RUTGERS
Robert Wood Johnson
Medical School

Biomarkers of MS therapies

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Disclosures

- *Consultant:* Bayer, Biogen, EMD Serono, Teva, Novartis, Acorda

- *Investigator/Research Grants:* TEVA, Biogen

Significance of biomarkers discovery in MS

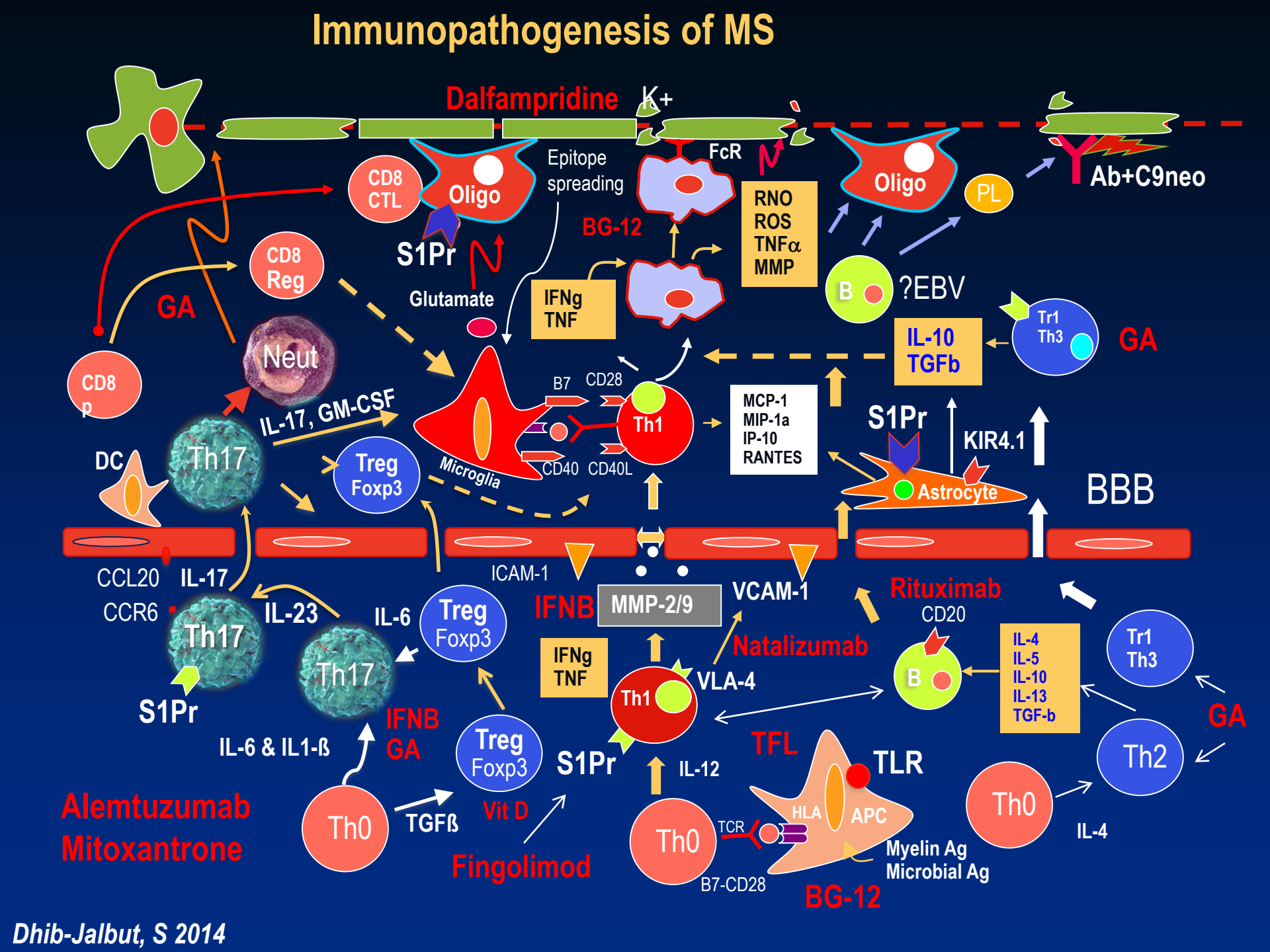
- Early diagnosis
- Predict clinical course (Benign, RR, PP)
- Assessment of disease activity
- Pathological phenotype
- Surrogate outcome in clinical trials
- Prediction of treatment response

Graber & Dhib-Jalbut. J Neurol Sci, 305:1-10, 2011

MS: Disease heterogeneity is a challenge to biomarkers discovery

- Variable clinical course
- Pathological heterogeneity
- Inconsistent immune findings
- Variable response to DMDs

Graber & Dhib-Jalbut. J Neurol Sci, 305:1-10, 2011


Immunopathogenesis of MS
Dalfampridine
K+
FcR
Epitope spreading
CD8 CTL
Oligo
S1Pr
Glutamate
BG-12
RNO
ROS
TNFα
MMP
Oligo
PL
Ab+C9neo
B
?EBV
CD8 Reg
GA
IFNg
TNF
Tr1
Th3
GA
IL-10
TGFb
CD8
p
Neut
IL-17, GM-CSF
B7
CD28
Th1
MCP-1
MIP-1a
IP-10
RANTES
S1Pr
KIR4.1
DC
Th17
Treg
Foxp3
Microglia
CD40
CD40L
Astrocyte
BBB
CCL20
CCR6
IL-17
ICAM-1
IFNB
MMP-2/9
VCAM-1
Rituximab
CD20
IL-23
IL-6
Th17
Th17
Treg
Foxp3
IFNg
TNF
Th1
VLA-4
Natalizumab
B
IL-4
IL-5
IL-10
IL-13
TGF-b
Tr1
Th3
GA
S1Pr
IL-6 & IL1-ß
IFNB
GA
Treg
Foxp3
S1Pr
IL-12
TFL
TLR
Th2
Alemtuzumab
Mitoxantrone
Th0
TGFß
Vit D
Th0
TCR
HLA
APC
Th0
IL-4
Fingolimod
B7-CD28
Myelin Ag
Microbial Ag
BG-12
Dhib-Jalbut, S 2014

BIOMARKERS OF TREATMENT RESPONSE

- Understand drug MOA
- Target molecules involved in the drug's MOA as potential biomarkers
- Genomics
- Proteomics

Interferons Biomarkers

Potential Mechanisms of Action of IFN-ß in MS

- Anti-proliferative effect
- Blocking T-cell activation
- Apoptosis of autoreactive T-cells
- IFN-g antagonism
- Cytokine shifts
- Blood-brain barrier
- Antiviral effect

Dhib-Jalbut S. Neurology. 2002(April 23);58(8 suppl 4):S3-S9

Journal of Neuroimmunology 254 (2013) 131–140

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Immune response during interferon beta-1b treatment in patients with multiple sclerosis who experienced relapses and those who were relapse-free in the START study

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[b] Bayer HealthCare Pharmaceuticals, 6 West Belt, Wayne, NJ 07470, USA

Overview of Exploratory Outcomes

Bayer HealthCare Pharmaceuticals

Exploratory Outcomes (Immune Parameters)			
Blood Brain Barrier Markers			
•sVCAM		•MMP-2	
•TIMP-1		•TIMP-1/MMP-2 Ratio	
Cytokines			
Pro-Inflammatory Cytokines	•IFN-gamma	•TNF-alpha	•IL-17
Anti-Inflammatory/Regulatory Cytokines	•TGF-beta •IL-4	•IL-10 •IL-13	•IL-27
Ratios	•IL-4/IFNg •IL-13/IFNg	•IL-10/IFN-g •IL-10/IL-17 ratio	
Neurotrophic factor expression			
• Brain-derived neurotrophic factor (BDNF)		• Nerve growth factor (NGF)	
Chemokines (Expression)			
• CXCR3 (Th1)		• CCR4 (Th2)	

Interferon therapy: Baseline molecules in relapsing and relapse-free subjects

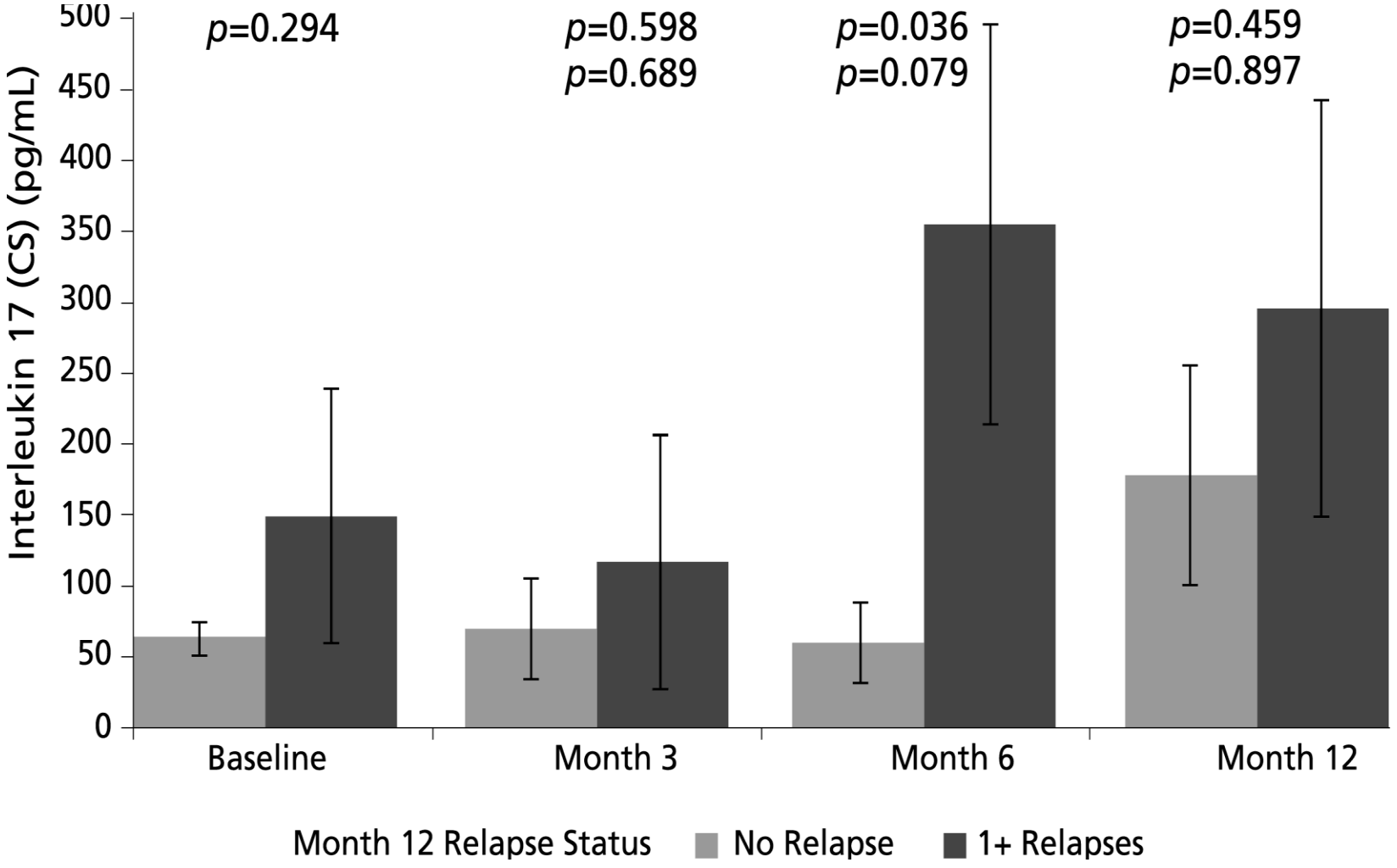
Immune Parameter	Time point	Statistical Parameter	Relapse Free Subset	≥1 Relapses Subset	*p*-value
IL-10 (CS)	Baseline	N	14	11	0.119
		mean	26.739	70.276	
		SD	21.112	98.268	
		median	20.360	47.450	
IL-10/IFN-gamma ratio (CS)	Baseline	N	14	11	0.074
		mean	0.067	0.380	
		SD	0.057	0.626	
		median	0.059	0.160	
IL-4/IFN-gamma ratio (CS)	Baseline	N	14	11	0.025
		mean	0.024	0.085	
		SD	0.021	0.091	
		median	0.018	0.056	
BDNF serum	Baseline	N	14	11	0.011
		mean	675.6	525.0	
		SD	137.86	132.11	
		median	666.3	554.5	

Dhib-Jalbut et al. J neuroimmunol. 254:131- 140, 2013

Immune markers during IFNβ-1b treatment in relapsing and relapse-free subjects

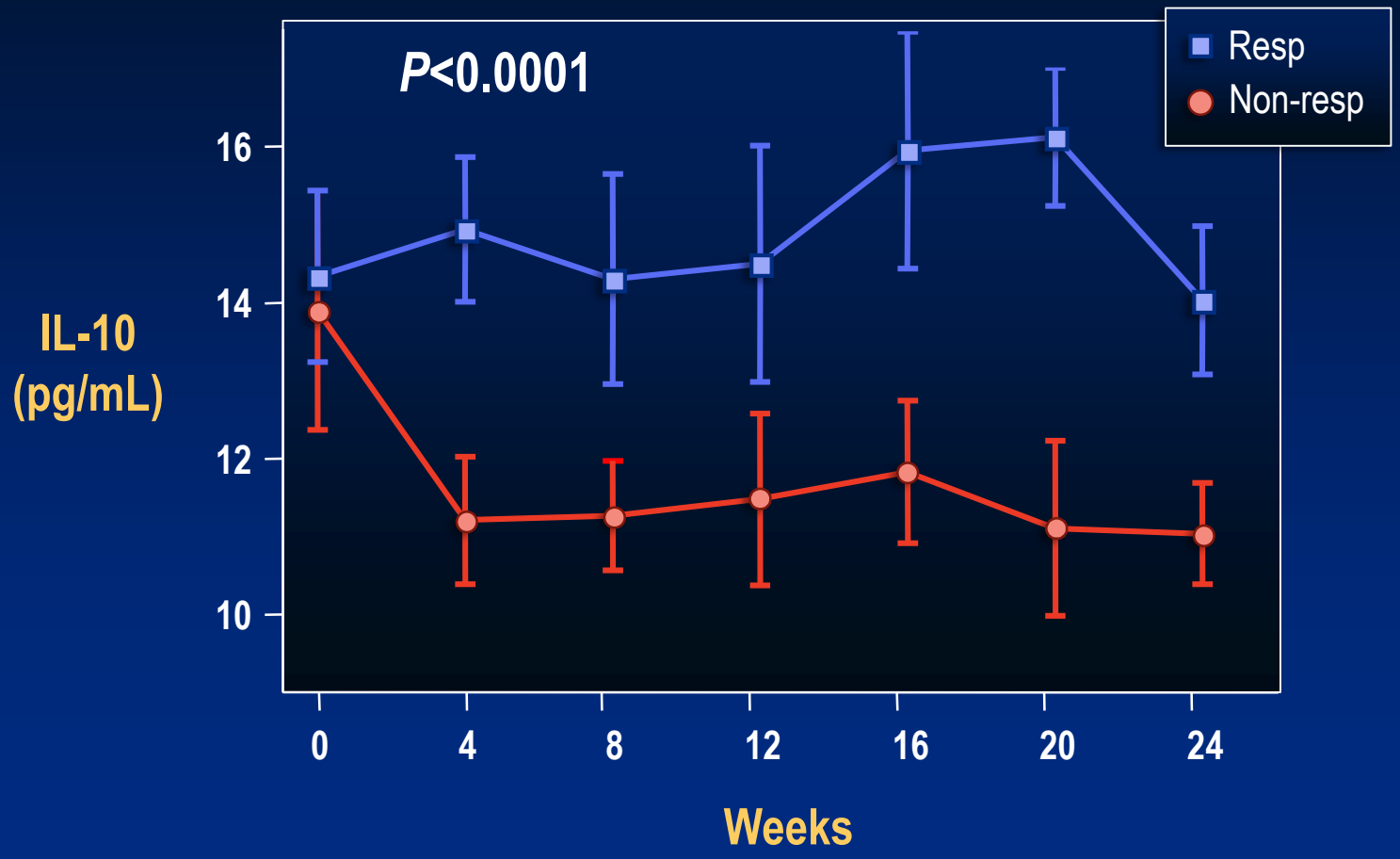
Immune parameter	Time point (months)	Relapse-free subset Mean (SD)	≥1 Relapse subset Mean (SD)	*p*-value
IL-17 (CS)	6	59.78 (100.91)	355.21 (466.87)	0.036
CXCR3 (PBMC)	3	24.9 (6.92)r	33.2 (12.25)	0.042
IL-4 (CS)	3	13.43 (7.05)	20.88 (31.60)	0.046
BDNF serum	3	768.9 (142.7)	631.8 (148.0)	0.028

IFN-B: Mean IL-17A Levels by Month 12 Relapse Status



Dhib-Jalbut et al. J neuroimmunol. 254:131- 140, 2013

Serum IL-10 Levels During 24 Weeks Of IFN-beta Therapy



Graber et al. *J Neuroimmunol*. 2007;185:168-174.

Association Between Expression of Multiple Biomarkers and IFN Treatment Response

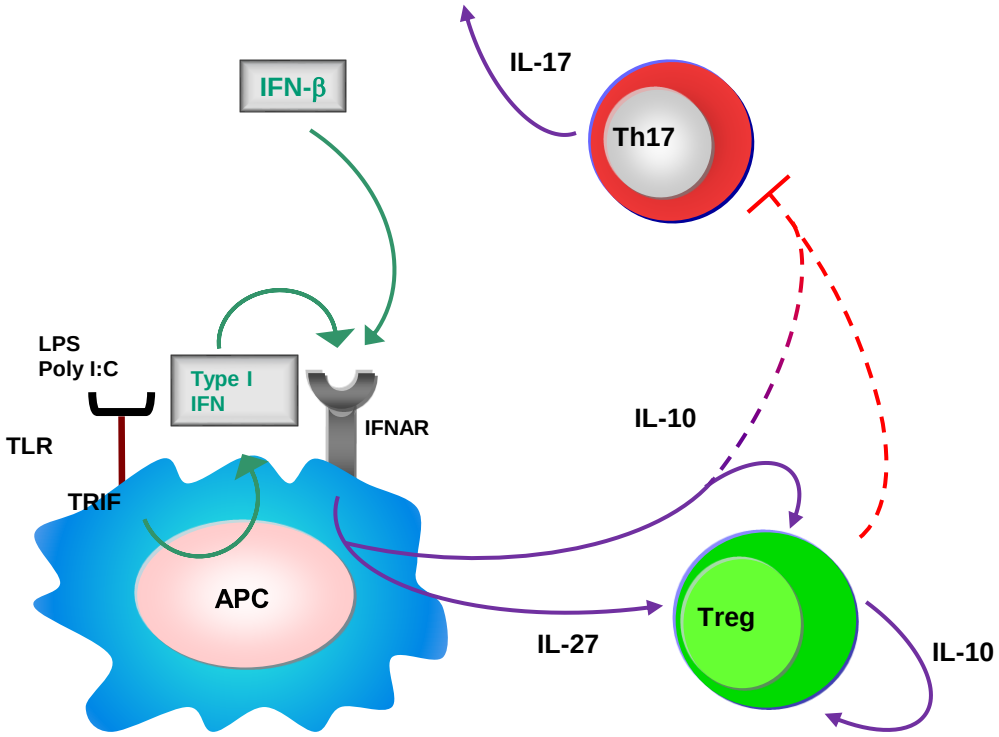
- Single biomarker expression is not a good predictor of therapeutic response
- Combinations of 2-3 biomarkers better predict therapeutic response
  - Gene combinations with high predictive value:
    - Caspase-2/IL-10/IRF-2
    - Caspase-2/IL-10/JNK-1
    - Caspase-2/IL-10
    - Caspase-2/IL-10/IL-12Rβ1

Caspase-2 involved in cell death; IL-10 involved in proliferation of T and B cells; IRF-2 involved in activation of IFNα and β; JNK-1 involved in T cell differentiation and cell death; IL-12 Rβ1 binds IL-12

IFN = interferon

Baranzini S et al. Presented at ECTRIMS / ACTRIMS 2011; Amsterdam, Netherlands. [P1126].

Signaling via IFN-β regulates Th17 differentiation



Prod'homme, T. and Zamvil, S.S. *Nat. Med*. 14: 614-616 (2008)

IFN-B Molecular Signatures Studies

- WeinstockGuttmanJ. Neuroimmunol. 2008 Dec 15;205(1-2):113-25. Epub 2008 Oct 23
- Comabella et al. Brain. 2009 Dec;132(Pt 12):3353-65.
- Rudick et al. *PloS one*. 2011;6:e19262
- Croze et al. Pharmacogenomics J. 2012

Small non-coding RNA signature in multiple sclerosis patients after treatment with interferon-β

Bruna De Felice[1,4*], Paolo Mondola[2], Anna Sasso[2], Giuseppe Orefice[2], Vincenzo Bresciamorra[2], Giovanni Vacca[2], Elio Biffali[3], Marco Borra[3] and Raimondo Pannone[3]

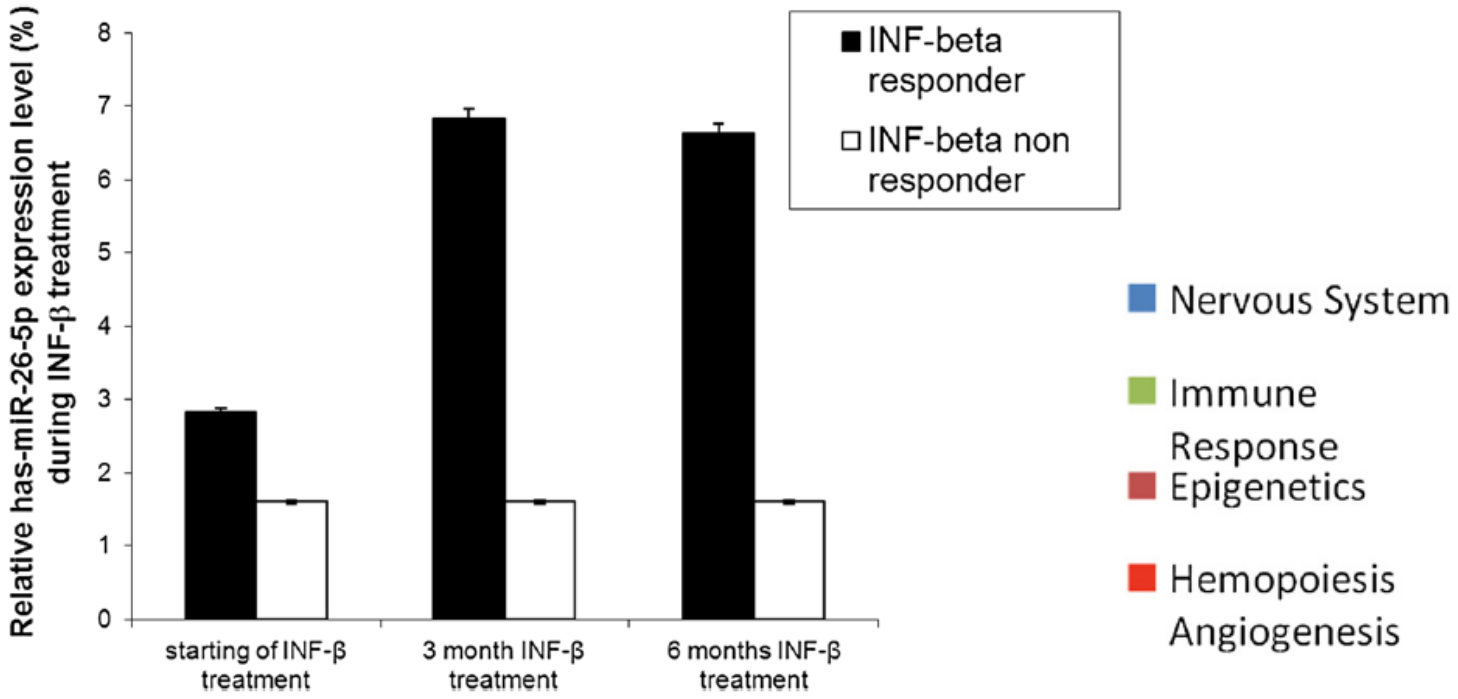
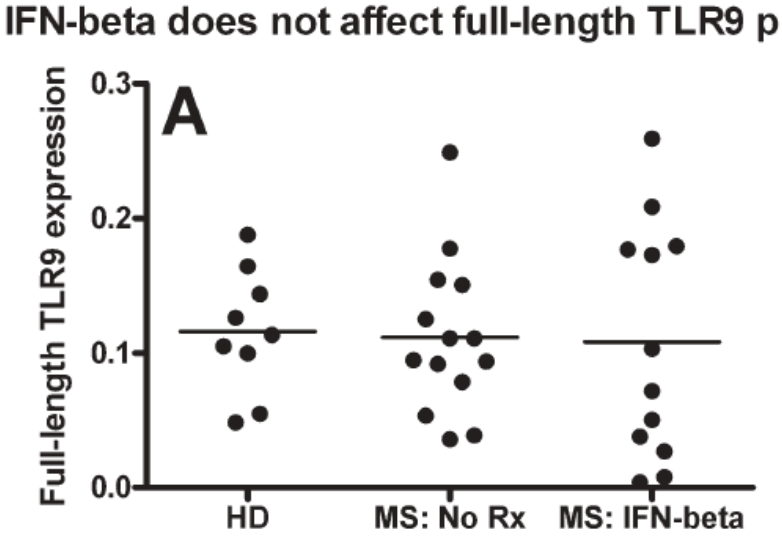
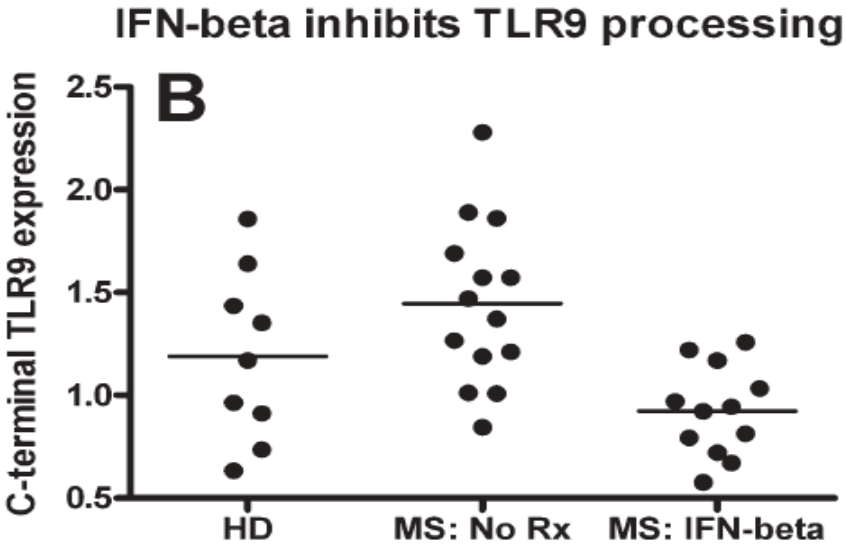


Figure 2 MiR-26a-5p expression levels in INF-β treated MS patient leukocytes. The expression of mir-26a-5p was studied in the peripheral blood from 40 IFN-β treated responder RRMS patients and from 10 IFN-β treated non-responder patients, at starting, at 3 and 6 months IFN-β treatment by microRNA assay-based quantitative RT-PCR following the $2^{-\Delta\Delta CT}$ method. RNU6B was utilized for an endogenous reference to standardize microRNA expression levels. The results were expressed as relative expression levels after calibration with the universal reference data. $P < 0.05$.

Interferon-beta Inhibits Toll-Like Receptor 9 Processing in Multiple Sclerosis

Konstantin E. Balashov, MD, PhD,[1,2] Latt Latt Aung, MD,[1]
Adi Vaknin-Dembinsky, MD, PhD,[2] Suhayl Dhib-Jalbut, MD,[1]
and Howard L. Weiner, MD[2]





Annals of Neurology 2010

Journal of Neuroimmunology 258 (2013) 27–31

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Effect of interferon beta-1a on B7.1 and B7.2 B-cell expression and its impact on T-cell proliferation

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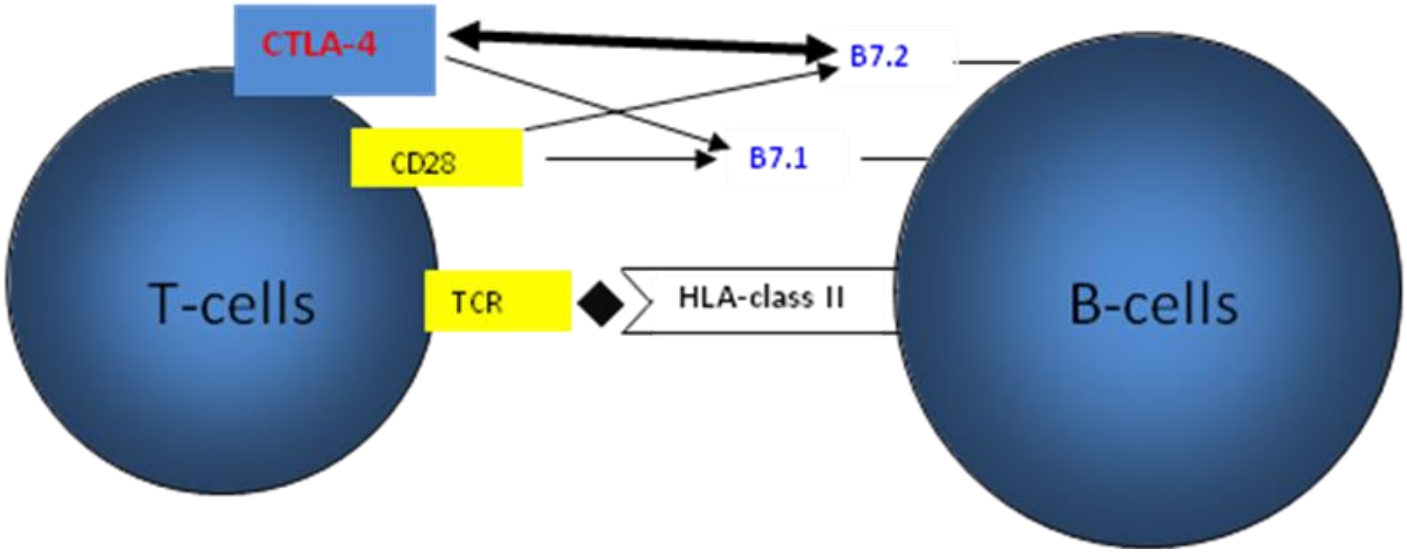
[b] EMD Serono, Inc., One Technology Place, Rockland, MA 02370, USA



Journal of Neuroimmunology 265 (2013) 68–74

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/jneuroim

Comparison of IFN-β inducible gene expression in primary-progressive and relapsing-remitting multiple sclerosis

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Department of Neurology, Rutgers-Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA

PPMS is unresponsive to IFN-B.

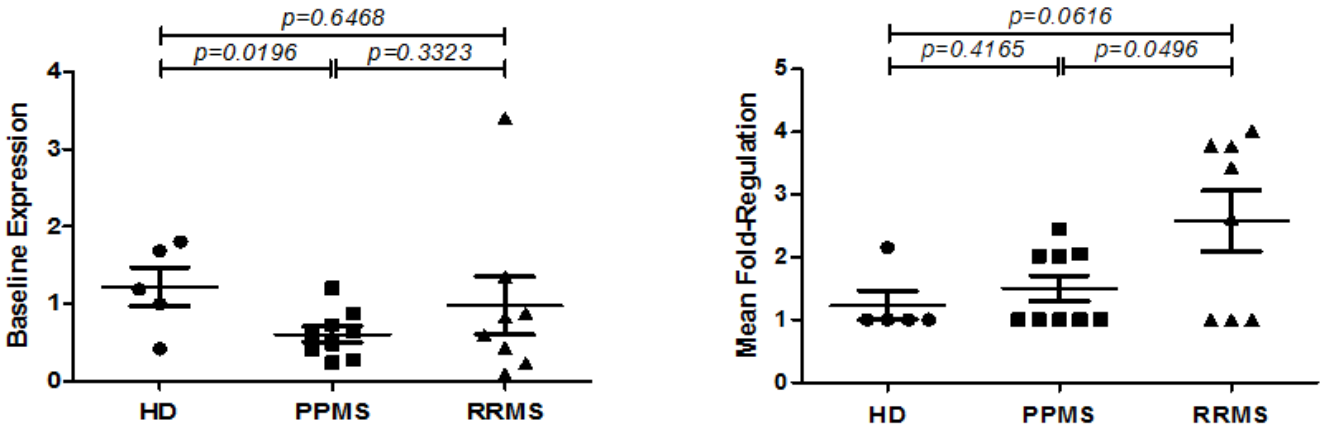
Why?

HLA-G is up-regulated in RRMS but not PPMS cells after IFN-β treatment *in vitro*

Table 3. Induction of IFN-β response genes upon *in vitro* treatment with IFN-β

Gene Symbol	HD (n=5)		PPMS (n=9)		RRMS (n=8)		p-value		
	Fold	SD	Fold	SD	Fold	SD	HD v. PP	PP v. RR	HD v. RR
...									
HLA-C	2.18	± 1.16	1.79	± 0.76	1.40	± 0.78	0.4643	0.3156	0.1748
HLA-F	1.21	± 0.47	1.59	± 0.77	1.66	± 0.96	0.3345	0.8745	0.3527
→ *HLA-G*	1.23	± 0.52	1.50	± 0.61	2.58	± 1.37	0.4165	**0.0496**	0.0616
IFI16	3.83	± 1.85	3.60	± 1.69	3.18	± 2.17	0.8169	0.6588	0.5894
IFI27	199.70	± 123.87	50.33	± 53.12	111.57	± 189.09	**0.0076**	0.3649	0.3781
IFI30	1.62	± 0.87	1.00	± 0.00	1.41	± 1.16	**0.0480**	0.3037	0.7350
...									

HLA-G



Boppana et al., *J Neuroimmunol*, 265:68-74, 2013

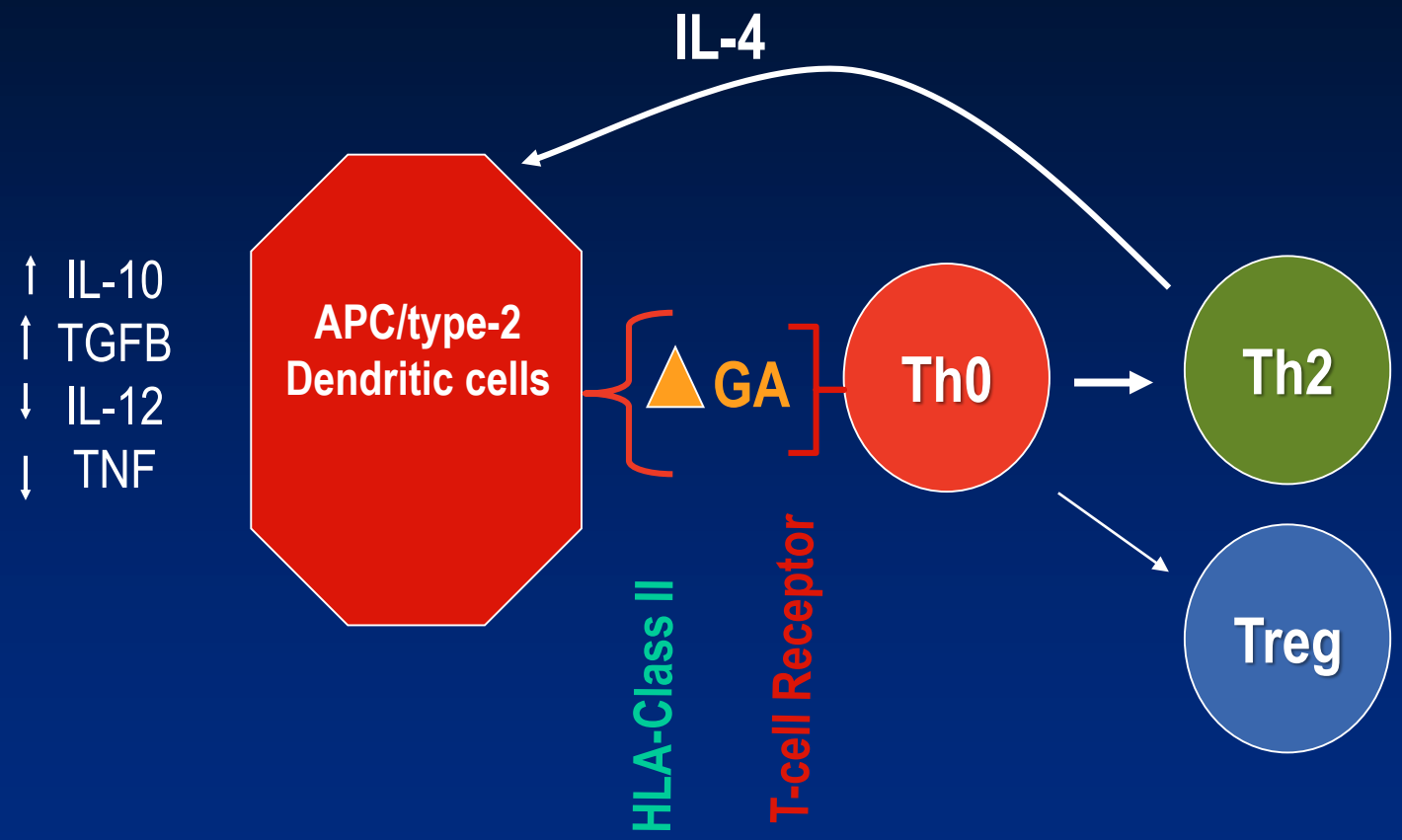
Potential IFN-β Serum Biomarkers

Responders	Non-responders
Increase in IL-10	IL-17F levels>200pg/ml
Reduction in Th1 cytokines	High baseline IFN-β levels
Increased in neurotrophic factors MicroRNA 26a-5p Increased monocytes IFN-I secretion in response to TLR	NAB SNPs (IRF8, IRF5) Increase PSTAT1 and IFNR1 on monocytes at baseline

Dhib-Jalbut S et al. *J Neuroimmunology.* 2013;254:131-140.
Comabella M et al. *Brain*. 2009;132:3353-3365.

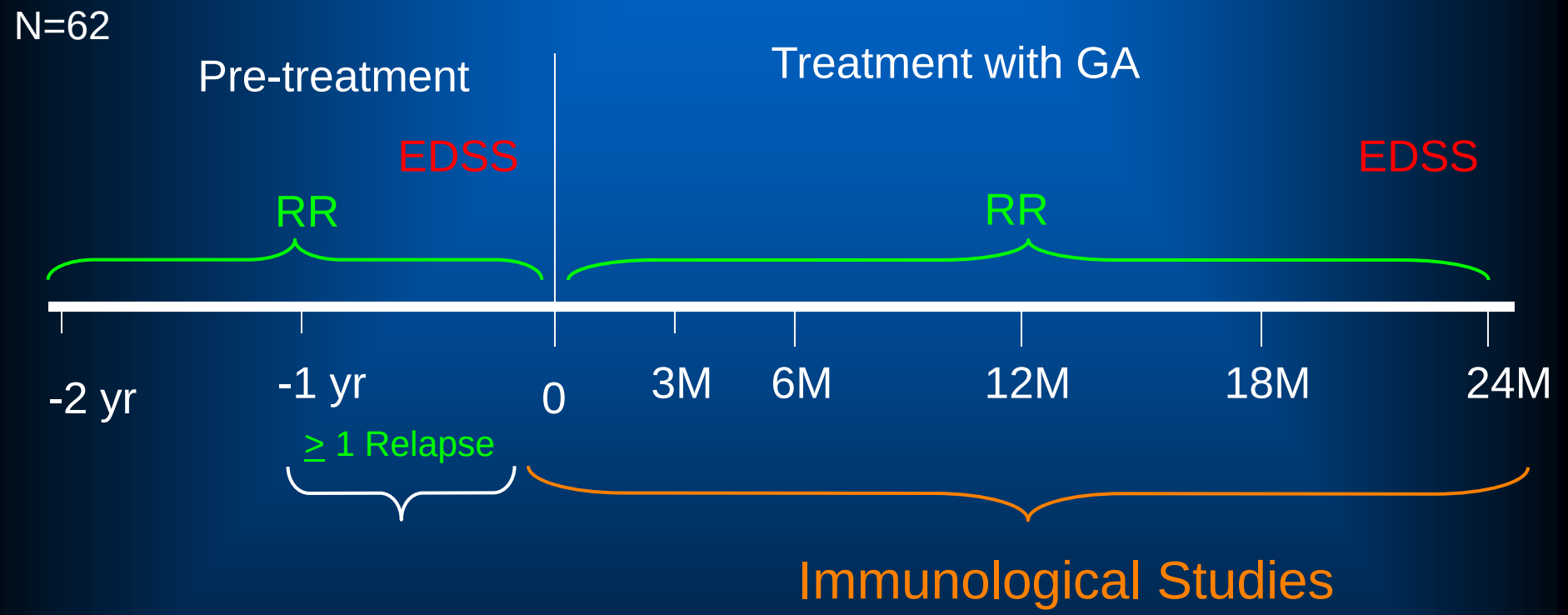
GLATIRAMER ACETATE

Glatiramer Acetate Binds to HLA Class II on Antigen Presenting Cells and Induces Type-2 APCs



Weber et al. *Nat Med*. 2007;13:935-43.

Biomarkers of Rx Response to GA: Prospective Study Scheme



Criteria for Clinical Response

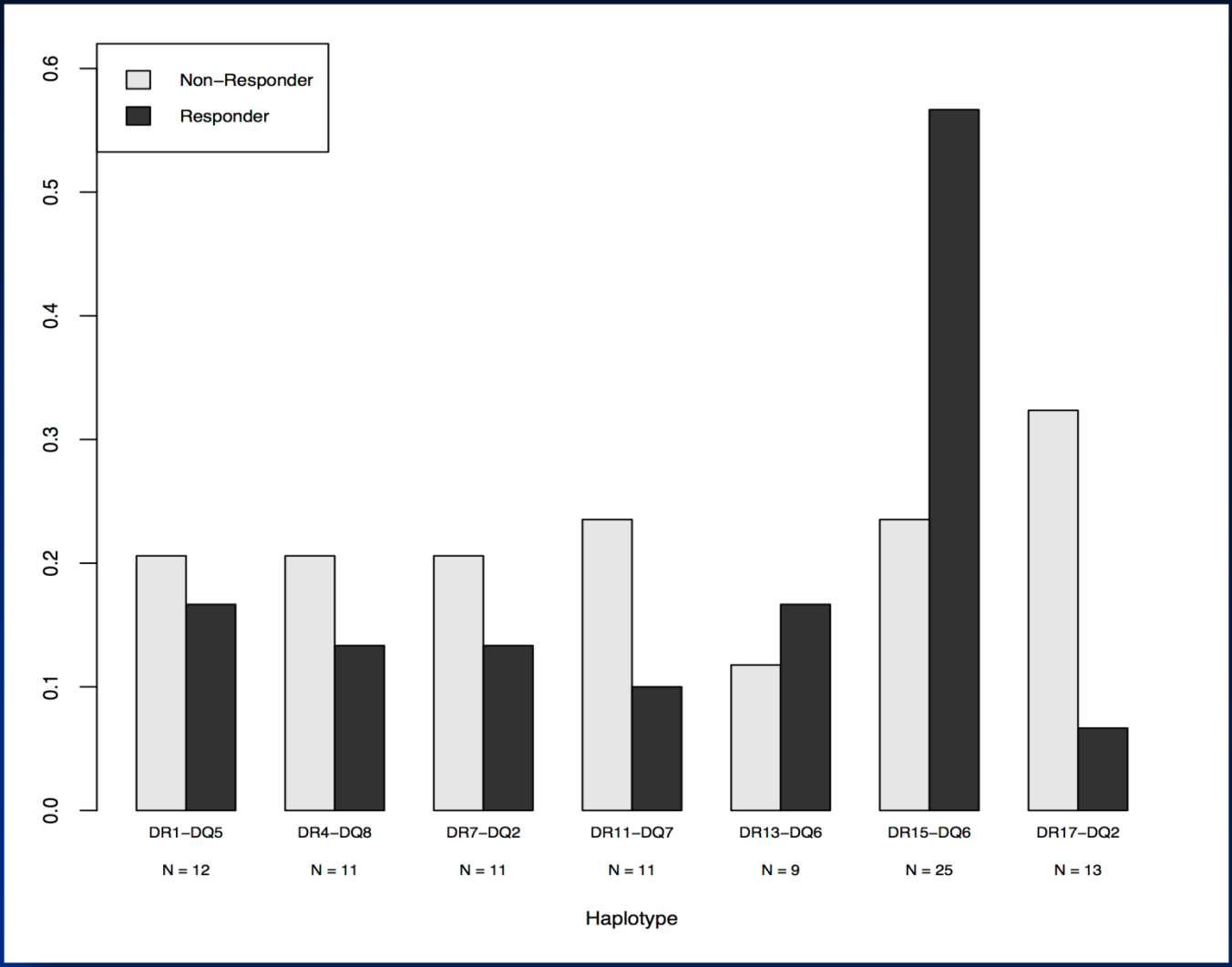
RESPONDERS

- Patients with no relapses
- With no evidence of disease progression as measured by EDSS

Non-RESPONDERS

- Patients with ≥1 Relapse
- With progression in the EDSS of at least 1 point sustained for 6 months

Response by Haplotype



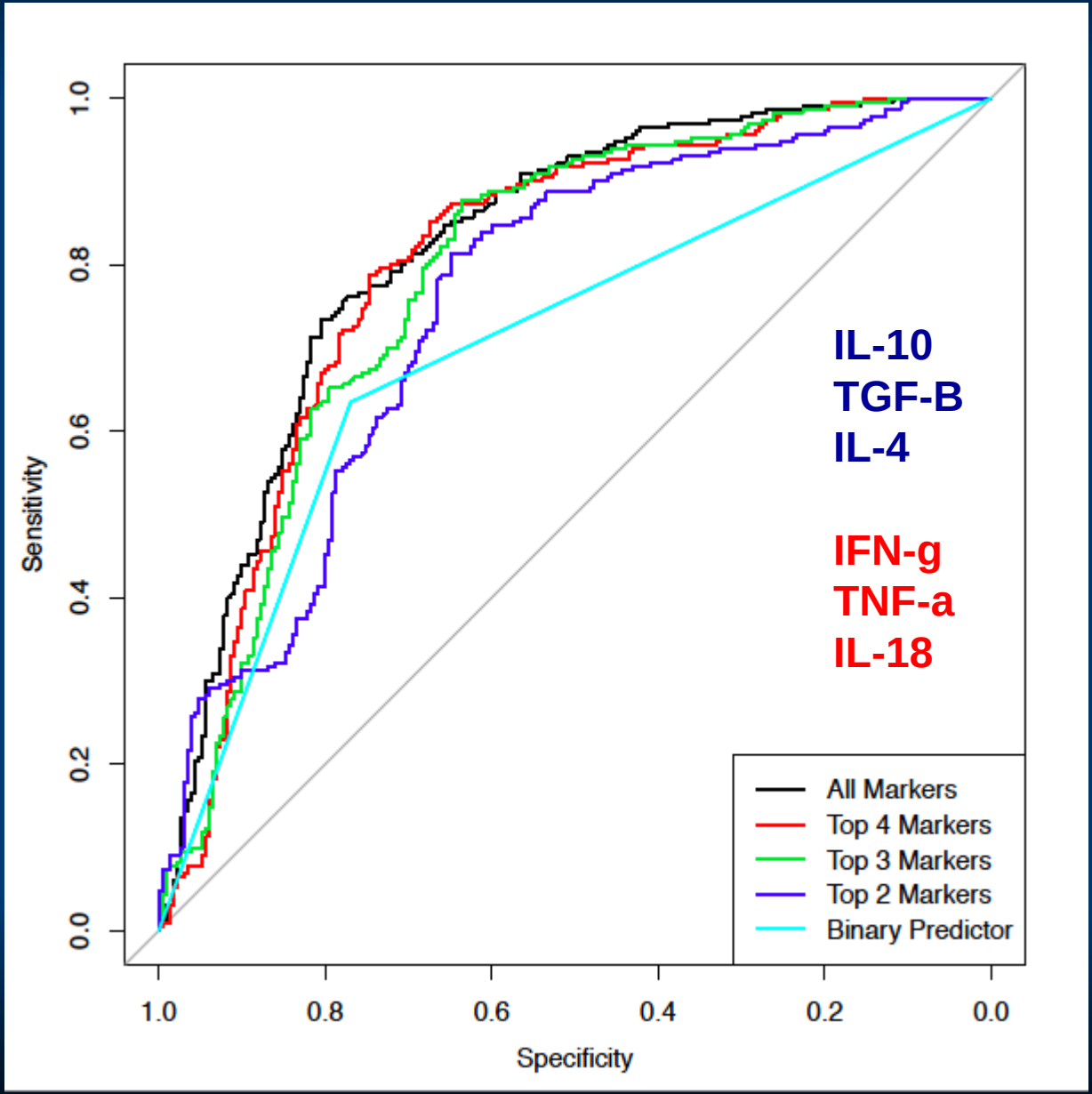
Dhib-Jalbut et al. MSARD 2:340-348, 2013

DR and DQ Haplotypes Predictors of Clinical Response to GA

PROGNOSTIC PROFILE	HAPLOTYPES	NR / R (%R)
Poor prognostic profile	DR15 - DQ6 absent DR17 - DQ2 present	10 / 2 (16.7%)
Neutral prognostic profile	DR15 – DQ6 present & DR17 – DQ2 present DR15 – DQ6 absent & DR17 – DQ2 absent	17 / 11 (39.5%)
Good prognostic profile	DR15 – DQ6 present DR17 - DQ2 absent	7 / 17 (70.8%)

Dhib-Jalbut et al. MSARD 2:340-348, 2013

GA induced Cytokine change from baseline; ROC analysis

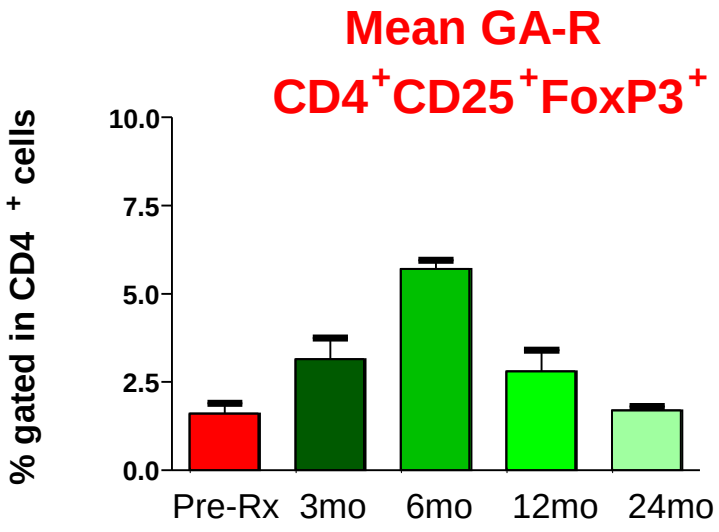


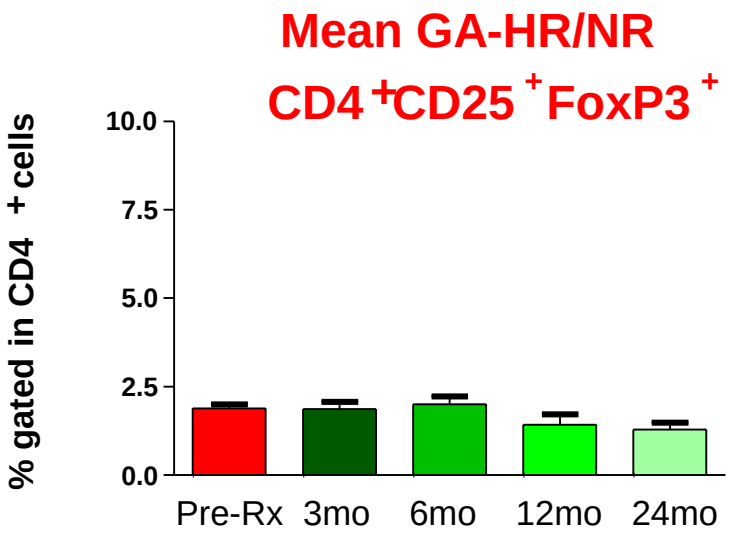
Binary Cytokine Predictors of response to GA

Clinical outcome	**Δ-IL-18>0 Δ-TGF-B<0**	**Δ-IL-18<0 Δ-TGF-B>0**
Responders	32.2%	73.4%
Non-Responders	67.8%	26.6%

Levels at 6 months compared to base-line
ROC=0.7

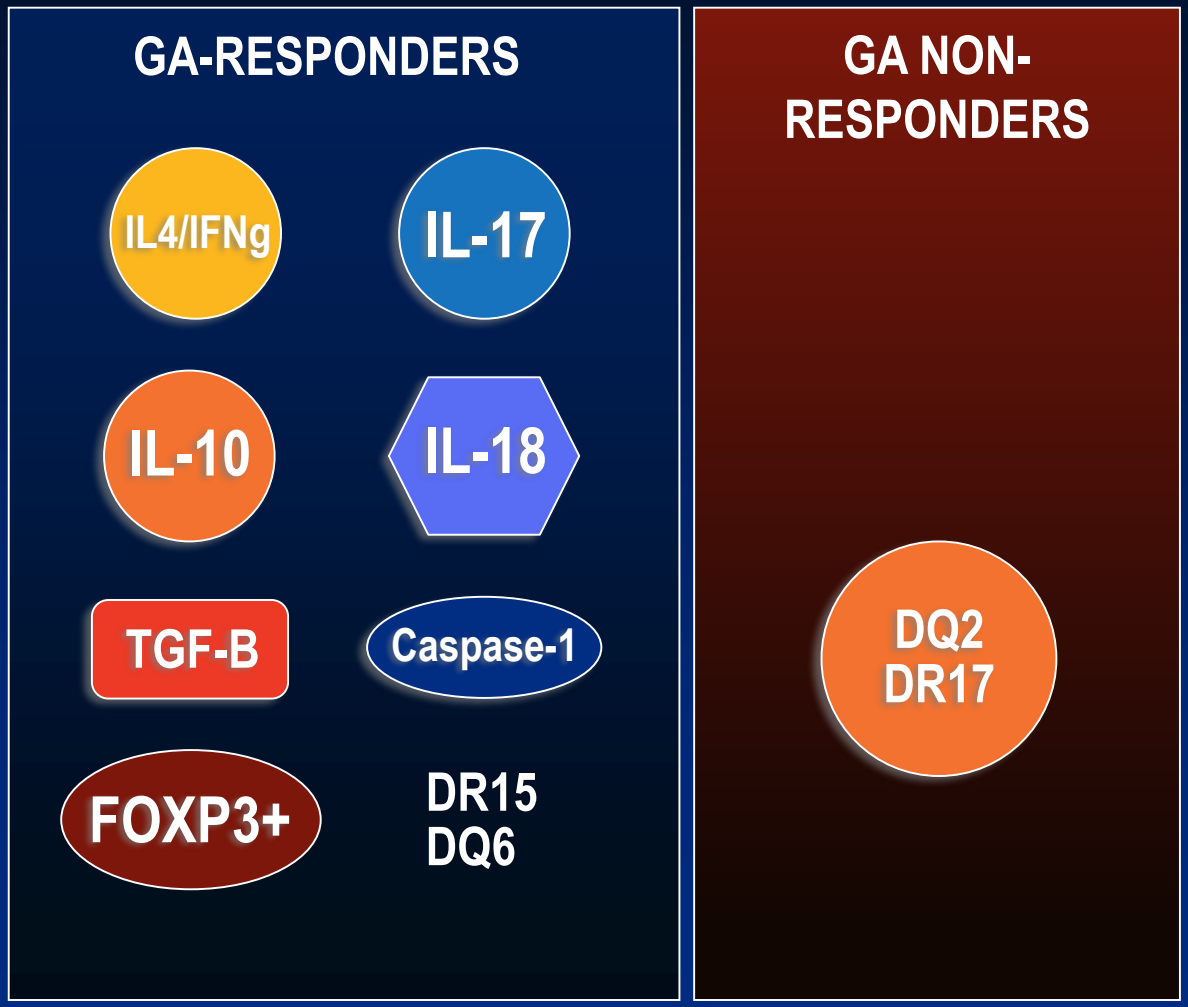
Induction of $CD4^+CD25^+FOXP3^+$ Tregs During Glatiramer Acetate Treatment





Dhib-Jalbut S, et al. 23rd Congress of ECTRIMS, 12th Conference of Rehabilitation in MS; October 11-14, 2007; Abstract ID 52136.

Summary of Potential GA-Biomarkers



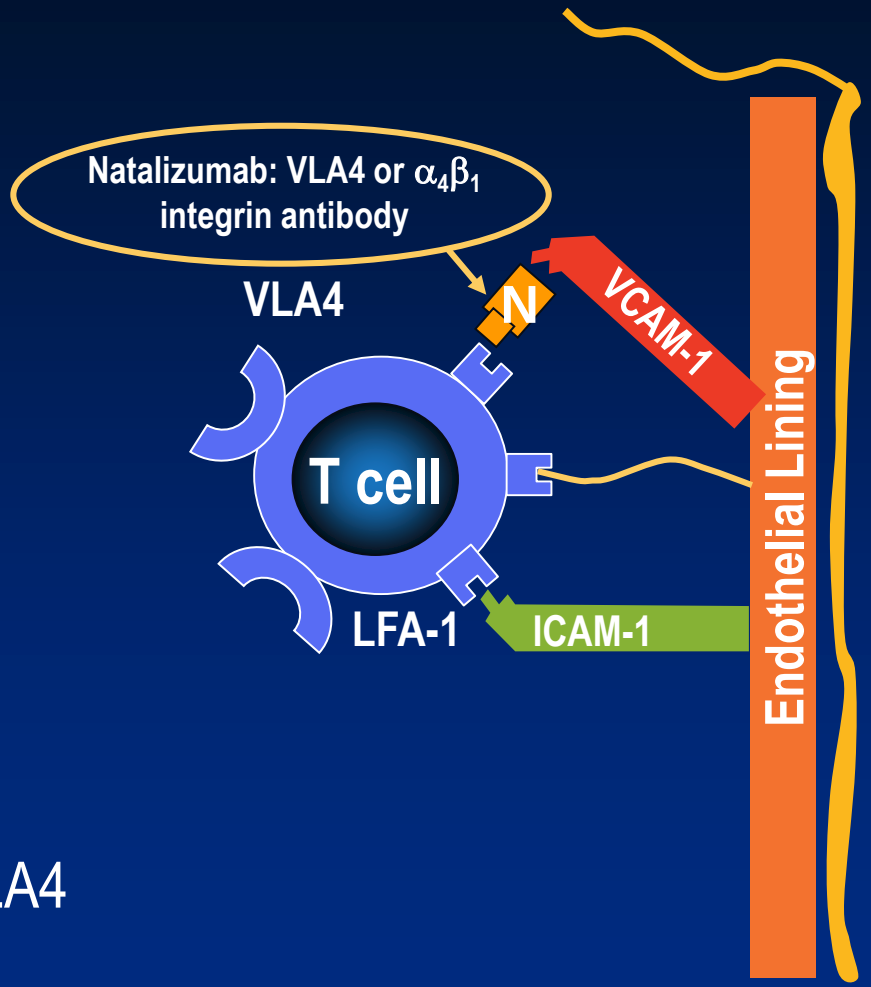
Dhib-Jalbut. Presented at ECTRIMS 2010.

NATALIZUMAB

Natalizumab: Mechanism of Action

Blood

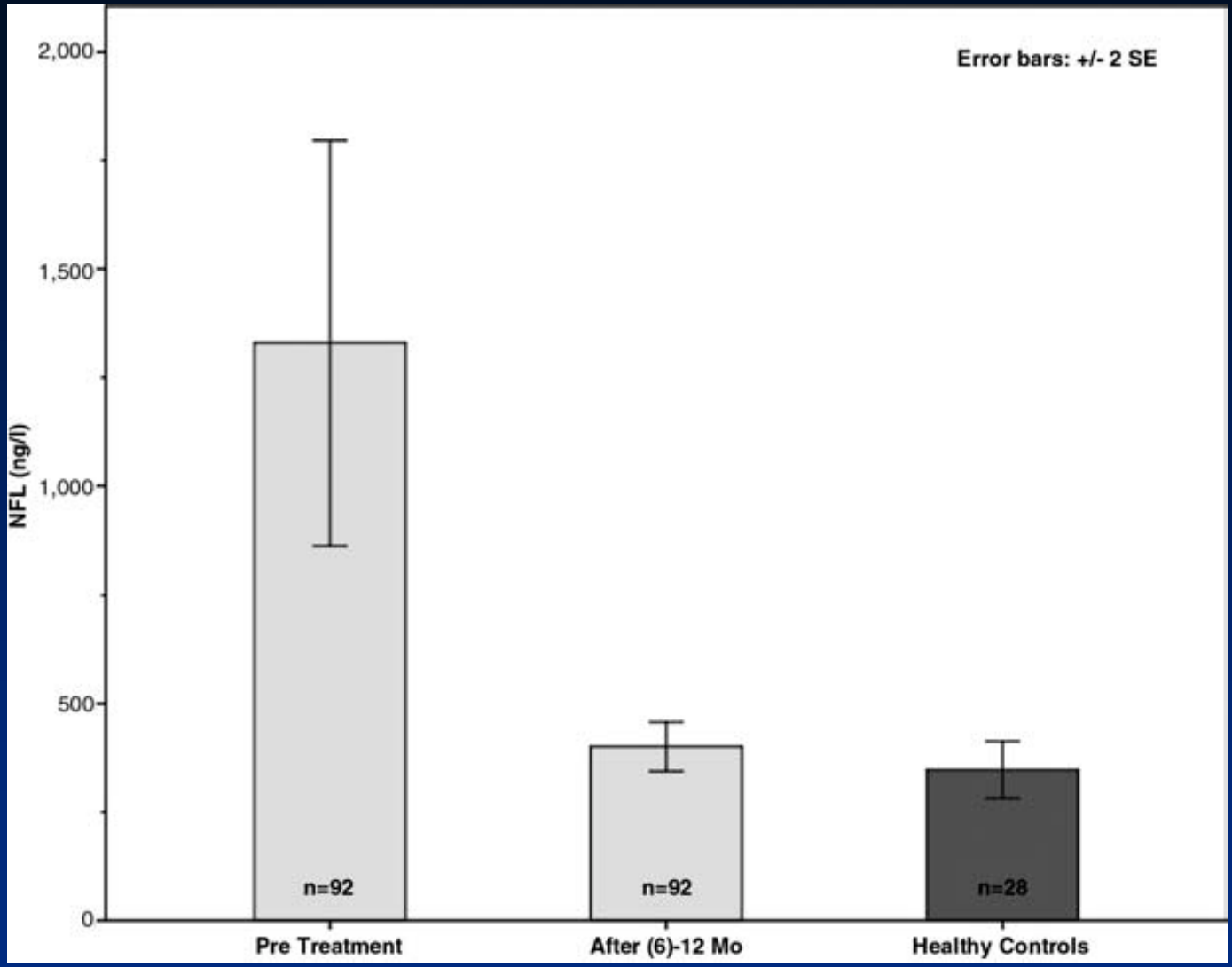
CNS



$\alpha_4\beta_1$ integrin = VLA4

Alter et al. *J Immunol.* 2003;170:4497-4505.

Axonal Damage in Relapsing Multiple Sclerosis is Markedly Reduced by Natalizumab



Gunnarsson et al.
ANN NEUROL
2011;69:83–89

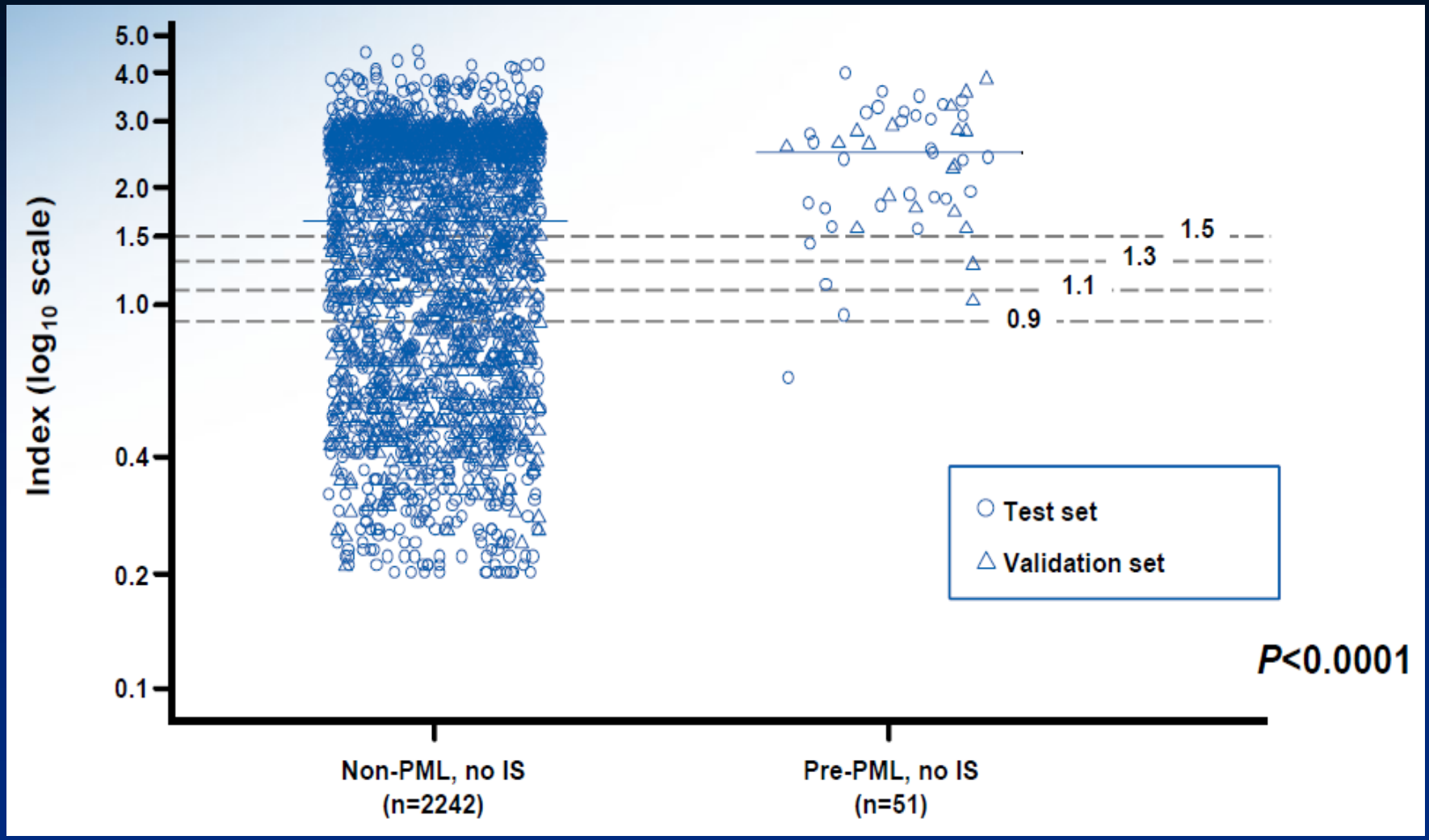
Potential Exploratory Biomarkers of Newer MS Therapies

Treatment	Tissue	Biomarker
Natalizumab	PB	VLA-4, CD34 cells
	CSF	NFL
Fingolimod	PB	Decreased Naïve and Tcm, Decreased CD4:Cd8 ratio, Decreased Th17, Decreased B-cells
	CSF	Decreased T-cells and CD4:CD8 ratio
Rituximab	CSF	Decreased T and B cells
Daclizumab	PB/CSF	Increased NKreg cells
BMT	PB	Decreased TH17

BP: Peripheral Blood; BMT: Bone Marrow Transplant

Biomarkers of Treatment Complications

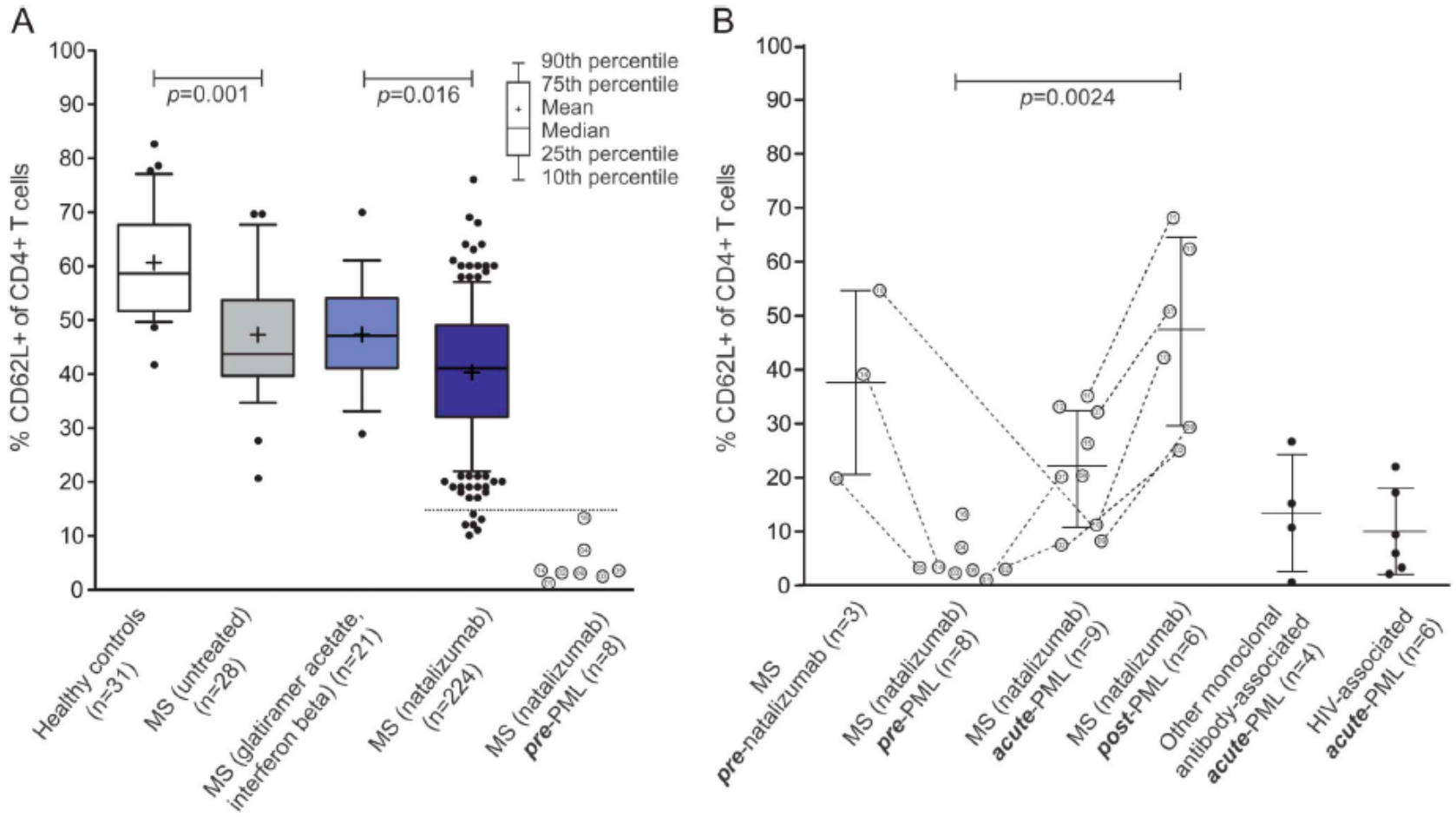
Anti-JCV Antibody Index Distribution in Anti-JCV Ab+ non-PML and pre-PML Patients with No Prior IS Use



Plavina T, et al. Use of anti-JC virus antibody index to further define risk of PML in anti-JCV antibody-positive TYSABRI-treated patients with MS. Platform presentation presented at 23rd Annual Meeting of European Neurological Society, June 9, 2013; Barcelona, Spain.

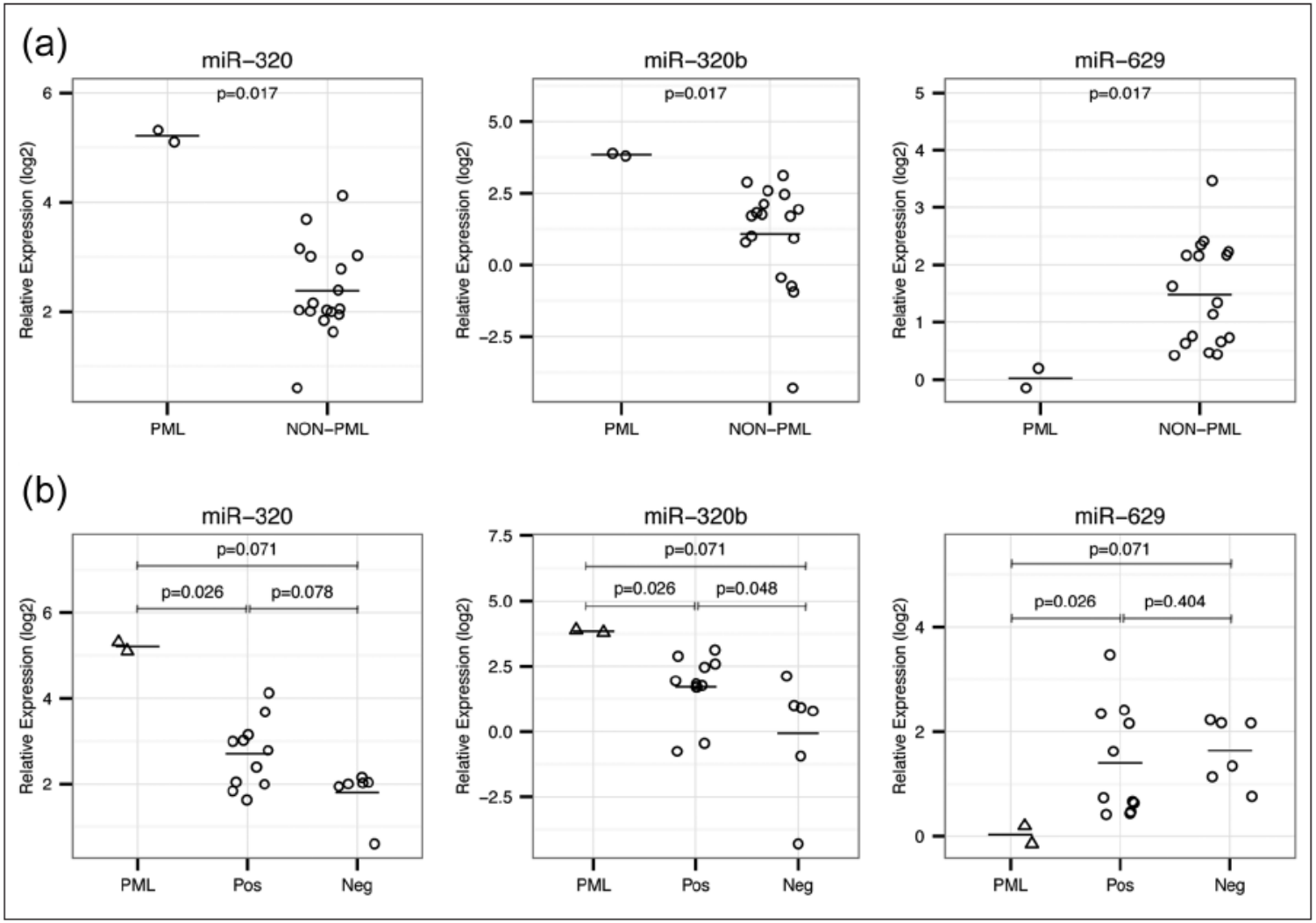
L-Selectin and Risk of PML in Natalizumab Treated MS Patients

Figure 1 Surface expression of CD62L and its correlation to progressive multifocal leukoencephalopathy development in multiple sclerosis patients receiving natalizumab therapy



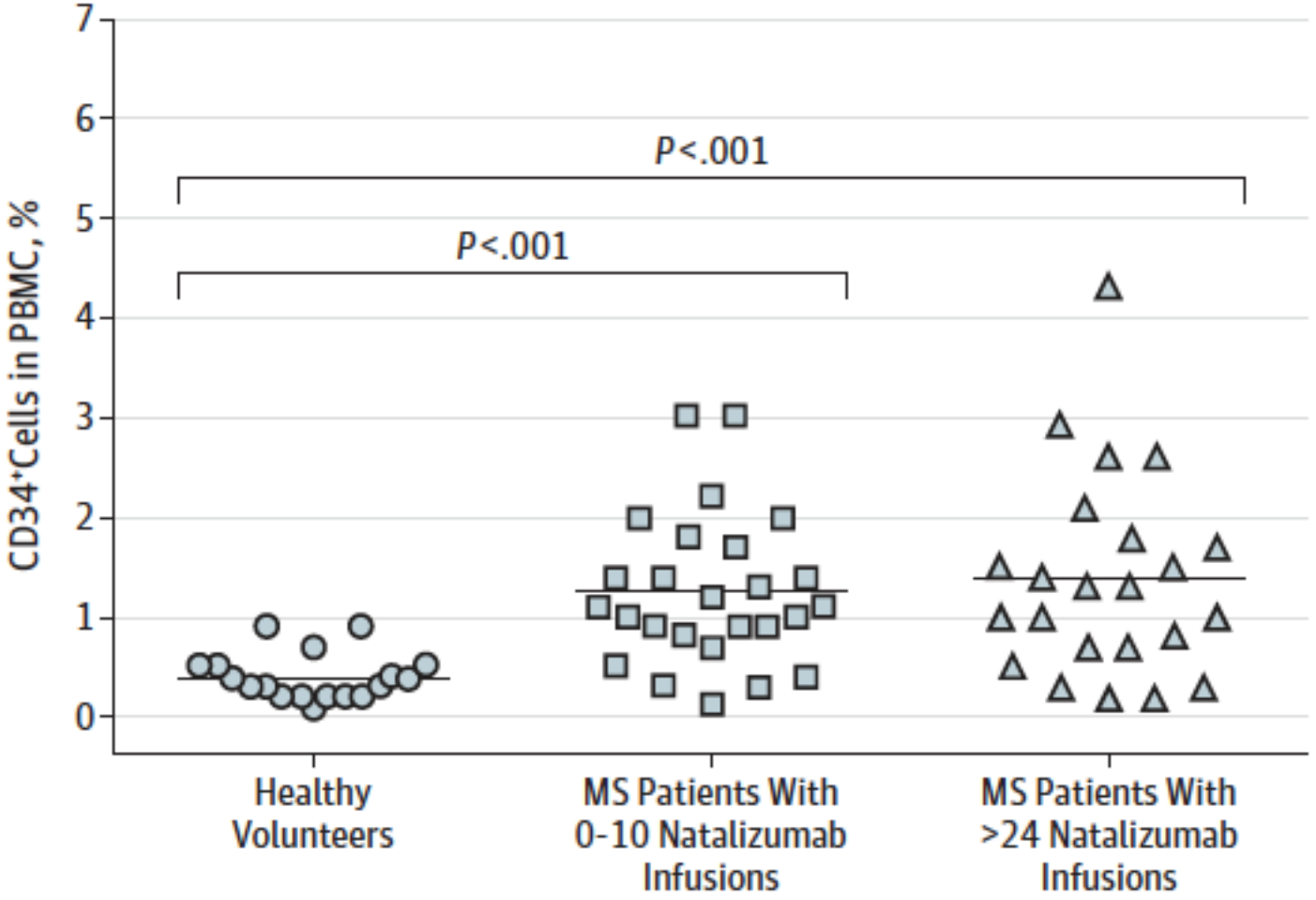
Schwab et al. *Neurology.* 2013;81:865–871.

Blood miRNA expression pattern is a possible risk marker for natalizumab-associated progressive multifocal leukoencephalopathy in multiple sclerosis patients



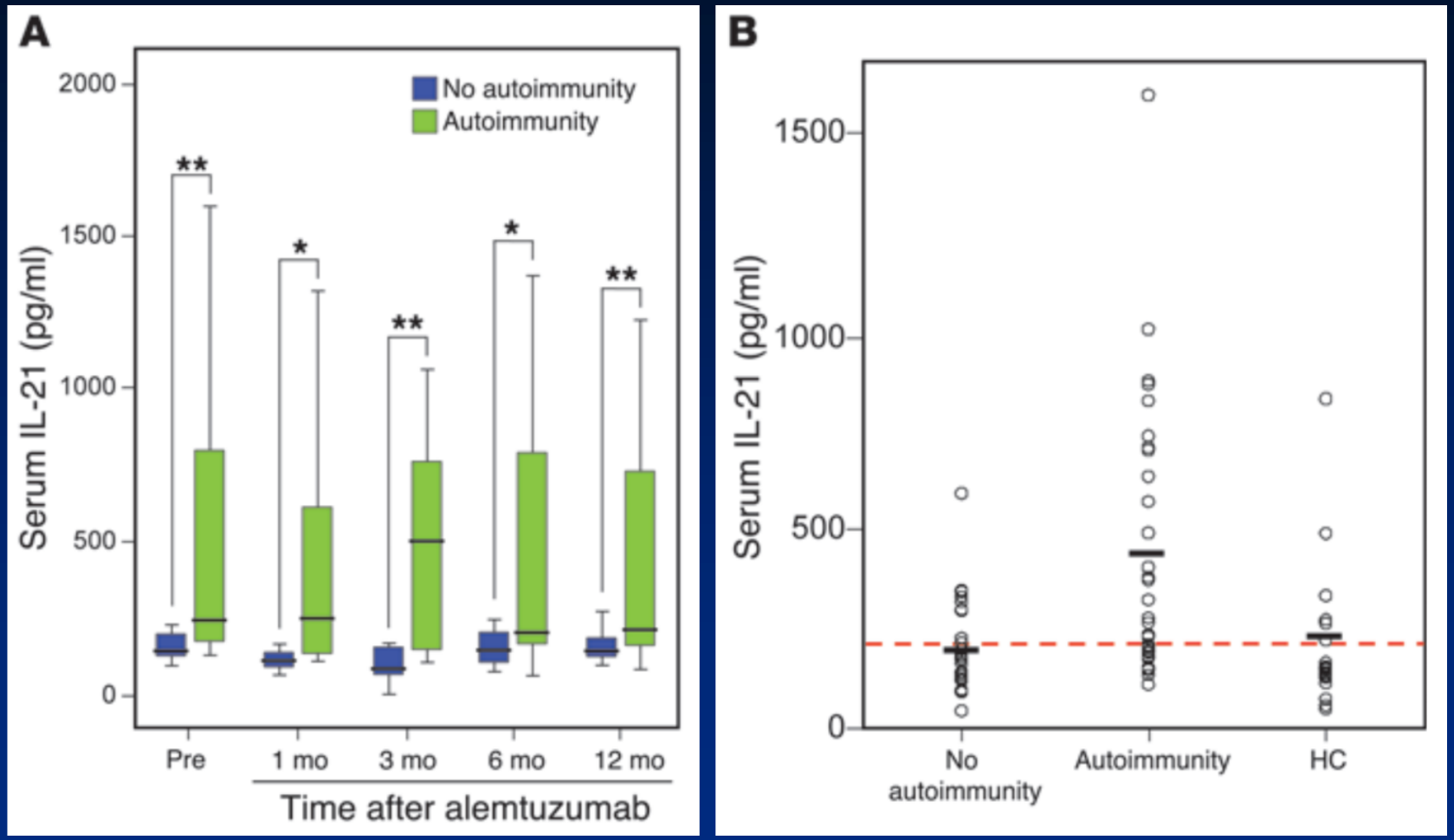
Munoz-Culla et al. MS Journal May 2014

JC Virus in CD34$^+$ and CD19$^+$ Cells in Patients With Multiple Sclerosis Treated With Natalizumab



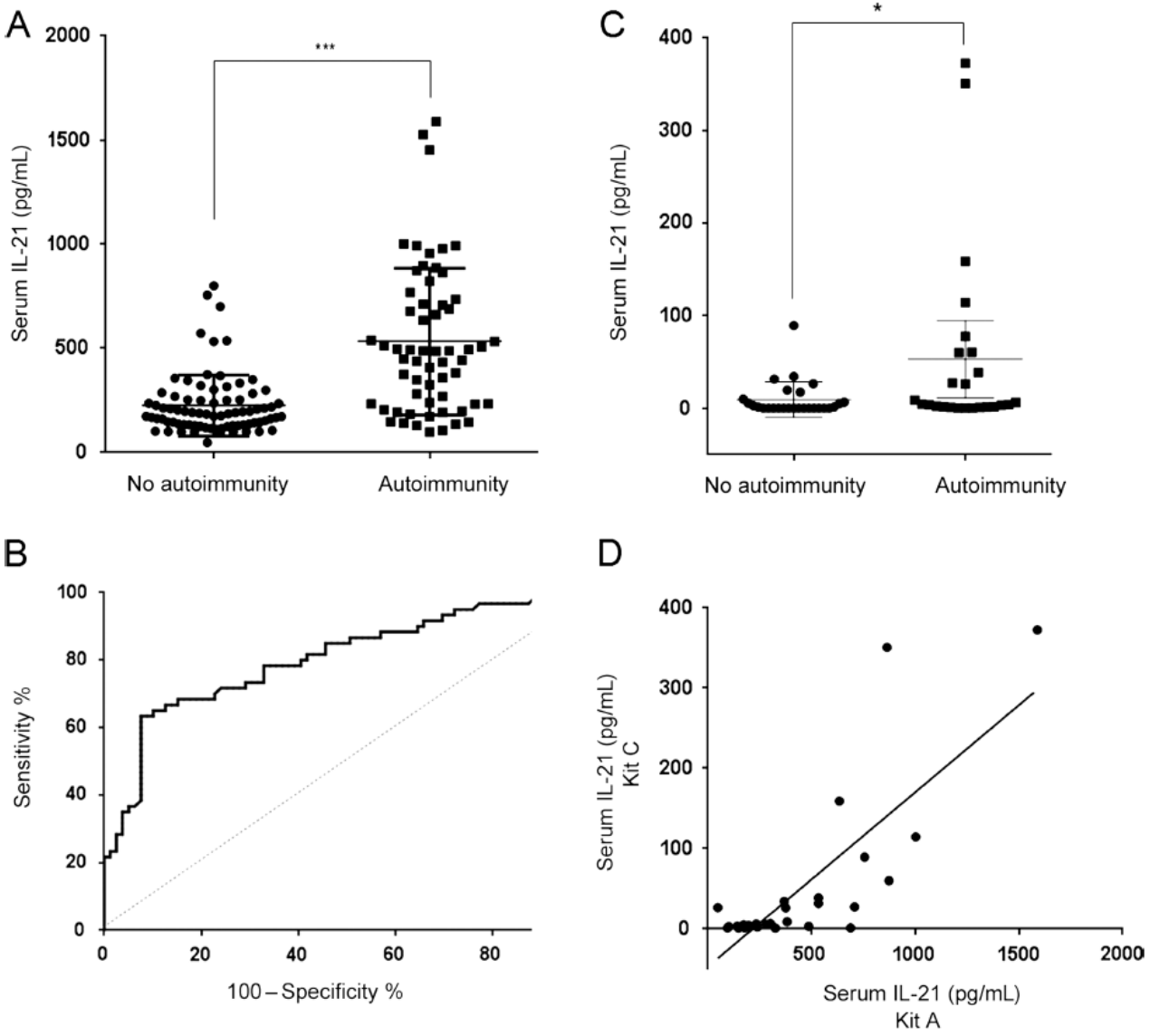
Frohman et al. JAMA Neurol. 2014;71(5):596-602.

Alemtuzumab, serum IL-21 and Autoimmunity



Jones et al. *J Clin Invest*. 2009;119:2052-2061.

Alemtuzumab: Predicting Autoimmunity



Azzopardi L, et al. J Neurol Neurosurg Psychiatry 2014;85:795–798.

Clinically Useful and Potential Biomarkers for Monitoring MS Therapy

Biomarker	Condition
JCV assay	PML risk with Natalizumab
NABs	IFN unresponsiveness
HLA DR & DQ; TGF-B/IL-18	Glatiramer response
IL-10/IL-17/SNIPS	Interferons response
IL-21	Alemtuzumab autoimmunity
CD 56^{Bright}	Daclizumab therapy
MRI	Interferon response

Dhib-Jalbut, S. 2014

Acknowledgements

- Reuben Valenzuela
- Man Chen
- Sumandeep Sumandeep
- Jerome Graber
- Steve Buyske
- Roland Martin
- Kouichi Ito
- Ken Johnson
- Kathleen Costello

- NIH/NINDS
- The Wadsworth Foundation
- NMSS
- DVA
- TEVA Pharmaceuticals
- EMD-SERONO
- BAYER Health
- BIOGEN Idec