

Immune monitoring of patients with multiple sclerosis

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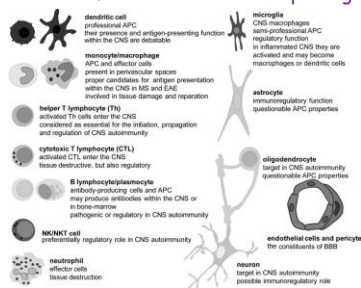
Conflict of Interest Disclosure

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This COI Disclosure Form can be viewed at the ESCCA 2019 Conference website www.escca.eu/norway2019 - Programme section / Accreditation page

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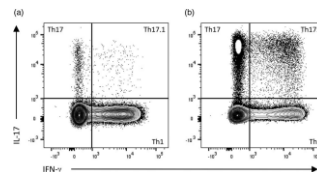
Contribution of immune cells in MS pathogenesis



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CD4+ lymphocytes

- combined IFN-g and IL-17-driven condition
- PB Th17 cells may also be indicative of relapse
- Higher proportions of total GM-CSF+, GM-CSF+/IFN-g and GM-CSF+IFN-g+CD4+ T cells
- Lower proportion of Tregs



A. P. Jones et al Clin and Exper. Immunology 2017

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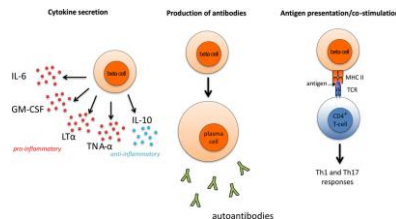
Hallmarks on the understanding of the role of the Th17 pathways in MS

- Increased IL-17 found in the blood and CSF of RRMS patients, especially during relapse
- IL-17-producing T cells identified in EAE
- Increased Th17 cells and IL-17 found in the brain of MS patients
- IL-17 production correlates with MRI activity
- Secukinumab (anti-IL-17A monoclonal antibody) reduces MRI lesions in a phase II clinical trial

Dos Passos GR et al. Mediators Inflamm 2016.

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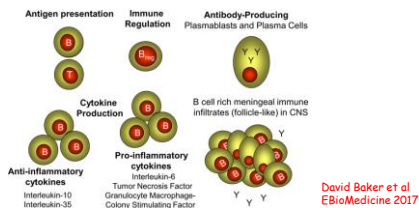
Role of B cells in MS



Ai-Lan Nguyen et al Br J Pharmacology 2017

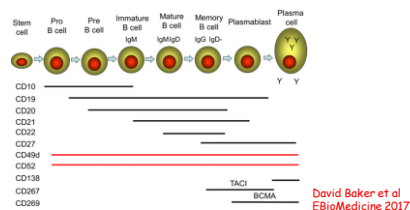
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Potential B cell functions in multiple sclerosis

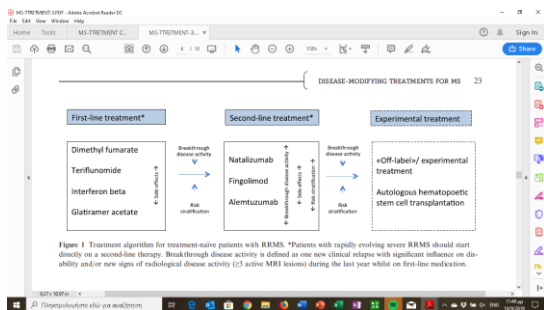


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B cell lineage and surface marker expression



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The role of cytometry lab?

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Biological drugs

A substance that is made from a living organism or its products and is used in the prevention, diagnosis, or treatment of cancer and other diseases. Biological drugs include antibodies, interleukins, and vaccines. Also called biologic agent and biological agent.

- Immune check point inhibitors (PD-1, PD-L1, and CTLA-4 targets)
- Immune Cell Therapy (also called Adoptive Cell Therapy or Adoptive Immunotherapy) (TILs, CAR T cells)
- Therapeutic antibodies
- Immune-Modulating Agents
- Therapeutic Vaccines

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Among the 10 top biologic drugs in USA

- **Humira TNF blocker** for Rheumatoid arthritis, plaque psoriasis, Crohn's disease, ulcerative colitis, ankylosing spondylitis, psoriatic arthritis, polyarticular juvenile idiopathic arthritis
rheumatologists, gastroenterologists
- **Rituxan (rituximab) anti CD20** for Non-Hodgkins lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, multiple sclerosis
rheumatologists, hematologists, neurologists
- **Anovex interferon β** for multiple sclerosis
neurologists

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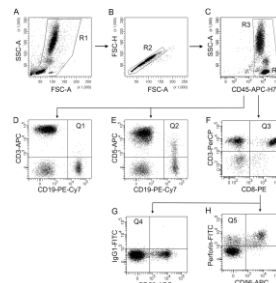
Response of MS patients to IFNβ

- Basal blood immune cell subsets contribute to identify MS patients with a high probability of showing an optimal response to IFN-beta.
- Percentages below 3% of CD19 + CD5 + cells
- or above 2.6% of CD8 + perforin + T cells
- increased the probability of achieving no evidence of disease activity status during treatment.

Raquel Alenda et al J of Neurology 2018

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Multicenter study for the gating strategy regarding CD19+CD5+ and CD8+perforin+ cells for the study of the MS patients before IFNβ treatment

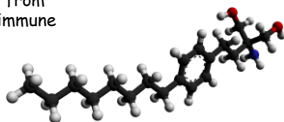


Noelia Villarrubia et al Clinica Chimica Acta 2019

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fingolimod

- sphingosine-1-phosphate receptor modulator,
- Immunomodulating drug
- It sequesters lymphocytes in lymph nodes, preventing them from contributing to an autoimmune reaction.



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fingolimod

- Significant decrease of CD3+CD4+ T lymphocytes, increasing with time on the drug.
- Significant increase in NK lymphocytes
- It can explain the adverse effects noted by clinicians, like susceptibility to infections

Julia Rudnicka et al Clinical Immunology 2015

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Monoclonal Antibodies

- Anti-B cell therapies

B-Cell Depletion Therapies (With Anti-CD20) Continue to Expand in the Treatment of Immune-Mediated Diseases

Approved Usages	Other Applications (Literature)
<ul style="list-style-type: none"> • Severe Rheumatoid Arthritis (Anti-TNF failures) • ANCA-mediated vasculitis • Granulomatosis with Polyangiitis (Wegener) and Microscopic Polyangiitis • Relapsing-Remitting MS • Primary Progressive MS • Renal and Extra-Renal SLE • ITP • Idiopathic Membranous Nephropathy • IgG4-Related Nephropathies • Optic Neuromyelitis • Cryoglobulinemic vasculitis • Anti-HLA Abs Removal in Transplants 	<ul style="list-style-type: none"> • Sjogrens Syndrome • Scleroderma • Myositis • Anti-Phospholipid Syndrome • MuSK-Mediated Myasthenia Gravis • TTP • Autoimmune Hemolytic Anemia • Inflammatory bowel disease • Chronic Graft-versus-Host disease • Pemphigus - Blistering skin disease • Pulmonary hypertension • Hepatitis C Cryoglobulinemia • IgM-associated polyneuropathy • Uveitis • Autoimmune paraneoplastic syndromes

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Examples of monoclonal antibodies

- **Rituximab anti-CD20**
- chimeric mouse-human IgG1k mAb that binds to the CD20 cell surface epitope on circulating B-cells
- Although rituximab has not been approved for the treatment of MS, it can be approved for off-label use in certain countries and it is definitely in use.
- More studies are to follow.

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Examples of monoclonal antibodies

- **Ocrelizumab anti-CD20**
- Recombinant humanized IgG1 antibody that binds to a different but overlapping epitope compared with rituximab
- **Approval 2017??**

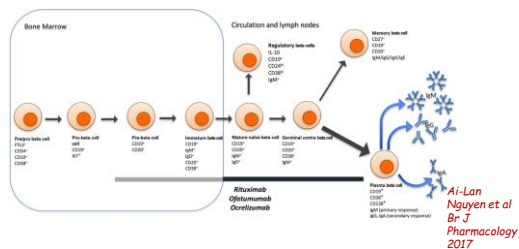
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Examples of monoclonal antibodies

- **Ofatumumab anti-CD20**
- Totally human IgG1 antibody that binds to a different but overlapping epitope compared with rituximab
- **Approval expected to be complete by July 2019**

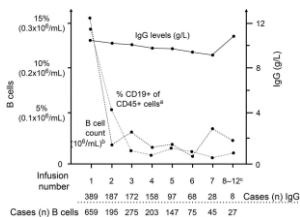
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B cell stages of differentiation- MoAbs action



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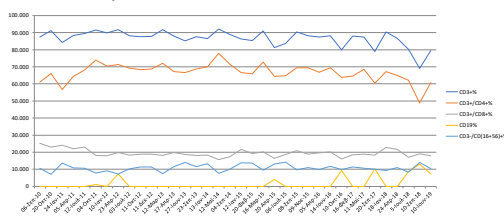
B-cell and immunoglobulin G (IgG) levels before and during rituximab treatment in multiple sclerosis cases



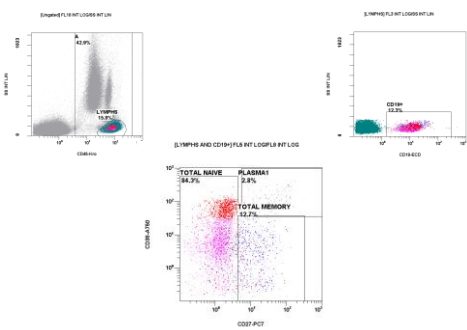
Jonathan Salzer et al
Neurology® 2016

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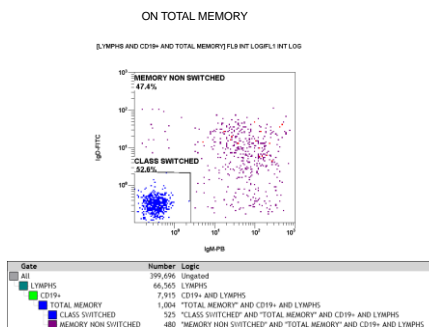
MS patient under Rituximab treatment follow-up



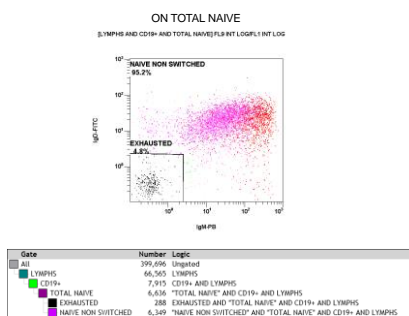
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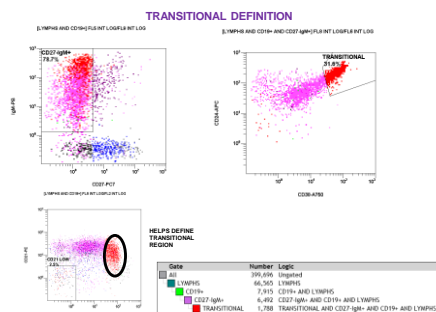
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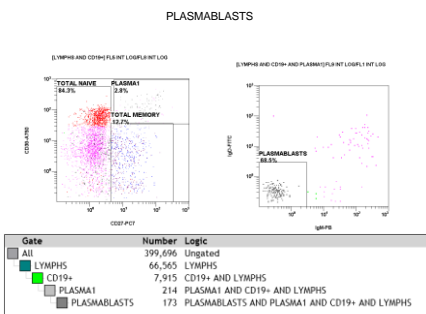
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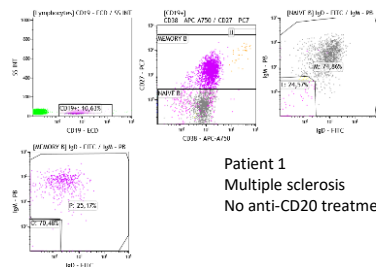
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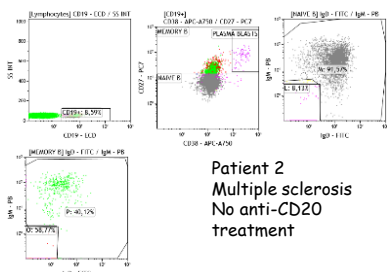


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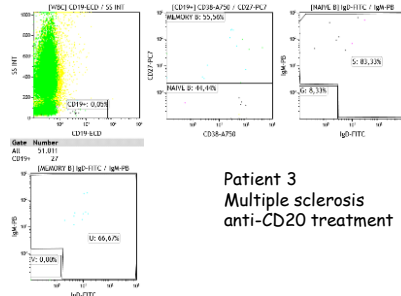
Patient 1
Multiple sclerosis
No anti-CD20 treatment

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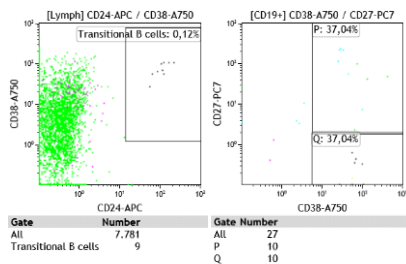
Patient 2
Multiple sclerosis
No anti-CD20
treatment

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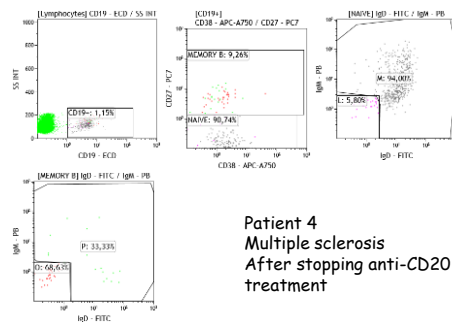


Patient 3
Multiple sclerosis
anti-CD20
treatment

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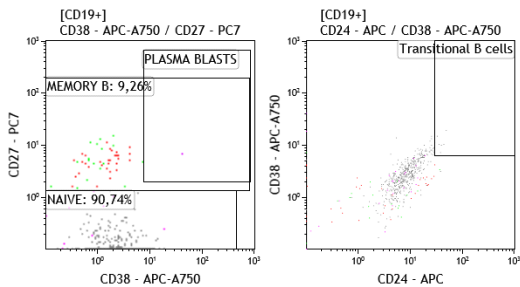


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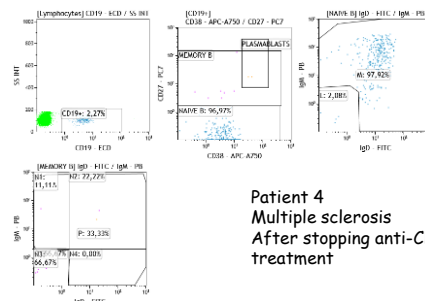


Patient 4
Multiple sclerosis
After stopping anti-CD20
treatment

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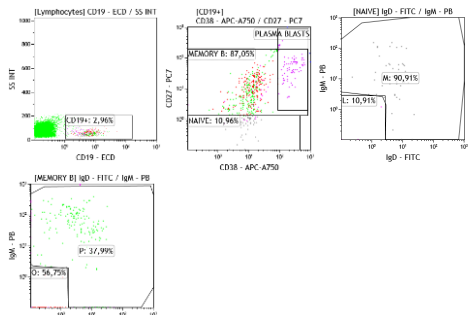


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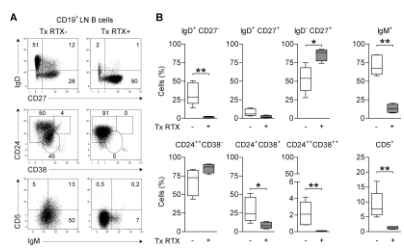
Patient 4
Multiple sclerosis
After stopping anti-CD20
treatment

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Phenotypic and functional characterization of lymph node (LN) B cells after a single Rituximab dose (no B cells in PB)



E. G. Kamburova et al American Journal of Transplantation 2013.

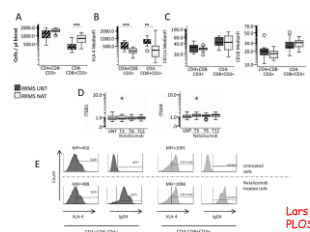
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Examples of monoclonal antibodies

- **Natalizumab for MS**
- humanized IgG4 mAb
- directed against the $\alpha 4$ subunit of the $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins
- prevents migration of leukocytes through the blood-brain barrier
- modulating leukocyte recruitment and activation in the CNS
- Increase of pre-B cells in PB (Krumbholz et al., 2008; Saraste et al., 2016)
- **Approved 2004 / suspended 2005 (PML)** by infection of oligodendrocytes by the John Cunningham Virus (JCV) / **approved 2006 FDA, EU under follow-up**

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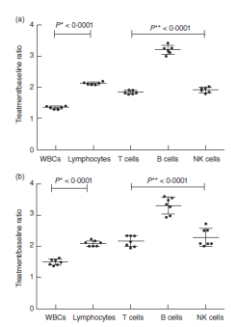
Natalizumab treatment selectively increased the effector memory T-cell pool but not the activation state of T-cells in the blood



Lars Bomsen et al PLOS one 2012

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Long term follow up of PB lymphocyte subsets after Natalizumab treatment



T. Kudriavtseva et al, Clin and Exper Immunology 2013

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Examples of monoclonal antibodies

- **alemtuzumab for MS**
- humanized IgG1 mAb
- directed against CD52
- rapid and profound depletion of CD52+ cells by three mechanisms:
- antibody dependent
- cell-mediated cytotoxicity (ADCC),
- complement dependent cytotoxicity (CDC) and
- induction of apoptosis (Freedman et al., 2013; Ruck et al., 2015), with ADCC being the most likely predominant mechanism (Knier et al., 2014; Lycke, 2015).
- This is by repopulation of peripheral T- and B-lymphocytes with an alteration in the number, proportions and functions of certain lymphocyte subsets, such as increased regulatory T-cell subsets and memory T-cells (Hartung et al., 2015; Milo, 2016).
- predominance of immature and, later, naive-memory B-cell subsets
- **Approved 2013, 2014**

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CASE REPORT Open Access

A case of Alectuzumab-induced neutropenia in multiple sclerosis in association with the expansion of large granular lymphocytes

A.G. Vekrellis^{1,2}, D. Tsanetaki¹, S. Valami¹, E. Gogosou², K. Papani¹, J. Triantafyllou¹, M. Anagnostou¹, S. Andrekou¹, M. P. Frangopoulou¹, G. Kousta¹, C. Chrysothanos¹, F. Galafos¹, A. Dimitrakopoulou¹, I. Sefiraki¹ and C. Koidris^{1*}

Background: Alectuzumab has been demonstrated to reduce the risk of relapse and accumulation of sustained disability in Multiple Sclerosis (MS) patients compared to interferon-β acting agent (IFNβ), leading to mainly to lymphopenia. Recent data have shown that mild neutropenia is observed in 14% of treated MS-patients whereas severe neutropenia occurred in 0.8%.

Case presentation: Herein we present the case of a 34-year-old woman with relapsing-remitting MS, with a history of treatment with glatiramer acetate and natalizumab, who subsequently received Alectuzumab (12 mg / 24 h x 5 days). Shortly after the last Alectuzumab administration, the patient displayed neutropenia (50k neutrophils/μl) with virtual absence of B-cells (B16 of total lymphocytes), low values of CD4+T-cells (8.8%) and predominance of CD8+T-cells (44%) and NK-cells (48%), while large granular lymphocytes (LGL) demonstrated in the blood smear examination. Due to prolonged neutropenia (5 days) the patient was placed on low-dose corticosteroids leading to sustained remission.

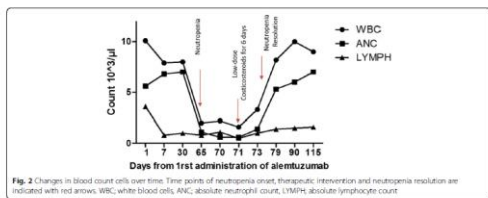
Conclusion: This is the first case of a patient with relapsing-remitting MS with neutropenia two months out-

Table 1 Studies showing the occurrence and disappearance of neutropenia following interferon-β treatment. Based findings in MS patients

Study	Number of patients	CD4+ T-cells	CD8+ T-cells	NK-cells	Neutrophils	Disappearance of neutropenia	Notes
Chen et al. (2012)	1752	52.8%	34.1%	13.1%	1.0%	100%	Case from MS patients treated with IFNβ
Miller et al. (2016)	1712	18%	34%	34%	14%	100%	Case from MS patients treated with IFNβ
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Miller et al. (2019)	1712	18%	34%	34%	14%	100%	Case from MS patients treated with IFNβ
Miller et al. (2020)	1712	18%	34%	34%	14%	100%	Case from MS patients treated with IFNβ
Miller et al. (2021)	1712	18%	34%	34%	14%	100%	Case from MS patients treated with IFNβ
Miller et al. (2022)	1712	18%	34%	34%	14%	100%	Case from MS patients treated with IFNβ
Miller et al. (2023)	1712	18%	34%	34%	14%	100%	Case from MS patients treated with IFNβ
Miller et al. (2024)	1712	18%	34%	34%	14%	100%	Case from MS patients treated with IFNβ
Miller et al. (2025)	1712	18%	34%	34%	14%	100%	Case from MS patients treated with IFNβ

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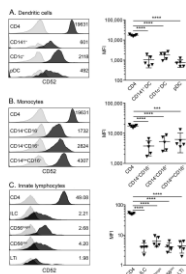
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Neutropenia with

- CD3-CD(16 + 56+): 47%
- CD3 + CD8+ T cells increased compared to baseline
- No B cells, very low CD3+CD4+ cells
- On 70th day 500/μl ANC, treatment discontinued
- Neutropenia resolved
- LGL (50% decrease) but % of NK cells high (48%), CD3+CD8+ % normal
- Immune derived neutropenia due to the MoAb treatment
- MS remission

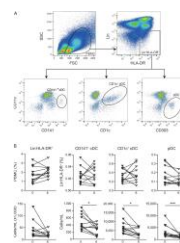
CD52 expression on innate lymphoid and myeloid cells



Catharina C. Gross et al. *Neuro Immunol Neuroinflamm* 2016

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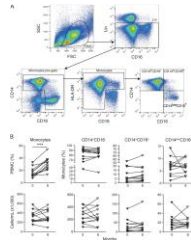
Alectuzumab-induced changes in the dendritic cell compartment



Catharina C. Gross et al. *Neuro Immunol Neuroinflamm* 2016

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Alemtuzumab-induced changes in monocytes

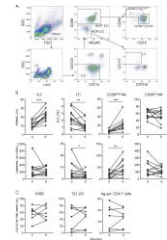


Catharina C. Gross et al
Neurol Neuroimmunol
Neuroinflamm 2016

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Alemtuzumab-induced changes in the innate lymphoid cell (ILC) compartment

6 months after alemtuzumab treatment, specific DC subsets are reduced, while CD56bright NK cells expanded in patients with MS



Catharina C. Gross et al
Neurol Neuroimmunol
Neuroinflamm 2016

Could it lead to autoimmunity?

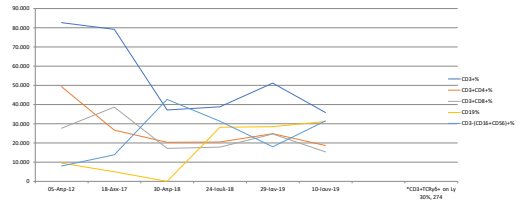
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Examples of monoclonal antibodies

- Daclizumab for MS
- humanized IgG1 mAb
- directed against CD25a
- expansion of immunoregulatory CD56 bright natural killer (NK) cells, which can utilize IL-2 via their low-affinity IL-2 receptor (Knierr et al., 2014).
- Approved 2014 FDA, EU

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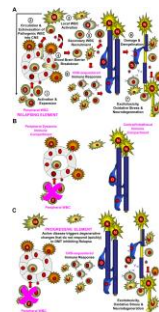
MS patient follow up



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Memory B Cells are Major Targets for Effective Immunotherapy in Relapsing Multiple Sclerosis

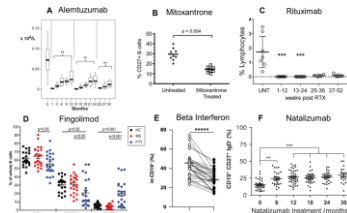
Therapies targeting CD4+ cells seem to decrease memory B cells as well.



David Baker et al
EBioMedicine 2017

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Active DMD in MS physically or functionally deplete memory B cell activity.



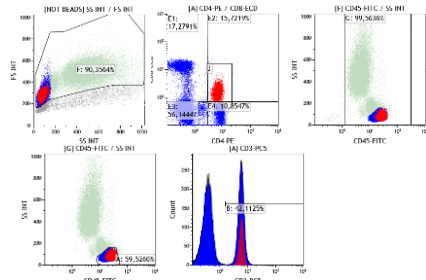
David Baker et al
EBioMedicine 2017

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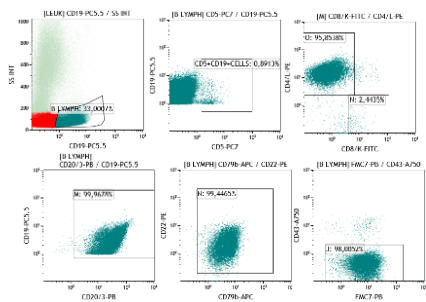
Other populations to be studied

- Th17 cells
- Tregs
- Proinflammatory CD20+ T cells
- Myeloid and DCs

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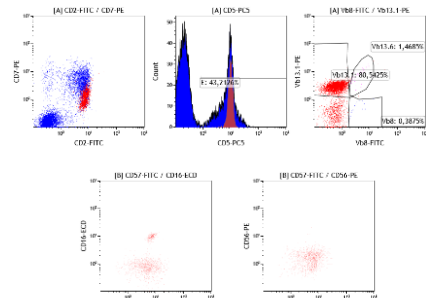


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Examples of monoclonal antibodies

- Humira TNF blocker
- Associated with lymphomas

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Many thanks to

- Serafeim Karathanos



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