# Best MS general summary

## Objective

The best-MS project (a better escalation therapy in Multiple sclerosis) objective was to determine the best composite criteria of response to Natalizumab (NTZ) during the first year of treatment that best predict disease activity during the second and third years of treatment with NTZ and serious adverse event.

## Material and methods

We collected 1500 NTZ treated patients stratified as responders and non-responders in a prospective cohort that gathered clinical radiological and biological multi-samples. Thirty patients developed a progressive multifocal leukoencephalopathy (PML) including more than 50 PML samples a unique sample collection worldwide. We applied innovative technology such as metabolomics, genomics, diverse cellular assays, and metagenomics (that explore the microbiome).

## Results

At the time of the final report we were able to patent a prediction test for PML using CD62L. This test is more effective than biological markers used so far for PML prediction since we need to stop NTZ in 10% of the patients in order to prevent 85% of PML. As a comparison with the index value 30% of patients need to stop NTZ in order to avoid 80% of the PML.
Further biological markers are now in the patent process such as MMP9, and NFK beta pathway
Regarding NTZ response prediction we found that genes in the GABA receptors family are associated with non-response to NTZ.

## Conclusion and perspective

Our project is an important step for precision medicine since we allow monitoring at the individual level the risk of developing PML. Beyond this result, our project is a paradigm for precision medicine in neurodegenerative disease, oncology internal medicine and HIV since all of them use biology therapy or develop PML

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| | |
|---|---|
| **CHUT cohort 1** | 1212 |
| **CHUT cohort 2** | 540 |
| **Biodonostia (Spain)** | 43 |
| **VHIR** | 44 |
| **Basel (Switzerland)** | 50 |
| **UKMuenster** | 95 |

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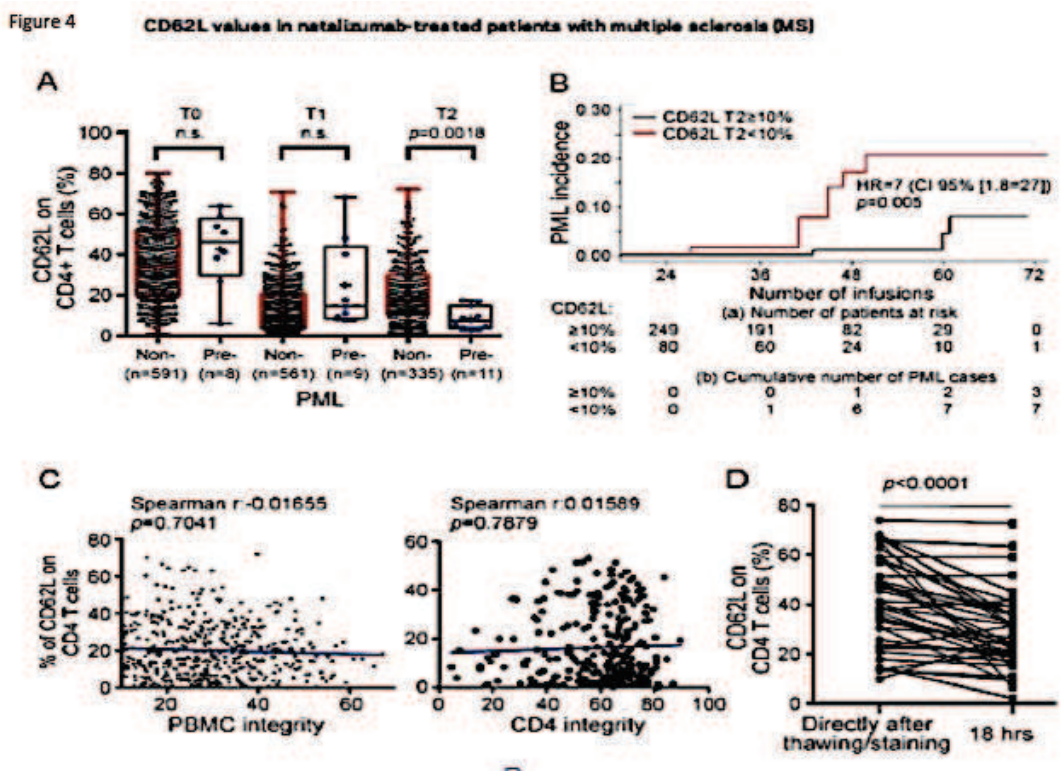



Figure 1 CD62L and PML prediction

CD62L values of CD4+ T cells were determined on frozen/thawed peripheral blood mononuclear cells (PBMC) as previously described.[1] (A) CD62L values evaluated in the Biomarkers and Response to Natalizumab for Multiple Sclerosis Treatment (BIONAT) cohort for non-progressive multifocal leukoencephalopathy (non-PML) patients vs patients who would later develop PML (pre-PML). At the initiation of the natalizumab (NTZ) treatment (T0), after 1 year of treatment (T1), and 2 years of treatment (T2) (n = 11; median [interquartile range] % CD62L = 7.2% [4.2; 12.2]) as compared to non-pre-PML patients (n = 335; median % CD62L = 16.8% [10.5; 28]; $p = 0.0018$). n.s. = not significant; Mann-Whitney test. (B) Kaplan-Meier analysis of time to PML stratified by 2 years at threshold of 10%. Number of PML cases after 72 infusions: CD62L ≥10% = 0, CD62L <10% = 1. (a) The number at risk is the number of participants who were still in the study and did not have the event at the end of the specified time. (b) Cumulative number of PML cases at the end of the specified time. CI = confidence interval; HR = hazard ratio. (C) Correlation between cellular integrity on either PBMC (n = 533) or CD4+ T cells (n = 290), assessed by forward scatter/side scatter gating strategy and % of CD62L. (D) Comparison of CD62L levels after assessment directly after thawing and staining, without PFA fixation, or after fixation, and 18 hours of storage before fluorescence-activated cell sorting analysis (paired t test). (E) A total of 358 NTZ-treated patients with MS were included in a prospective cohort to longitudinally follow their CD62L values on CD4+ T cells. The 358 patients presented at least one measure of CD62L.

## Conclusion and perspective

Our project is an important step for precision medicine since we allow monitoring at the individual level the risk of developing PML. Beyond this result, our project is a paradigm for precision medicine in neurodegenerative disease, oncology internal medicine and HIV since all of them use biology therapy or develop PML

## WP1 - Establishment of a database with 1500 MS patients

- Objectives

To establish a database with clinical, radiological and biological information based on 1500 patients and made available to all Best-MS participants through the internet.

TABLE 1 : Patients' origins that should be included in the database.

| | |
|---|---|
| **CHUT** | 1212 |
| **CHUT** | 540 |
| **Biodonostia (Spain)** | 43 |
| **VHIR** | 44 |
| **Basel (Switzerland)** | 50 |
| **UKMuenster** | 95 |

- CNIL consent:

CNIL (the French Regulatory Agency for information technology and liberty: commission nationale informatique et liberté) authorization for this database has been obtained by **November 2015**

- Web access and database sharing:

The BIONAT data has been shared between all Best-MS partners via the Renater website using FileSender. Indeed, Renater proposes a rich and hosted solution via the FileSender free software. This solution permits to share heavy weight (several Go) documents in a safe manner.

- Conclusion

*this database allows to share all data generated by the consiortium. As we will see we probably work during the next 4 to 5 years with the raw data generated. New patents may be asked. This is why so far the data are not publicaly available. Nevertheless at one point we may give open the database online to anybody that would be interested to work with.*

**WP2 – Defining treatment response to NTZ**

- Background and objective:

In patients with RRMS, different score systems based on new MR active lesions, relapses, and sustained disability progression after one-year treatment with IFNβ have been used to predict disability progression over time. These scores, however, have not been tested in patients receiving natalizumab (NTZ). Thus, the objective of this study is to evaluate whether previous scores can also be applied to patients treated with NTZ.

- Methods:

This is a prospective and longitudinal study of 4293 patients treated with NTZ. Patients were classified based on the presence of new lesions on MRI, relapses, confirmed disability increase or combinations of all these variables after one year of therapy. Regression analysis was performed in order to identify variables of response.

- Results:

A prediction model analysis showed that Rio score ≥1 and or any evidence of disease activity (EDA) during the first year of treatment with NTZ predict new clinical activity during the follow-up.

- Conclusions:

The presence of any Evidence of disease activity in patients treated with NTZ identifies those patients with a higher risk to develop new clinical activity and disability progression in the ensuing years. Thus, we need to consider alternative therapies in this group of patients.

- Remarks: A paper will be submitted by the beginning of January with the following authors: J Rio, M Comabella, S OteroB Pignolet, ,OOutteryck, , JC Ongagna, Jde Sèze, B Brochet, JCOuallet, M Debouverie, S Pittion, G Defer, N Derache, P Hautecoeur,, ATourbah, P Labauge, G Castelnovo, P Clavelou, EBerger, J Pelletier,A Rico, H Zéphir, D Laplaud, S Wiertlewski, W Camu, E Thouvenot, O Casez, TMoreau, AFromont,,C Papeix, M Cohen, D Biotti, R Bernard-Valnet, F Bucciarelli, L Scandella, F Umuhoza, J Ciron, P

Vermersch,C Lebrun-Frenay, S Vukusic, and D Brassat,on behalf of OFSEP, BIONAT, SFSEP and BEST-MS networks

Table 2: Risk of activity during the period of follow-up (month 12 to 36) according to positivity for different variables after 12 months of therapy

| | Odds ratio (CI) | Significance |
|---|---|---|
| RS =1 | 1.9 (1.4– 2.8) | <0.001 |
| RS>1 | 1.8 (0,96 – 3.6) | 0.07 |
| EDA | 2.7 (1.4 – 5.1) | 0.003 |

MRI: Magnetic resonance imaging, RS: Rio's Score, EDA: any evidence of disease activity

**WP3 – A Phase IV, multi-centre, open-label trial intended to test the difference in efficacy of Natalizumab versus Fingolimod, 2 medicinal products with marketing authorisation for the treatment of multiple sclerosis.**

- Inclusion update and patients needed re-calculation

Originally we estimated that 600 patients will be necessary for demonstrating a significant natalizumab superiority. In a second step, based on a recent retrospective study (Laplaud et al neurology 2015) where 326 patients have been treated by NTZ and 303 by FGL, we showed that

- Probability for a relapse during the first year was higher in the FGL group vs NTZ (27.1% vs 21.2%, relative risk of 1.3)
- probability for a new active MRI lesionishigher in the FGL group 21% vs 8.2%, (relative risk of 2.6).

With these new data we re-calculated how many patients should be included for:

- a relative risk of 1.8 (instead of 1.5 considered in the first step).
- 60% of the patients with a positive JC virus serology.
- A 1 year follow-up
- We included 5 to 15% treatment choice variability (not related to JC virus)
- fingolimod arm with no disease activity between 40% and 50%
- 5% alpha risk and 80% power
- Calculation with the PASS 11 software

and found that a number of 183 to 255 patients overall should be sufficient to demonstrate the previous points.

A significant delay in recruiting patients has been observed and was the main reason for asking the EU a fourth year extension of the project. This was accepted. Further since the last patient was included by September 2016, our scientific officer accepted that the WP3 final report will consist in a demographic description of the patients at baseline

As a result, 223 patients have been included.

Table 3: 223 inclusions by september 2016.

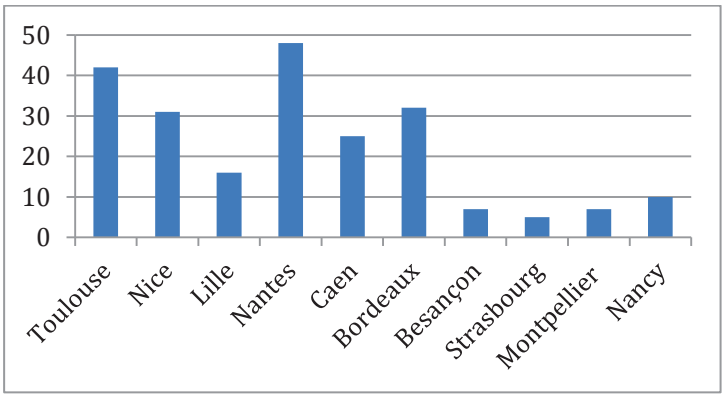
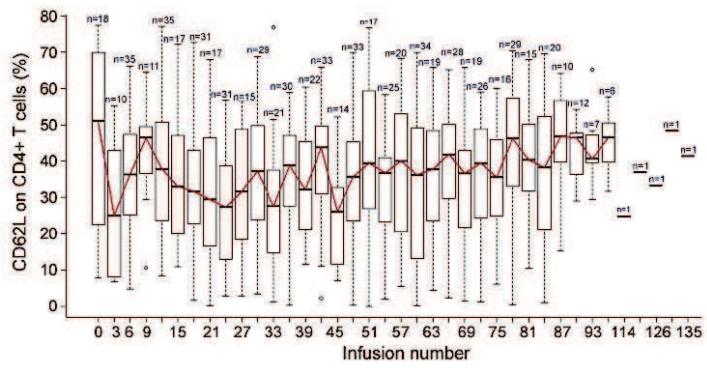


Table 4 : Demographic Data at WP3 baseline based on the first 127 patients

| | NTZ | FGL | Total population |
|---|---|---|---|
| **Number** | **60** | **67** | **127** |
| **Age (in years)** | | | |
| **Mean (SD)** | **34.75 (8.49)** | **39.07 (9.32)** | **37.03 (9.16)** |
| **Median [min ; max]** | **34.5 [18 ; 54]** | **39 [18 ; 63]** | **37 [18 ; 63]** |
| **Sex** | | | |
| **Female (%)** | **48 (80%)** | **51 (76.12%)** | **99 (77.95%)** |
| **Duration of disease** | | | |
| **Mean (SD)** | **4.77 (5.11)** | **7.51 (6.90)** | **6.22 (6.25)** |
| **Median [min ; max]** | **2.5 [0 ; 19]** | **5 [0 ; 32]** | **4 [0 ; 32]** |
| **EDSS score** | | | |
| **Mean (SD)** | **2.58 (1.23)** | **2.41 (1.43)** | **2.49 (1.34)** |
| **Median [min ; max]** | **2.5 [0 ; 5.5]** | **2.5 [0 ; 5.5]** | **2.5 [0 ; 5.5]** |
| **Brain MRI** | | | |
| **Patients with gadolinium-enhanced lesion** | **32 (53.33%)** | **30 (44.78%)** | **62 (40.82%)** |
| | | | |
| **Patients receiving immunostimulants** | **37 (66.67%)** | **50 (76.73%)** | **87 (68.50%)** |
| **Number receiving immunostimulants (ATC L03A)** | | | |
| **Mean (SD)** | **0.93 (0.94)** | **1.13 (0.92)** | **1.03 (9.27)** |
| **Median [min ; max]** | **1 [0 ; 3]** | **1 [0 ; 3]** | **1 [0 ; 3]** |
| | | | |
| **Patients receiving immunosuppressants** | **4 (6.67%)** | **4 (5.97%)** | **8 (6.30%)** |
| **Number receiving immunosuppressants (ATC L04A)** | | | |
| **Mean (SD)** | **N/A** | **N/A** | **0.08 (0.30)** |
| **Median [min ; max]** | **N/A** | **N/A** | **0 [0 ; 2]** |

- WP3.2: CD62L cohort: *ancillary study:*

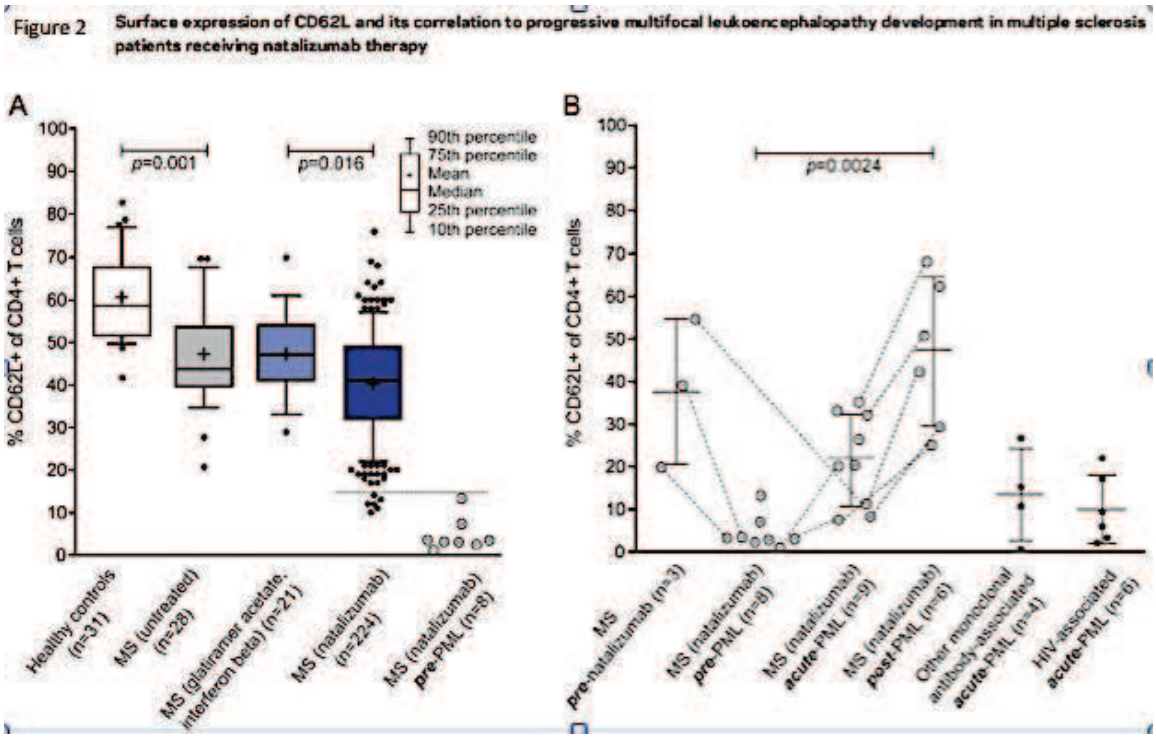
Results from these ancillary study has been included in the following paper published
The aim of this ancillary study is to confirm in a prospective study the role of CD62L in a new patient's cohort. See WP4
Figure 1: CD62L prospective follow up



## WP4 – Biological treatment response definition and PML risk prediction

- PML risk prediction marker

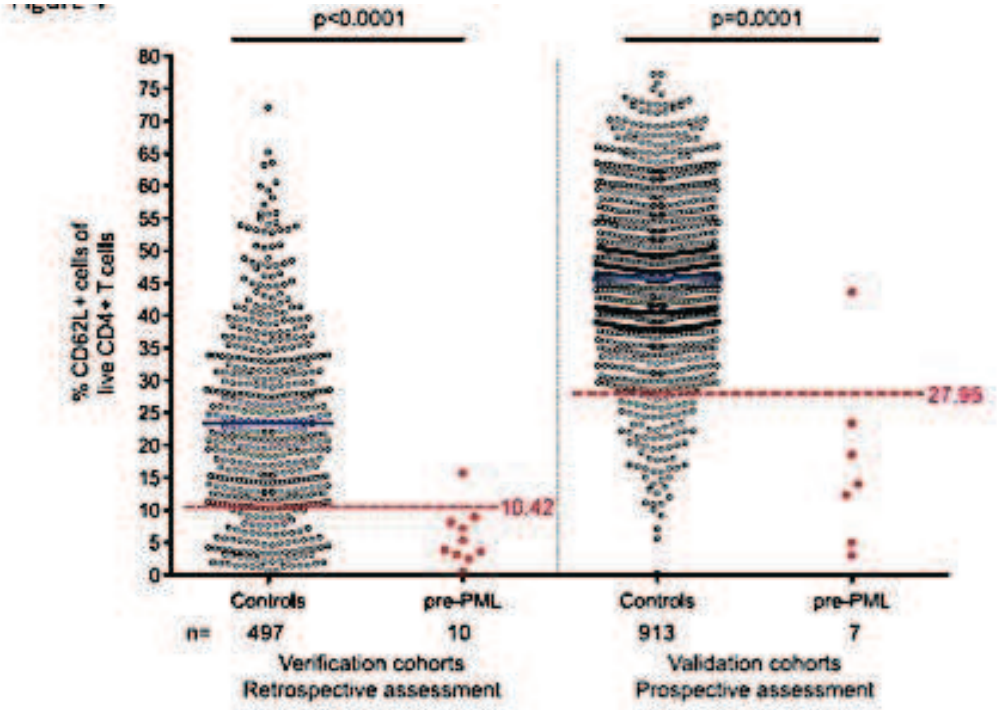
A first study had for Objective: To find biomarkers identifying patients at risk for the development of progressive multifocal leukoencephalopathy (PML) during natalizumab treatment. Methods: Patients were recruited from 10 European and US cohorts. Of 289 patients with multiple sclerosis (MS), 224 had been treated with natalizumab (18–80 months), 21 received other immune-modulatory treatments, and 28 were untreated. We had access to samples from 16 natalizumab PML patients. Eight of these patients had given blood before the diagnosis of PML. We also analyzed non-natalizumab-treated patients who developed PML (n 5 10) and ageand sex-matched healthy donors (n 5 31). All flow cytometric assessments were done on previously cryopreserved, viable peripheral blood mononuclear cells. Results: The percentage of L-selectin-expressing CD41 T cells was significantly lower in patients treated long-term with natalizumab (40.2%) when compared with patients not receiving natalizumab treatment (47.2%; p 5 0.016) or healthy controls (61.0%; p , 0.0001). An unusually low percentage (9-fold lower; 4.6%) was highly correlated with the risk of developing PML in the patient group with available pre-PML samples when compared with non-PML natalizumab-treated patients (p #0.0001). Samples were gathered between 4 and 26 months before PML diagnosis.Conclusions: The cell-based assessment of the percentage of L-selectin-expressing CD4 T cells could provide an urgently needed biomarker for individual PML risk assessment.




A second study had the following Objective: This study aimed at verifying and integrating both

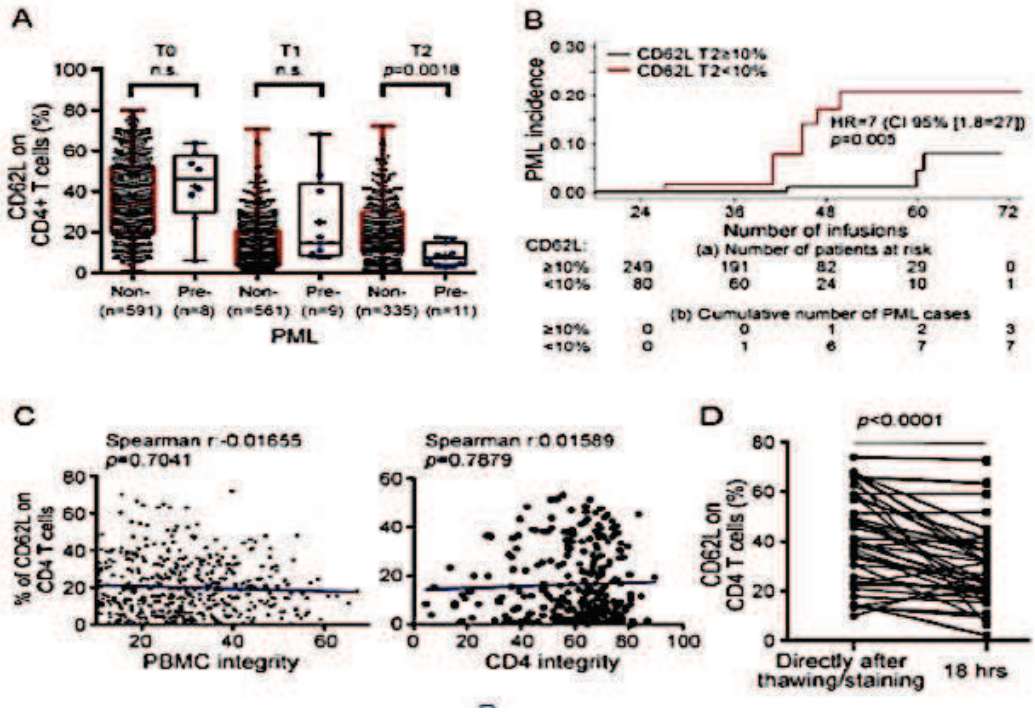
parameters into one algorithm for risk stratification. Methods: Multicentric, international cohorts of natalizumab-treated MS patients were assessed for JCV Index (1921 control patients and nine pre-PML patients) and CD62L (1410 control patients and 17 pre-PML patients).Results: CD62L values correlate with JCV serostatus, as well as JCV index values. Low CD62L in natalizumab-treated patients was confirmed and validated as a biomarker for PML risk with the risk factor “CD62L low” increasing a patient’s relative risk 55-fold ($p < 0.0001$). Validation efforts established 86% sensitivity/91% specificity for CD62L and 100% sensitivity/59% specificity for JCV index as predictors of PML. Using both parameters identified 1.9% of natalizumab-treated patients in the reference center as the risk group. Conclusions: Both JCV index and CD62L have merit for risk stratification and share a potential biological relationship with implications for general PML etiology. A risk algorithm incorporating both biomarkers could strongly reduce PML incidence.

Figure 3 :




A third study provide new data from the Biomarkers and Response to Natalizumab for Multiple Sclerosis Treatment (BIONAT) and Difference in Efficacy of Natalizumab Versus Fingolimod for the Treatment of Multiple Sclerosis (BEST-MS) cohorts (1,220 and 358 NTZ-treated patients, respectively. The determination of CD62L levels at 2 years of NTZ treatment was a reliable marker of risk of PML, diagnosed up to 35 infusions later (sensitivity 80%, specificity 87%, hazard ratio 7). We are now performing a prospective study (BEST-MS) in highrisk patients (JCV index >0.9 and more than 18 months NTZ) in order to replicate the results found in the retrospectively analyzed BIONAT cohort and to determine optimal thresholds.

Figure 4 **CD62L values in natalizumab-treated patients with multiple sclerosis (MS)**




- Biomarkers of Response to NTZ treatment:

**1. Sodium Intake and disease severity (response to treatment):**

**Background:** The association of Sodium Intake (SI) with multiple sclerosis (MS) is controversial. Evidence from preclinical studies suggested a link between high SI and disease severity through salt-sensing Th17 or a higher blood-brain-barrier permeability in genetically susceptible animals. In one adult-onset MS cohort, this link was confirmed at the clinical and radiological level. On the other hand, two studies in paediatric MS found no association with MS onset or severity.

**Objectives:** To determine whether high SI is associated with MS severity in an adult-onset MS cohort (BIONAT) of 393 MS patients.

**Methods:** Spot urine samples from 393 MS patients of the BIONAT cohort were prospectively collected. SI was evaluated using the Tanaka formula. Disease severity was defined by the MSSS (the multiple sclerosis severity score) as the primary criterion. Secondary criteria for disease severity were brain MRI parameters and patients with no evidence of disease activity

(NEDA). Univariate and multivariate analyses were performed, together with an equivalence study, to determine whether sodium intake was associated with disease severity.

**Results:**We found no association (p=0.07) between SI and MSSS, MRI parameters (confluent lesions, p=0.775; more than 9 lesions, p=0.346; black holes, p=0.086; gadolinium enhancing lesion, p=0.151) or response to treatment (p=0.313).

**Conclusions:** In this study, SI was not associated with multiple sclerosis severity.

| Variable | ≤ 3.4 g/day | 3.4 - 4 g/day | > 4g/day | p |
|---|---|---|---|---|
| **Age** | N=127 | N=121 | N=132 | |
| Median (IQR) | 26 (21-30) | 28 (21-35) | 29 (24-35) | 0·02 |
| **Gender** | N=128 | N=123 | N=136 | |
| F, n(%) | 101 (78·91) | 85 (69·11) | 101 (74·26) | 0·208 |
| M, n(%) | 27 (21·09) | 38 (30·89) | 35 (25·74) | |
| **BMI** | N=127 | N=118 | N=133 | |
| Median (IQR) | 23 (20-25) | 23·5 (21-26) | 24 (21-29) | 0·013 |
| ≤ 25, n(%) | 98 (77·17) | 87 (73·73) | 81 (60·9) | 0·01 |
| > 25, n(%) | 29 (22·83) | 31 (26·27) | 52 (39·1) | |
| **Lymphocytes T0** | N=107 | N=105 | N=112 | |
| Median (IQR) | 1·9 (1·6-2·5) | 1·9 (1·4-2·5) | 1·9 (1·5-2·2) | 0·595 |
| **CD4 T0** | N=107 | N=105 | N=109 | |
| Median (IQR) | 896 (716·5-1186·5) | 905 (639-1248) | 948 (7486-1132) | 0·715 |
| **CD8 T0** | N=107 | N=104 | N=110 | |
| Median (IQR) | 401 (274-586) | 430·5 (277·2-593·2) | 400·5 (308·2-548·5) | 0·953 |
| **CD19 T0** | N=91 | N=95 | N=97 | |
| Median (IQR) | 271 (185-388·5) | 270 (198-415) | 255 (203-348) | 0·595 |

IQR: interquartile range; BMI: Body Mass Index.

**Table 5: Sodium intake, demographic and biological association.**

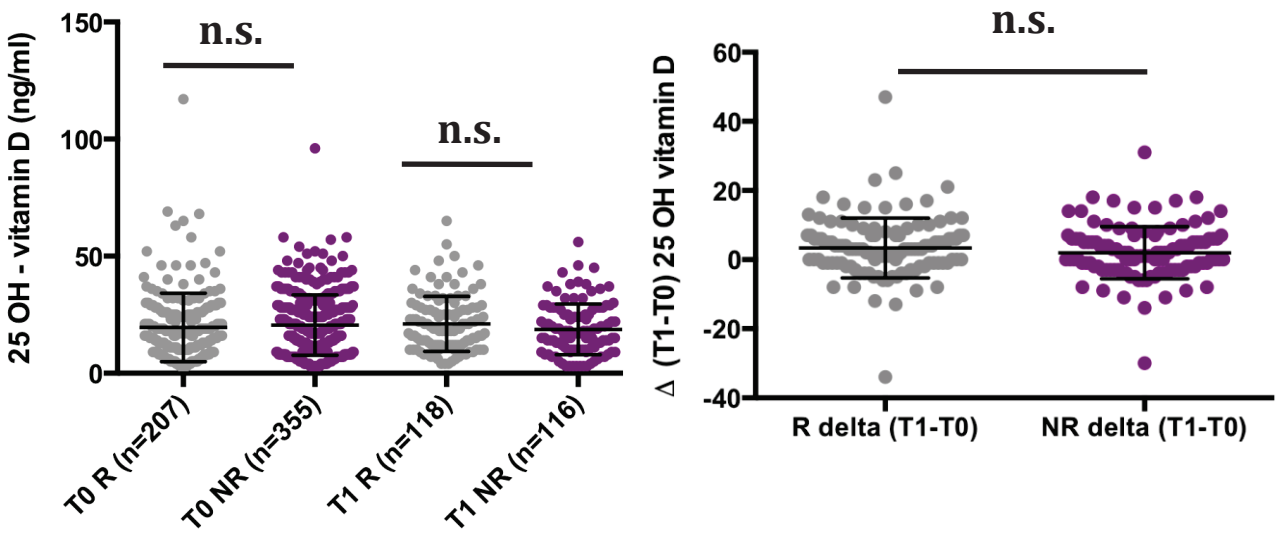
**2. Vitamin D:**

**Rational:**

Lower vitamin D level has also been associated with higher rates of MS relapse and higher MS-specific disease activity and disability (Mowry et al., 2010 and 2012). Circulating level of vitamin D, as measured by the level of 25-hydroxyvitamin D (25OHD, is the best clinical determinant of vitamin D. We raised the question whether or not vitamin D level could be correlated to NTZ response status at 2 years. To that aim we determined serum level of circulating 25OHD in NTZ patients and we will analyze its correlation with NTZ response status at 2 years of treatment.

25-OHD measurement has been finished by the end of October 2015 (Isabelle Gennero, IFB - CHUT)

**Figure 5 : Preliminary results**:




Vitamin D appeared to be not predictive of response treatment at T2

**3 IL-17 :**

In these study We investigate levels of IL-17 in serum and CSF in patients diagnosed at different stages of demyelinating diseases (RIS, CIS, relapsing remitting (RR) or activemultiple sclerosis patients:AMS) as a marker of inflammatory condition. Methods: 1417 sera has been tested for IL-17A (1177 from active MS, 80 RRMS, 35 RIS, 35 CIS, 10 IIH: idiopathic intracranial hypertension, and 80 controls) and 240 CSF from RIS, CIS, IIH and controls. Results: No difference has been found between RIS who early clinically converted and CIS patients who rapidly evolve in McDonald or clinically definite MS, nor active MS. No correlation was found with usual MRI or CSF criteria. Conclusion: Our results do not confirm that IL-17 can be considerate as a reliable marker of inflammation in the demyelinating spectrum disorders, either in blood or CSF.

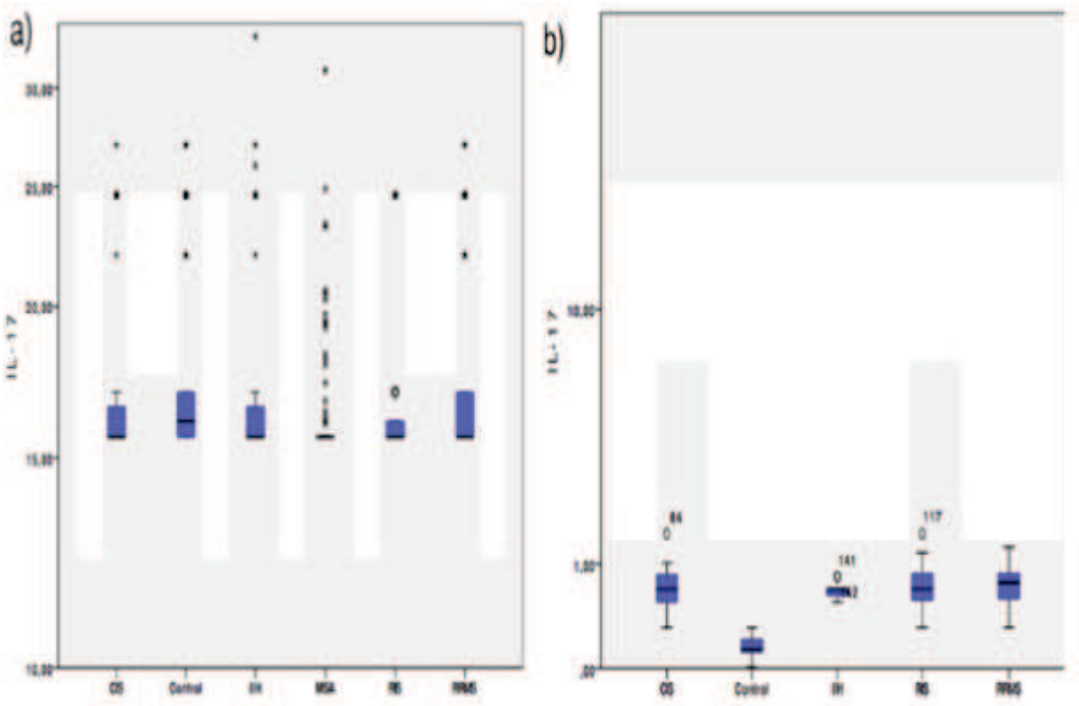



Figure 6 IL-17A (pg/ml) in sera and CSF in different patients status of demyelinating diseases, IIH and controls. RIS: radiologically isolated syndrome; CIS: clinically isola syndrome; KRMS: relapsing remitting multiple sclerosis; AMS: active multiple sclerosis; IIH: Idiopathic Intracranial Hypertension; controls.

## 4. Biomarkers of Fingolimod response

Since one of the main objective of the BEST-MS project relies on the constitution of a Fingolimod treated MS patients cohort, we would take advantage of this cohort and the related biobanked biological samples to determine Fingolimod response predictive markerssuch as determination of Fingolimod (FTY720 and its active phosphorylated derivate) quantity in plasma of treated patients and in depth immunophenotyping of blood cells using a new high promising technology (CYTOF). These tasks have been performed by Bertin-Pharma company but no results are available at the moment of the report

## WP5 – Genetic basis of NTZ efficacy in MS patients

### *5.1. Genome wide association study for natalizumab response*

- **Objectives:**

To determine if genetic variants are associated with response to treatment especially natalizumab and fingolimod

- Methods

Here we will present the results from the first Genome wide association study of 1200 natalizumab patients stratified as responders and non-responders to the drug. Response was defined as primary criteria as no evidence of disease activity based on the absence of relapse, EDSS evolution and new MRI lesions. A secondary criteria was designed based on a biological marker related to natalizumab target (the CD49d). The Analysis was performed with a gene by gene approach and pathway approach.

Results

The strongest association signal to treatment response was observed with a SNP (rs6784509, P=7.44x10$^{-7}$, OR=0.58, [95% CI=0.36-0.79]) located ~69kb downstream of the *Phospholipid Scramblase Family Member 5* (*PLSCR5*) gene. A second analysis was performed with a gene set approach. One gene set only was found significant after Bonferroni correction (REACTOME set "Gaba A Receptor Activation") and under both PC-Magma (P=4.64x10$^{-5}$) and SW-Magma (P=4.58x10$^{-5}$) models. The Self-contained test was also found significant under PC-Magma. The set includes 10 genes clustering on 3 chromosomes 4 (3 genes), 5 (4 genes) and 15 (3 genes).The 2$^{nd}$ most associated gene set ("Ion Channel Transport") was identified under both models and failed short the significance threshold under SW-Magma (P=7.29x10$^{-5}$). Nonetheless, it is worth noting that the results obtained from these sets of genes are not providing fully independent information. The overlap among them is indeed substantial (for instance, all genes in the set "Gaba A Receptor Activation" also belong to the other 2 top-ranked gene sets). Altogether, these results indicate that this set is enriched for genes associated to TR.

Conclusion

The gene set Gamma ammino butyric acid receptor is associated with non response to tysabri. This may be of interest for MS physicians by helping them to determine the which patients have the best benefit to risk ratio. A patent is ongoing that is a reason for delaying the paper publication

**Table 6: GSA results for TR and CD49d**

| Phenotype | Gene Model | Pathway Set | Set Name | #Genes | Comp-P | Self-P | Main genes in set |
|---|---|---|---|---|---|---|---|
| TR | PC-Magma | REACTOME | GABA A RECEPTOR ACTIVATION | 10 | **4.64E-05** | **1.34E-05** | GABRA4, GABRB1, GABRA2, GABRB2, GABRA6, GABRG2 |
| | | REACTOME | GABA RECEPTOR ACTIVATION | 50 | 6.32E-04 | 0.02 | |
| | | REACTOME | ION CHANNEL TRANSPORT | 48 | 1.28E-03 | 1.77E-04 | |
| | SW-Magma | | | | | | |
| | | REACTOME | GABA A RECEPTOR ACTIVATION | 10 | **4.58E-05** | 1.16E-04 | |
| | | REACTOME | ION CHANNEL TRANSPORT | 48 | 7.29E-05 | 1.55E-04 | |
| | | REACTOME | APOPTOTIC EXECUTION PHASE | 48 | 3.38E-04 | 0.01 | |
| | | REACTOME | APOPTOTIC CLEAVAGE OF CELLULAR PROTEINS | 34 | 6.49E-04 | 0.02 | |
| CD49d | PC-Magma | REACTOME | ACTIVATION OF IRF3 IRF7 MEDIATED BY TBK1 IKK EPSILON | 13 | 3.81E-04 | 0.02 | |
| | | REACTOME | DEPOSITION OF NEW CENPA CONTAINING NUCLEOSOMES AT THE CENTROMERE | 60 | 7.23E-04 | 0.06 | |
| | | REACTOME | P75 NTR RECEPTOR MEDIATED SIGNALLING | 71 | 8.88E-04 | 0.05 | |
| | SW-Magma | KEGG | PENTOSE AND GLUCURONATE INTERCONVERSIONS | 28 | 2.01E-04 | 4.37E-03 | |
| | | KEGG | STARCH AND SUCROSE METABOLISM | 52 | 4.57E-04 | 9.71E-04 | |

Table 6 Gene set results for genes associated with response to natalizumab
TR : treatment response

### *5.2. Metagenomic: Microbiome, MS and response to NTZ*

Background

The microbiome has been implicated in different autoimmune diseases including MS. Natalizumab is one of the most efficacious treatment in MS. Its mode of action is poorly understood.. Interestingly the effect of natalizumab on bacterial translocation has been documented in HIV infection and this effect has been shown

to stablize central nervous system injury (PLoS Pathog. 2014 Dec 11;10(12):e1004533.). Whether this effect on gut microbiota and bacterial translocation should contribute to the effect on natalizumab in multiple sclerosis is unknown.

- Objective

To explore this hypothesis, we sought to investigate the effect of natizulimab on blood microbiota in 1200 ntz-treated patients with multiple sclerosis.

- Material and method

The microbiote RNA 16s Sequencing :The microbial population present in the samples has been determined using next generation high throughput sequencing of variable regions of the 16S rRNA bacterial gene. This workflow, established by Vaiomer includes the following steps:

PCR amplification was performed using 16S universal primers targeting the V3-V4 region of the bacterial 16S ribosomal gene (Vaiomer universal 16S primers). The joint pair length was set to encompass 476 base pairs amplicon thanks to 2 x 300 paired-end MiSeq kit V3. For each sample, a sequencing library was generated by addition of sequencing adapters. The detection of the sequencing fragments was performed using MiSeq Illumina® technology.

Bioinformatics pipeline : The targeted metagenomic sequences from microbiota were analysed using the bioinformatics pipeline established by Vaiomer from the FROGS guidelines (details below). Briefly, after demultiplexing of the bar coded Illumina paired reads, single read sequences are cleaned and paired for each sample independently into longer fragments. Operational taxonomic units (OTU) are produced with via single-linkage clustering and taxonomic assignment is performed in order to determine community profiles.

Satistical approach Non-parametric Mann-Whitney tests or t-tests and Fisher's exact tests were conducted on quantitative and categorical variables respectively using the software PRISM v6.05 (GraphPad software, CA, USA), with NTZ status as independent variable — a p-value < 0.05 was considered significant. The output matrix containing the relative abundance of OTUs per sample was processed with the linear discriminant analysis effect size (LEfSe) algorithm[30] using an alpha cutoff of 0.05 and an effect size cutoff of 2.0. Then, since the LEFSe analysis carried out showed that the clostridia class was the only that correlated with response to NTZ, we explored further the relationship between class clostridia and response to treatment. Because of the high percentage of null values (xx%), we categorized subjects into 3 groups according to the percentage of clostridia in the plasma microbiota: null, not zero but less than the median, and above the median. Statistical analyses were conducted using the software STATa.

- Results

A decrease of alpha diversity (richness) is observed between T0 and T2.Bacterial taxa differences are observed in the relative abundance barplots between T0 and T2, and further highlighted by the LEfSe analysis. These differences might be induced by the treatment itself.

Responder status - responders(R+) / non-responders (R-) at each time point. At T0, bacterial taxa differences are reported by LEfSe between R+ and R- patients. At T1 and T2, few bacterial differences are reported by LEfSe. Potential biomarkers should be further investigated at T0 to discriminate R+ and R- patients with appropriate statistical tools and including the other clinical parameters. At T1 and T2, the modifications induced by the treatment itself might be masking the more subtle differences that could exist between R+ and R- patients.

- Conclusion

In these study, we observed:

A decrease of alpha diversity (richness) before and 2 years after natalizumab treatment.

**Responder status** - responders(R+) / non-responders (R-) at each time point

At baseline before treatment, bacterial taxa differences are seen between responders and non-responders to natalizumab giving the opportunity to develop a predictor marker for natalizumab response.

## WP6 – Transcriptomic study

### Objective

This WP aims to determine the difference of expression profile between NTZ responders and non-responders using a gene expression study and thus to identify RNA-linked markers associated with the response to NTZ treatment. In summary, we aim at determining if an expression signature is associated with response to NTZ.

### Material and methods

25 patients with an extreme good response and 25 with an extreme bad response were selected. RNA was extracted from PBMC at 2 time points: baseline and 1 year after natalizumab start. After quality controls steps, Libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit (Illumina), following the manufacturer's instructions. Sequencing was performed, first after amplification by the cBot automated cluster generation system (Illumina), and second, the obtained clusters were sequenced by the HiSeq 2000 system (Illumina) using the 'reversible terminator' chemistry. The bioinformatics steps were conducted as follow:  FastQC v.0.10.1(http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/)., TopHat v.2.0.9 [1], ConDeTri [2]., SAMtools v.0.1.19 [3], VarScan v.2.3.5 [4], SnpEff v.3.5C, PLINK/SEQ (http://atgu.mgh.harvard.edu/plinkseq).

### Results:

When stratified by response to NTZ, we found the above listed top genes called response signature

Table 7 gene expression signature associated with natalizumab response

| | logFC | P.Value | FDR P.Val |
|---|---|---|---|
| **PDPR** | 0,788126419 | 2,53E-07 | 0,001289981 |
| **SNRPD1** | -0,944105082 | 2,62E-07 | 0,001289981 |
| **LLPH** | -0,65469227 | 9,67E-07 | 0,003170926 |
| **TCEAL4** | -0,704009897 | 1,42E-06 | 0,003496953 |
| **NPM1** | -0,738955984 | 2,90E-06 | 0,00570412 |
| **SSBP1** | -0,572416342 | 5,64E-06 | 0,009241123 |
| **HNRNPH3** | -0,301888885 | 1,16E-05 | 0,016288914 |
| **GIMAP7** | -0,811007242 | 1,63E-05 | 0,020007379 |
| **POLR3GL** | -0,548775004 | 2,05E-05 | 0,020810037 |
| **SSB** | -0,622249033 | 2,30E-05 | 0,020810037 |
| **LTV1** | -0,481432094 | 2,33E-05 | 0,020810037 |
| **NDUFA1** | -1,059110587 | 3,56E-05 | 0,029195997 |
| **SHFM1** | -0,9129375 | 4,14E-05 | 0,031302086 |
| **METAP2** | -0,52190193 | 5,72E-05 | 0,039021823 |
| **METTL5** | -0,645281734 | 5,95E-05 | 0,039021823 |
| **LSM5** | -0,60294483 | 6,92E-05 | 0,041336777 |
| **CFDP1** | -0,623305853 | 7,14E-05 | 0,041336777 |
| **HMGN3** | -0,624622617 | 7,71E-05 | 0,04214509 |

| | | | |
|---|---|---|---|
| **DLST** | 0,290374466 | 9,75E-05 | 0,047820214 |

### Conclusion

A scientific paper was delayed since these results may give us the opportunity to develop a patent for treatment response

## WP7 – Genomic basis of PML risk

### Exome Sequencing

D7.1. A scientific paper submission: Exome sequencing. A scientific paper with PML clinical description will be written. If positive a patent application will be considered.

***Work hypothesis:***
Herpes simplex virus-1 (HSV-1) can result from a single-gene immunodeficiency that does not compromise immunity to most pathogens. Since PML in NTZ-treated patients shared common features with HSV encephalitis, we postulated that some MS-patients could carry a mutation responsible for host susceptibility to PML. To confirm our hypothesis we will sequence patients and controls by the **exome sequencing technique**, in order to determine if PML affected patients do indeed carry a mutation responsible to this susceptibility.

**-a- The discovery cohort:**

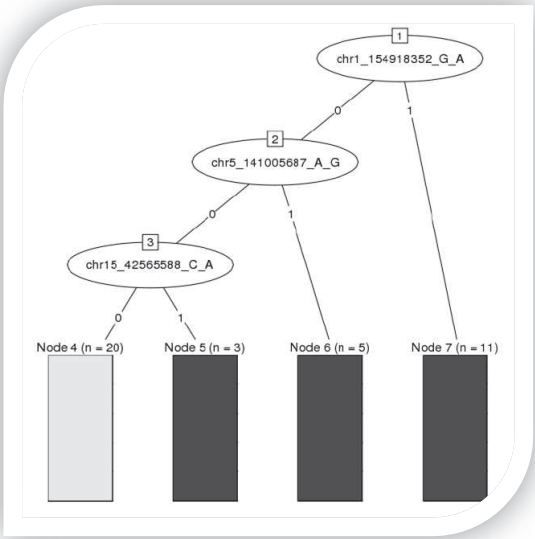
Cohort:
18 (20 samples with 2 doublets) NTZ-treated MS patients that developped PML vs20 long-term NTZ-treated MS patients (see following table).

Technic:
Sure Select XT$^{TM}$ Human All Exon V5 (Agilent)Sequenced by the HiSeq 2000 system (Illumina) with a 80X Depth and read length 100 nt .
This exome sequencing has been performed by PGK. (see**WP7-Appendix 1**).

Analysis:
A first analysis has been performed by Norman Breuil and the Genosplice company (see **WP7-Appendix 2**, **WP7-Appendix 3**). However, nothing interesting has emerged.

In order to confirm the absence of positive results, we asked for a new way of analysing the data performed by Sistemas Genomicos, a company already implicated in the network and more specifically in the Best-MS Task 7.3. (see**WP7-Appendix 4**)

Briefly, the use of J48 algorithm allows identifying the most important variants for the correct classification between controls and patients.
Using these interesting variants is possible correctly classify the 100% of cases and control (see following cartoon).




All variants are functionally related. For this study, system biology methodologies was applied. Results are presented in the following figure.

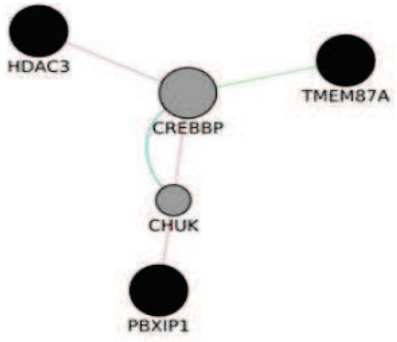



Figure 7 : exome sequencing results in the first study

The interesting genes are very close to NF-kB, this transcription factor is related with inflammatory response. Some authors have correlated the functional interaction between CREBBP and HDAC3.

**-b- Confirmatory study:**

Confirmation of the presence of the - 1798C>T variant in the *PBXIP1 gene, the - 921-49T>C variant in the HDAC3 gene* and the *- 9G>T variant in the TMEM87A gene.* Using an alternative technics: the Sanger sequencing. In addition, we add a confirmatory cohort. This task has been performed by Sistemas Genomicos (see **WP7-Appendix 5**)

Table 8 sanger analysis of the 3 genes of interest in the first and second cohorts

| Type | Category | Total | Positives | % Prev | Comments | Sum_Prev |
|---|---|---|---|---|---|---|
| Case | NEW PML NATALIZUMAB | 16 | 6 | 37* | | 59,6 |
| Case | previously exome PML NATALIZUMAB | 20 | 18 | 90 | Initial_Hypotesis | |
| Case | other biotherapies related PML | 3 | 0 | 0 | | |
| Case | PML-non MS | 1 | 0 | 0 | | |
| Case | VIH-related PML | 12 | 7 | 58 | | |
| Control | previously exome control | 20 | 0 | 0 | Initial_Hypotesis | 23,0 |
| Control | Long term Nat treated JC+ | 32 | 12 | 37* | | |

Red = new cohort

-> not confirmed in the new cohort (see *)

**c- Exome sequencing on the new PML NTZ-cohort:**

The discrepancy between the discovery cohort and the new NTZ-related PML, forced us to investigate in more detail this phenomenon by performing a whole exome sequencing experiment on the new cohort.

36 samples :
- 16 PML from the new cohort
- 16 long-term NTZ-treated JC+ MS patients (controls of the new cohort)
- 4 controls of RNAseq (M. Comabella , task 7.3)

**d-Conclusion**

At the time of this report the results are non conclusive since we were not able to find genes that carry a host genetic susceptibility to the JC virus infection. Nevertheless further analysis are ongoing with a new exome sequencing performed with this FP7 project and new statistical analysis in collaboration with the rockfeller university at New York.

- **GWAS and JC serostatus:**

GWAS of JC serostatus (positive vs negative) has been conducted using this imputed map. The results show a strong association signal (P=2x10-12) within HLA Class II region (Fig 2). We thus confirm the results reported by the, so far, single published association study of JC serostatus within the HLA region. This study was conducted in MS (1,352) and controls (465) subjects of Scandinavian and German origins (Sundqvist et al., Plos Pathogens, 2014). As in this study, our top association signals are located a few kb of the HLA-DRB1 locus, the major MS-risk locus. We have further performed conditional analyses within the HLA-Class II region. We found that most top association signals are, indeed, not independent of the signal at the DRB1 locus (Fig 2). Our GWAS study also identifies association signals, although of lower statistical significance, outside the HLA Class II region. These preliminary results are encouraging and, unless other updates/revisions of the databases are planned, we think INSERM will be able to complete this study within the next 2 months.
Fig 2: GWAS of JC serostatus (posvsneg): Association results in the HLA region*

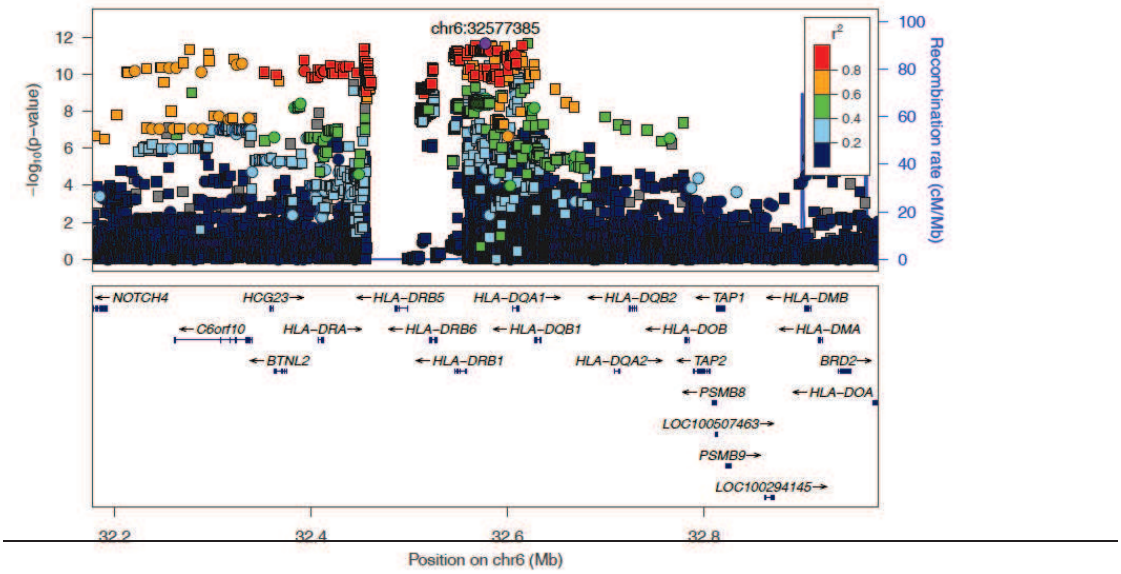



Figure 8 Manhattan plot of HLA and JC virus GWAS

The GWAS of JC status has been conducted and our results confirm the positive association with HLA-class II loci. GWAS of JC Index value will start afterwards. These GWAS are based on imputed data (post-QC map= 9,723,756 autosomal imputed and genotyped data)

### RNAseq and PML prediction

**Objective**: To identify biomarkers associated with the development of progressive multifocal leukoencephalopathy (PML) in multiple sclerosis (MS) patients treated with natalizumab (NTZ).

**Methods**: Relapsing-remitting MS (RRMS) patients who developed PML under NTZ therapy (pre-PML) and non-PML natalizumab-treated patients (NTZ-ctr) were included in the study. Cryopreserved peripheral blood mononuclear cells (PBMC) and serum samples collected at baseline, at one- and two-year treated time points, and during PML were analyzed for gene expression by RNA-sequencing and for serum protein levels by LUMINEX and ELISA assays respectively.

**Results:** Among top differentially expressed genes in the RNA-sequencing between pre-PML and NTZ-ctr patients, pathway analysis revealed a high representation of genes belonging to the following categories: pro-angiogenic factors (MMP9, VEGFA), chemokines (CXCL1, CXCL5, IL8, CCL2), cytokines (IL1B, IFNG), and plasminogen- and coagulation-related molecules (SERPINB2, PLAU, PLAUR, TFPI, THBD). Serum protein levels for these candidates were measured in a two-step manner in a screening cohort and a combined validation cohort of pre-PML and NTZ-ctr patients. Only MMP9 was validated and, in pre-PML patients MMP9 protein levels were significantly reduced at baseline compared with NTZ-ctr patients and levels remained unchanged during NTZ treatment, whereas in NTZ-ctr patients MMP9 levels were down-regulated by the effect of NTZ treatment.

**Conclusions:** The results from this study suggest that the pro-angiogenic factor MMP9 may play a role as biomarker associated with the development of PML in MS patients treated with NTZ.

## WP8: COMPOSITE TEST

1. OBJECTIVES

• Investigate the relationship between different clinical, radiological, biological and genetic variables and response to NTZ

• Explore different statistical models that can best explain this relationship

• Evaluate and compare the performance of these models to find the best candidate prediction model

### PREDICTIVE MODEL OF NTZ RESPONSE WITH ADDITIONAL DATA (2016)

In this study, the following data were added vitamin D levels, smoking, JC virus serology and DR2 status.

In addition, since additional clinical, radiological data has been collected also collected from 2014, a total of 905 NTZ-treated MS patients has been now included in the study.

Off note, the same NTZ response criteria have been applied to this study (see paragraph 2.2, “response definition”).

### 3.1.Dataset : Patients characteristics and response to NTZ

➔ **Clinical and genetic characteristics**

| CLINICAL AND GENETIC CHARACTERISTICS | | | ALL PATIENTS 905 | RESPONDERS 468 (52%) | NON-RESPONDERS 437 (48%) | P-VALUE | |
|---|---|---|---|---|---|---|---|
| Gender | | | | | | 0.6721 | X-squared = 0.1791, df = 1 |
| | NA | | - | - | - | | |
| | female | n (%) | 688 (76%) | 359 (52%) | 329 (48%) | | |
| | male | n (%) | 217 (24%) | 109 (50%) | 108 (50%) | | |
| EDSS at treatment onset | | | | | | 0.05513 | W = 105318 |
| | NA | | 18 | 0 | 18 | | |
| | mean (sd) | | 3.34 (1.66) | 3.44 (1.71) | 3.22 (1.60) | | |
| | median (range) | | 3.5 (0, 8) | 3.5 (0, 8) | 3 (0, 7.5) | | |
| Number of relapses one year prior treatment onset | | | | | | 0.09608 | W = 95595 |
| | NA | | 2 | 0 | 2 | | |
| | mean (sd) | | 2.07 (1.09) | 2.03 (1.14) | 2.10 (1.03) | | |
| | median (range) | | 2 (0, 8) | 2 (0, 6) | 2 (0, 8) | | |
| Disease duration at treatment onset (years) | | | | | | 0.09504 | W = 108088.5 |
| | NA | | 3 | 1 | 2 | | |
| | mean (sd) | | 8.84 (6.83) | 9.16 (6.85) | 8.50 (6.81) | | |
| | median (range) | | 7 (0, 42) | 8 (0, 41) | 7 (0, 42) | | |
| Previous immuno-suppressant use | | | | | | 0.3785 | X-squared = 0.7756, df = 1 |
| | NA | | - | - | - | | |
| | yes | n (%) | 209 (23%) | 102 (49%) | 107 (51%) | | |
| | no | n (%) | 696 (77%) | 366 53%) | 330 (47%) | | |
| Previous immuno-modulatory use | | | | | | 0.6719 | X-squared = 0.1794, df = 1 |
| | NA | | - | - | - | | |
| | yes | n (%) | 813 (90%) | 418 (51%) | 395 (49%) | | |
| | no | n (%) | 92 (10%) | 50 (54%) | 42 (46%) | | |
| Smoking | | | | | | 0.865 | X-squared = 0.0289, df = 1 |
| | NA | | 271 (30%) | 128 | 143 | | |
| | yes | n (%) | 221 (24%) | 117 (53%) | 104 (47%) | | |
| | no | n (%) | 413 (46%) | 223 (54%) | 190 (46%) | | |
| Vitamin D | | | | | | 0.3252 | W = 48723 |
| | NA | | 265 | 129 | 136 | | |
| | mean (sd) | | 20.50 (13.31) | 20.20 (12.62) | 20.85 (12.97) | | |
| | median (sd) | | 18 (3, 117) | 17 (3, 117) | 19 (3, 96) | | |
| MSSS at treatment onset | | | | | | 0.8509 | W = 93254.5 |
| | NA | | 43 | 12 | 31 | | |
| | mean (sd) | | 4.80 (2.49) | 4.82 (2.49) | 4.78 (2.50) | | |
| | median (sd) | | 4.82 (0.16, 9.68) | 4.72 (0.21, 9.84) | 4.82 (0.16, 9.88) | | |
| Age at disease onset | | | | | | 0.9925 | W = 98231.5 |
| | NA | | 3 | 1 | 2 | | |
| | mean (sd) | | 28.13 (8.77) | 27.87 (8.65) | 28.41 (8.90) | | |
| | median (sd) | | 27 (6, 60) | 27 (6, 58) | 27 (8, 60) | | |
| Age at treatment onset | | | | | | 0.9128 | W = 102688.5 |
| | NA | | - | - | - | | |
| | mean (sd) | | 36.97 (9.82) | 37.01 (9.92) | 36.93 (9.71) | | |
| | median (sd) | | 37 (15, 66) | 37 (15, 65) | 36 (16, 66) | | |
| DR2 | | | | | | 0.03856 | X-squared = 4.2801, df = 1 |
| | NA | | 124 (14%) | 68 | 56 | | |
| | DR2 | n (%) | 375 (41%) | 207 (55%) | 168 (45%) | | |
| | No DR2 | n (%) | 406 (45%) | 193 (48%) | 213 (52%) | | |

NOTE: Chi-square test used for categorical variables, Mann-Whitney test used for numeric variables. NA: Not available.

Table 9: Clinical and genetic characteristics and responses to NTZ.

➔ **Biological characteristics**

| BIOLOGICAL CHARACTERISTICS | | ALL PATIENTS 905 | RESPONDERS 468 (52%) | NON-RESPONDERS 437 (48%) | P-VALUE | |
|---|---|---|---|---|---|---|
| **IgG at treatment onset** | | | | | 0.1747 | W = 77524.5 |
| NA | | 138 | 66 | 72 | | |
| mean (sd) | | 9.86 (2.53) | 9.90 (2.56) | 9.81 (2.51) | | |
| median (range) | | 9.70 (0.90, 19.70) | 10.00 (0.90, 17.60) | 9.60 (3.00, 19.70) | | |
| **IgA at treatment onset** | | | | | 0.1499 | W = 77773.5 |
| NA | | 138 | 66 | 72 | | |
| mean (sd) | | 2.01 (0.81) | 2.04 (0.86) | 1.98 (0.75) | | |
| median (range) | | 1.90 (0.10, 9.40) | 2.00 (0.10, 9.40) | 1.90 (0.30, 5.20) | | |
| **IgM at treatment onset** | | | | | 0.9791 | W = 72498.5 |
| NA | | 143 | 69 | 74 | | |
| mean (sd) | | 1.35 (0.96) | 1.35 (1.05) | 1.36 (0.85) | | |
| median (range) | | 1.20 (0.10, 18.00) | 1.20 (0.10, 18.00) | 1.20 (0.20, 10.90) | | |
| **Lymphocytes at treatment onset** | | | | | 0.0560 | W = 69442.5 |
| NA | | 128 | 69 | 59 | | |
| mean (sd) | | 2.31 (2.76) | 2.40 (3.32) | 2.21 (2.01) | | |
| median (range) | | 1.80 (0.20, 33.80) | 1.80 (0.60, 33.80) | 1.90 (0.20, 27.20) | | |
| **CD3 count at treatment onset** | | | | | [illegible] | W = 20078.5 |
| NA | | 469 | 237 | 232 | | |
| mean (sd) | | 1428 (570.24) | 1374 (594.02) | 1489 (537.08) | | |
| median (range) | | 1332 (314, 4970) | 1287 (314, 4970) | 1413 (420, 3252) | | |
| **CD4 count at treatment onset** | | | | | 0.0725 | W = 72487 |
| NA | | 113 | 55 | 58 | | |
| mean (sd) | | 965.20 (460.54) | 948.50 (431.58) | 983.50 (490.08) | | |
| median (range) | | 881 (133, 7100) | 842 (237, 4596) | 920 (133, 7100) | | |
| **CD8 count at treatment onset** | | | | | [illegible] | W = 70403 |
| NA | | 114 | 55 | 59 | | |
| mean (sd) | | 449.70 (217.24) | 437.90 (224.15) | 462.50 (208.97) | | |
| median (range) | | 404 (38, 1850) | 381 (106, 1850) | 416 (38, 1600) | | |
| **CD19 count at treatment onset** | | | | | 0.0520 | W = 52343 |
| NA | | 228 | 127 | 101 | | |
| mean (sd) | | 292.60 (233.32) | 273 (152.95) | 312.40 (292.10) | | |
| median (range) | | 246 (21, 4442) | 231 (21, 912) | 254 (33, 4442) | | |
| **JC Virus** | | | | | 0.4627 | X-squared = 0.5394, df = 1 |
| NA | | 50 (6%) | 29 | 21 | | |
| positive | n (%) | 564 (62%) | 284 (50%) | 280 (50%) | | |
| negative | n (%) | 291 (32%) | 155 (53%) | 136 (47%) | | |

NOTE: Chi-square test used for categorical variables, Mann-Whitney test used for numeric variables. NA: Not available.

Table 10: Biological characteristics and responses to NTZ

➔ **Radiological characteristics**

| RADIOLOGICAL CHARACTERISTICS | | ALL PATIENTS | RESPONDERS | NON-RESPONDERS | P-VALUE | |
|---|---|---|---|---|---|---|
| | | 905 | 468 (52%) | 437 (48%) | | |
| GD+ enhancing lesions at treatment onset | | | | | 0.03493 | X-squared = 4.4485, df = 1 |
| NA | | 25 (3%) | 5 | 20 | | |
| yes | n (%) | 498 (55%) | 278 (56%) | 220 (44%) | | |
| no | n (%) | 382 (42%) | 185 (48%) | 197 (52%) | | |
| T2 superior to 9 at treatment onset | | | | | 0.4143 | X-squared = 0.6664, df = 1 |
| NA | | 38 (4%) | 6 | 32 | | |
| yes | n (%) | 800 (88%) | 430 (54%) | 370 (46%) | | |
| no | n (%) | 67 (7%) | 32 (48%) | 35 (52%) | | |
| T2 confluent lesions at treatment onset | | | | | 0.3384 | X-squared = 0.9164, df = 1 |
| NA | | 40 (4%) | 8 | 32 | | |
| yes | n (%) | 69 (8%) | 41 (59%) | 28 (41%) | | |
| no | n (%) | 796 (88%) | 419 (53%) | 377 (47%) | | |

NOTE: Chi-square test used for categorical variables, Mann-Whitney test used for numeric variables. NA: Not available.

Table 11: Radiological characteristics and responses to NTZ

### 3.2. LR and BT models of NTZ response prediction

The performance metrics obtained for each of the two modeling approaches are illustrated in **Table 11** below. The number of repetitions (100 or 1000) had little impact on the results.

| | 100 Repetitions | | 1000 Repetitions | |
|---|---|---|---|---|
| | LR | BT | LR | BT |
| **Sensitivity** | 0.46 | 0.51 | 0.47 | 0.53 |
| **Specificity** | 0.55 | 0.50 | 0.55 | 0.48 |
| **PPV** | 0.49 | 0.53 | 0.49 | 0.53 |
| **NPV** | 0.51 | 0.49 | 0.52 | 0.49 |
| **Accuracy** | 0.26 | 0.25 | 0.25 | 0.25 |

Table 12: Sensitivity, specificity, PPV, NPV and overall accuracy for the logistic regression (LR) and the binary trees with recursive partitioning (BR) models averaged over 100 or 1000 repetitions of two-fold cross validation runs.

Lastly, **Table 13** below illustrates the ranking of the predictors selected by each approach

from most to least often over 100 repetitions.

| 100 Repetitions | | | | | |
|---|---|---|---|---|---|
| | LR | | | BT | |
| | Count | Rate (%) | | Count | Rate (%) |
| MRIGd_T0 | 105 | 53 | CD8_T0 | 105 | 53 |
| CD19_T0 | 96 | 48 | CD4_T0 | 101 | 51 |
| Lymphocytes_T0 | 92 | 46 | CD19_T0 | 95 | 48 |
| DR2 | 74 | 37 | IgG_T0 | 87 | 44 |
| IgG_T0 | 56 | 28 | IgA_T0 | 84 | 42 |
| CD8_T0 | 46 | 23 | Lymphocytes_T0 | 52 | 26 |
| EDSS_T0 | 38 | 19 | EDSS_T0 | 49 | 25 |
| ImmunoMod | 33 | 17 | IgM_T0 | 45 | 23 |
| ImmunoSup | 32 | 16 | MRIGd_T0 | 31 | 16 |
| IgM_T0 | 31 | 16 | NumberRelapses_T0 | 29 | 15 |
| MRIT2Confluent_T0 | 29 | 15 | MSDuration_T0 | 26 | 13 |
| MRIT2Sup9_T0 | 29 | 15 | ImmunoSup | 14 | 7 |
| CD4_T0 | 26 | 13 | ImmunoMod | 7 | 4 |
| NumberRelapses_T0 | 23 | 12 | DR2 | 4 | 2 |
| IgA_T0 | 21 | 11 | MRIT2Sup9_T0 | 3 | 2 |
| MSDuration_T0 | 20 | 10 | Gender | 2 | 1 |
| Gender | 15 | 8 | MRIT2Confluent_T0 | 1 | 1 |

| 1000 Repetitions | | | | | |
|---|---|---|---|---|---|
| | LR | | | BT | |
| | Count | Rate (%) | | Count | Rate (%) |
| MRIGd_T0 | 1071 | 54 | CD4_T0 | 1052 | 53 |
| CD19_T0 | 998 | 50 | CD8_T0 | 997 | 50 |
| Lymphocytes_T0 | 994 | 50 | IgG_T0 | 990 | 50 |
| DR2 | 658 | 33 | CD19_T0 | 980 | 49 |
| IgG_T0 | 566 | 28 | IgA_T0 | 736 | 37 |
| CD8_T0 | 397 | 20 | IgM_T0 | 602 | 30 |
| MRIT2Sup9_T0 | 394 | 20 | Lymphocytes_T0 | 513 | 26 |
| ImmunoMod | 362 | 18 | MSDuration_T0 | 395 | 20 |
| ImmunoSup | 330 | 17 | EDSS_T0 | 348 | 17 |
| EDSS_T0 | 321 | 16 | NumberRelapses_T0 | 263 | 13 |
| IgM_T0 | 304 | 15 | MRIGd_T0 | 262 | 13 |
| MSDuration_T0 | 272 | 14 | ImmunoSup | 146 | 7 |
| CD4_T0 | 223 | 11 | ImmunoMod | 65 | 3 |
| IgA_T0 | 223 | 11 | DR2 | 57 | 3 |
| MRIT2Confluent_T0 | 221 | 11 | MRIT2Sup9_T0 | 46 | 2 |
| NumberRelapses_T0 | 217 | 11 | Gender | 27 | 1 |
| Gender | 165 | 8 | MRIT2Confluent_T0 | 22 | 1 |

Table 13: Predictors ranked by the percentage of times over the 100 repetitions that they were selected when using the logistic regression with backward selection (LR) or the binary tree with recursive partitioning model (BT).

### 3.3. Concluding remarks

Interestingly, NTZ responders and non responders could be discriminate at base line (T0)

- In the first study : based on IgG levels (p=0.039), CD8 count (0.0491), and at the MRI level on the GD+ criteria (0.02323)
- In the second study: based on DR2 (p=0.03856), CD3 count (p=0.0061), CD8 count (p=0.0171) and also at the MRI level on the GD+ criteria (0.03493)

When going to the LR and BT methodologies, in the 2 presented studies, none of the 2 different approaches appears to be able to predict NTZ response. However, we can notice that among all variables used, the top 5 variables recurrently used by the repetitions (100 or 1000) are the following:

- Logistic Regression: MRIGd_T0, CD19_T0, Lymphocytes_T0, DR2, IgG_T0
- Binary Trees: CD4_T0, CD8_T0, IgG_T0, CD19_T0, IgA_T0

In particular, in the both approaches, we can find CD19_T0 et IgG_T0 which could be of high interest.

**Best-MS society impact**

- **Background : Multiple sclerosis, Neurodegenerative diseases, biologic drugs HIV and precision medicine**

The best-MS project (a better escalation therapy in Multiple sclerosis) objective was to determine the best composite criteria of response to Natalizumab (NTZ) during the first year of treatment that best predict disease activity during the second and third years of treatment with NTZ and serious adverse event.

NTZ is a biologic drug and multiple sclerosis (MS) a neurodegenerative disease of the young adult, mainly driven by autoimmune mechanisms. The main strength of the project was to use a biological database of 1200 patients prospectively collected and available at the beginning of the project.

Therefore this project was a paradigm for how precision medicine could be use in the near future especially in the field of neurodegenerative diseases (Alzheimer's, Parkinson's, diseases, Amyotrophic lateral sclerosis, Multiple sclerosis, or biologic drugs (oncology, internal medicine). Further serious adverse events with biologic drugs are opportunistic infection especially the progressive multifocal leukoencephalopathy (PML). PML is a common problem in the HIV field that will be impacted by this project

Precision medicine, (formerly called personalized medicine), importance has been pointed out recently by president Obama, when launching the precision medicine initiative in 2015. The FP7 program was pioneering and our project was clearly related to this field.

The concept of precision medicine — prevention and treatment strategies that take individual variability into account — is not new: blood typing, for instance, has been used to guide blood transfusions for more than a century. But the prospect of applying this concept broadly has been dramatically improved by the recent development of large-scale biologic databases (such as the human genome sequence), powerful methods for characterizing patients (such as proteomics, metabolomics, genomics, diverse cellular assays, and even mobile health technology), and computational tools for analyzing large sets of data. What is needed now is a broad research program to encourage creative approaches to precision medicine, test them rigorously, and ultimately use them to build the evidence base needed to guide clinical practice. (See A New Initiative on Precision Medicine Francis S. Collins, M.D., Ph.D., and Harold Varmus, M.D. N Engl J Med 2015; 372:793-795.

- **Impact 1 :** METHODS OF RISK ASSESSMENT OF PML AND RELATED APPARATUS patent WO2013057092 (A1) — 2013-04-25

The invention provides a method of assessing the risk of occurrence of progressive multifocal leukoencephalopathy (PML) in a subject as well as a method of stratifying a subject undergoing VLA-4 blocking agent treatment for suspension of VLA-4 blocking agent treatment. These methods comprise detecting the level of L-selectin (CD62L) and optionally LFA-1 expressing T cells in a sample from the subject

In this patent we are able to predict the occurrence of PML in NTZ treated patients but also in HIV patients

### Impact 2 : Pattent project with other biological marker for predicting PML

Preliminary data are promising for the development of biomarker beyond CD62L. Afetr an exome sequencing of PML patients versus controls we are confident that genes of the NFKappa B pathway are of interest for the prediction

Based on a RNA sequencing we believe that information from the MMP-9 pathway may help us in PML prediction and diagnosis

Based on a metagenomic approach we found that clostridium bacteria family are involved in PML prediction

A combination of the 4 makers developed during the project (CD62L, MMP9, NFKb) and those already used (index value and JC virus serology) is also ongoing

### Conclusion impact 1 and 2

With our project we are confident that PML a severe adverse drug reaction due to an opportunistic infection and may cause death will be decrease. Therefore we increased the safety of a usefull drug. Our findings also impact the HIV field and PML related to other biologic drugs.

### Impact 3 prediction of NTZ response

During the best-MS project with a GWAS of responders versus non responders we found that genes of the GABA receptors family are associated with non response to natalizumab. This is an important findings since we are designing a response prediction test that will allow to better choose between the 15 biologic drugs available in MS. Further this response test with be an important complementary test to adverse event prediction test.

### A global prediction test for NTZ

Even if this goal was not done at the end of the project a mathematical model is now possible and we will answer to the H2020 call: SC1-PM-17-2017: Personalised Computing Models and In-silico Systems for well-being. Our Project with now a 10 year follow up is a rare database for precision medicine

### A paradigm for neurodegenerative diseases

We have now a 10 year follow up since our first patient has been included in the database. It is a unique database compelling clinical radiological and biological data. Since 5% of our relapsing remitting patients develop under NTZ a progressive form of the disease we gathered a rare cohort that will allow us to explore the immunology to neurodegeneration interplay.