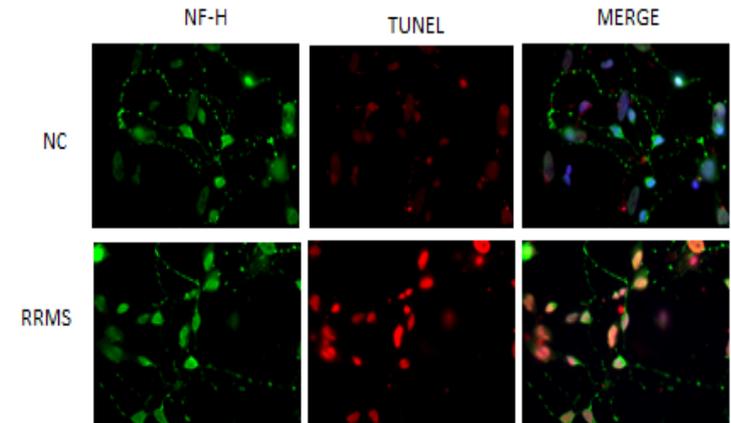
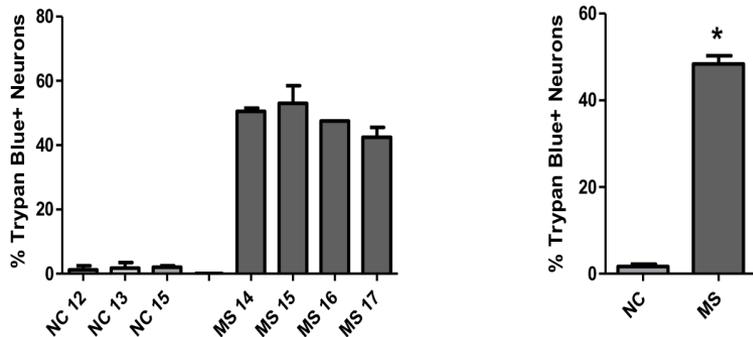


# B cell soluble products from MS patients are also more toxic to neurons than normal B cell products

Proportion of dead Rat neurons



Proportion of dead Human neurons



## Onset of multiple sclerosis before adulthood leads to failure of age-expected brain growth



Béregère Aubert-Broche, PhD, Vladimir Fonov, PhD, Sridar Narayanan, PhD, Douglas L. Arnold, MD, David Araujo, MD, PhD, Dumitru Fetco, MD, Christine Till, PhD, John G. Sled, PhD, Brenda Banwell, MD and D. Louis Collins, PhD On behalf of the Canadian Pediatric Demyelinating Disease Network

*Neurology* January 17, 2012 vol. 78 no. 3 194-201

## Reduced head and brain size for age and disproportionately smaller thalami in child-onset MS

A. Kerbrat, MD, B. Aubert-Broche, PhD, V. Fonov, PhD, S. Narayanan, PhD, J.G. Sled, PhD, D.A. Arnold, MD, B. Banwell, MD and D.L. Collins, PhD

## Take aways:

Complex inflammatory mechanisms contribute to MS

Distinct compartments, evolving over time, overlap

Significant intra- and inter- individual heterogeneity

**Blue** (relapsing): integrated dynamic cell-based, to inform more readily accessible biomarker

**Red** (non-relapsing): in vivo measures of meningeal-based inflammation: molecular imaging  
CSF based (limited but part of integrated approach)

## Thanks to:



### Laboratory

Ina Mexhitaj

Rui Li

Ayman Rezk

Ina Mexhitaj

Giulia Fadda

Leslie Scarffe

Hanane Touil

Mahtab Ghadiri

Luke Healy

Farzaneh Jalili

### Experimental Therapeutics

Sandeep Vanamala

Ada Vilalobos

Boli Fan

Julien Sirois

Marie-Noelle Boivin

Sonia Lai

### Patients, Families, volunteers

### MS Clinic at MNI

### CRU at MNI

### Canadian B cell Team in MS

Jennifer Gommerman - U of T

Alexandre Prat - CHUM

Simon Fillatreau – Berlin

Frauke Zipp - Mainz

Felix Luessi

### Canadian PD ADS/MS

Brenda Banwell – CHOP/SickKids

Doug Arnold - MNI

Louis Collins - MNI

Dessa Sadovnick – UBC

Ruth-Ann Marrie – U Manitoba

### Canadian BMT/MSCT Group

Mark Freedman - Ottawa

Harry Atkins - Ottawa

### Glial-Immune interaction

Jack Antel – MNI

Craig Moore – Memorial U

Alyson Fournier – MNI

Tim Kennedy – MNI

Bob Lisak – Wayne state

Valeria Ramaglia - Amsterdam

### 'Deep' Immune Monitoring

Heinz Wiendl – Munster

Sven Meuth - Munster

### anti-CNS Antibodies

Edgar Meinl – Munich

Luisa Villar – Madrid

Kevin O' Connor – Yale

Chris Linington - Glasgow

### Virology/Vit D

Carmen Yea – U of T

Reinhold Vieth – U of T

Heather Hanwell - U of T

# Centre for Neuroinflammation and Experimental Therapeutics, and the Comprehensive MS Center

University of Pennsylvania Pearlman School of Medicine

