

# Viral- and myelin-specific cellular immune response in MS patients treated with natalizumab: a cross-sectional and a longitudinal study

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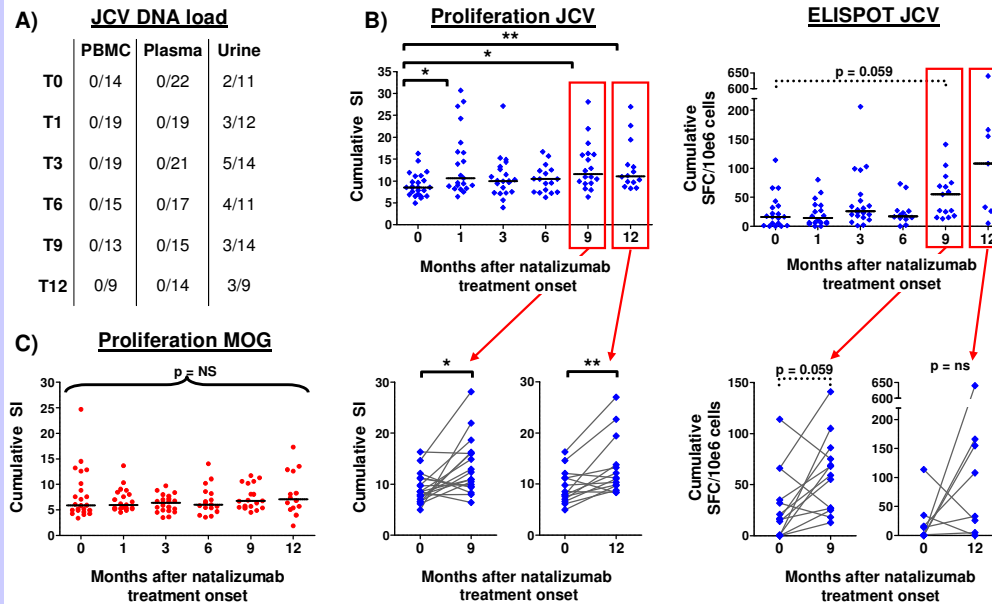
## INTRODUCTION:

Natalizumab (Tysabri®) is a monoclonal antibody which binds to the  $\alpha 4$  integrins, molecules that are expressed on the surface of activated lymphocytes and allow them to cross the blood-brain barrier. By preventing the rolling and diapedesis of CNS auto-antigen activated lymphocytes, this medication has been shown to be highly efficient in decreasing the relapses rate and the progression of disability in multiple sclerosis (MS) patients (1,2). However, since its approval, eight MS patients treated with natalizumab suffered from progressive multifocal leukoencephalopathy (PML), on a total of approximately 57'000 treated patients (as of end of May 09).

PML is a severe demyelinating disease of the central nervous system. The polyomavirus JC (JCV) infects 60-85% of the normal adult population (3-4), and its reactivation in the setting of immuno-suppression leads to a lytic infection of oligodendrocytes (3). Yet, since the blood cell counts are not decreased and since it does not seem to be associated with other opportunistic infections, natalizumab cannot be considered as a classical immunosuppressant. Therefore, other mechanisms have to be looked for.

In this ongoing cross-sectional and longitudinal study, we are examining the JCV activity in the peripheral blood of Tysabri-treated patients over a one-year period. Using RT-PCR specific for JCV, we are determining their JCV DNA load in different compartments. At the same time-points, we are assessing the cellular immune response against JCV as well as against other viruses used as controls (EBV and CMV) and against the myelin oligodendrocyte glycoprotein (MOG), a recognized target in the CNS. We compare these responses with a cohort of patients treated with IFN- $\beta$ .

## Increased proliferation and IFN- $\gamma$ secretion of JCV-specific T cells in the blood of MS patients on natalizumab



**JCV DNA quantification.** JCV DNA load stays undetectable in PBMC and plasma of patients undergoing natalizumab therapy. Detectable JCV DNA is found in urines of some patients, but does not correlate with the JCV-specific immune responses (A).

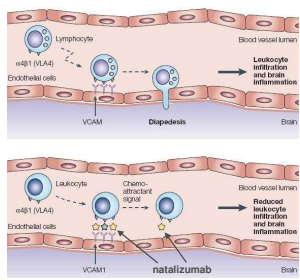
**Cellular immune response against JCV.** The number of JCV VP1-responding MS patients in proliferation assays increases nine months after natalizumab onset, from 25% of the MS patients before natalizumab onset up to 80% of the MS patients, and stays stable up to one year (data not shown).

The proliferation of the JCV VP1-specific T cells is increased, already one month after onset of the treatment (B; upper left panel) and at nine and twelve month (B; upper and lower left panels). Secretion of IFN- $\gamma$  by JCV VP1-specific T cells is increased at nine months after the onset of natalizumab treatment (B; upper right and lower right panels).

**Cellular immune response against myelin antigen and control virus.** Myelin oligodendrocyte glycoprotein (MOG)-specific T cells are found in about 22% of all MS patients on natalizumab without significant change over time (proliferation assays; data not shown). The MOG-specific T cell proliferation is similar through the period of observation (C). Similarly, there are no change in the EBV- or CMV-specific T cell proliferation or IFN- $\gamma$  secretion from through the study period (data not shown).

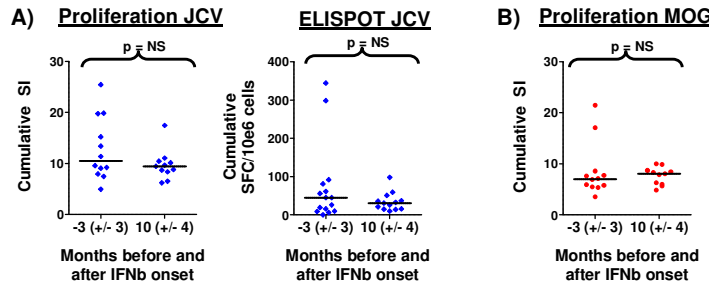
We enrolled 24 MS patients receiving monthly injections of natalizumab. So far, 14/24 patients have reached 12 months of natalizumab treatment (T12). Urine and plasma were collected and frozen until further use. PBMC were isolated and immediately processed. JCV viral load was determined by quantitative PCR as described previously (5). Proliferation and IFN- $\gamma$  secretion of antigen-specific T cells were assessed in PBMC of MS Tysabri-treated patients at different time-points after onset of treatment (up to one year). PBMC were stimulated with i) 15-mer peptides overlapping by 10 aa and encompassing the VP1 protein of JCV, ii) similar overlapping 15-mer peptides for MOG protein, iii) EBV and CMV lysates - to elicit a CD4+ T cell responses - or a pool of nonamer peptides - to elicit a CD8+ T cell responses. Data are presented as cumulative data, where results for each pool of peptide (SI or SFC/10e6 cells) are added to each other (JCV, 9 pools; MOG, 6 pools). NS, non significant (Kruskal-Wallis ranked test); \*p<0.05, \*\*\*p<0.005 (Wilcoxon paired ranked test).

## Physiological steps in lymphocyte adhesion : natalizumab blocks the blood-brain barrier



Lymphocytes flowing in the blood vessels are induced via chemokines to slow down, roll along the blood vessel walls and finally arrest their movement and attach to the endothelium through a complex sequence of events. After attachment to the blood vessel wall via an interaction between  $\alpha 4 \beta 1$  integrin on the lymphoid cell and vascular cell adhesion molecule 1 (VCAM1) on the endothelial cell, the process of diapedesis begins. When  $\alpha 4 \beta 1$  integrin is blocked by the natalizumab, lymphocyte adhesion to the endothelial wall is disrupted as  $\alpha 4 \beta 1$  integrin VCAM1 interactions are impaired.

## Control patients receiving IFN- $\beta$ immunomodulatory treatment do not have increased JCV-specific T cell response



**Cellular immune response against JCV.** Some MS patients receiving IFN- $\beta$  exhibit a JCV-specific T cell response, however no difference is found between the two time-points either for proliferation assays or for the secretion of IFN- $\gamma$  (A).

**Cellular immune response against myelin antigen and control virus.** Similarly the proliferation of MOG-specific T cells does not differ between the two time-points (B). The same is true for EBV- and CMV-specific T cells proliferation and IFN- $\gamma$  secretion (data not shown).

Fifteen MS patients receiving IFN- $\beta$  were enrolled and PBMC obtained before and after IFN- $\beta$  treatment onset. Proliferation and IFN- $\gamma$  secretion of antigen-specific T cells were assessed in PBMC using exactly the same conditions as described in the legend of the above figure (natalizumab-treated patients). Data are presented as cumulative data, where results (SI or SFC/10e6 cells) for each pool of peptide were cumulated (JCV, 9 pools; MOG, 6 pools). SFC, spot forming cell; NS, non significant (Wilcoxon paired ranked test).

## CONCLUSION:

- There is no increase of the JCV viral load in MS patients on natalizumab over time. These data confirm those from others (6).
- However, there is an enhancement of the JCV-specific cellular immune response after 9-12 months of treatment, as assessed by proliferation and ELISPOT assays. By contrast, the responses against the control viruses (EBV and CMV) or against the myelin antigen MOG remain unchanged.
- Contrasting with patients on natalizumab, there is no increase of the JCV-specific cellular immune response in patients on IFN- $\beta$ .
- Altogether, these results suggest that natalizumab may enhance the polyomavirus-specific cellular immune response in the peripheral blood.

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## ACKNOWLEDGEMENTS:

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