# Molecular analysis of blood memory CD8+T cells at the single-cell level reveals a specific pattern of clonally expanded cells in multiple sclerosis patients

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## INTRODUCTION AND PURPOSE

A body of evidence highlights the involvement of CD8⁺T cells in Multiple sclerosis (MS). We have recently demonstrated that oligoclonally expanded CD8⁺T cells found at lesion sites, thought to be driven by local cognate antigens, are the same overrepresented T cells found in the CerebroSpinal Fluid (CSF) of the same patients and represent up to 47% of the overrepresented CD8⁺T cells in the blood. Based on these previous results the blood can be used as a source of T cells involved in the disease process. However, to date we have not identified yet in the periphery the culprit CD8⁺T cells driving autoimmune inflammation nor their phenotype and/or function. Our working hypothesis is that the cells able to provoke damages in the Central Nervous System (CNS) are also present in the blood and may have a specific phenotypic or functional pattern.

The purpose of this project is to **study CD8⁺ T cells in the CSF and blood of MS patients at a single cell level to identify their specificity** compared to healthy volunteers (HV) or patient with other neurological disease (CTRL), to **decipher their roles in the MS pathophysiology** and if **oligoclonally expanded clones are linked to a specific phenotype of CD8⁺ T cells**.

## METHODS

To analyze single-cells molecular signatures, we isolate single memory CD8⁺T cells from the blood or CSF of MS and CTRL patients and from blood of Healthy Volunteers (HV) using an ARIA cell sorter cytometer. We then isolate 96 cells per samples with the C1 Single-Cell Auto Prep System (Fluidigm) and performed a pre-amplification of 96 well-chosen genes together with their TCR Vβ chain.

Subsequently, we used the pre-amplified samples to performed, on one hand, qPCR of the 96 genes using the Biomark systems (Fluidigm) and on the other hand, a PCR to amplified the TCR Vβ chain followed by a sequencing.

In parallel, we performed a TRBV deep immunosequencing on a pool of memory CD8⁺T cells to identify the expanded clones in the samples.

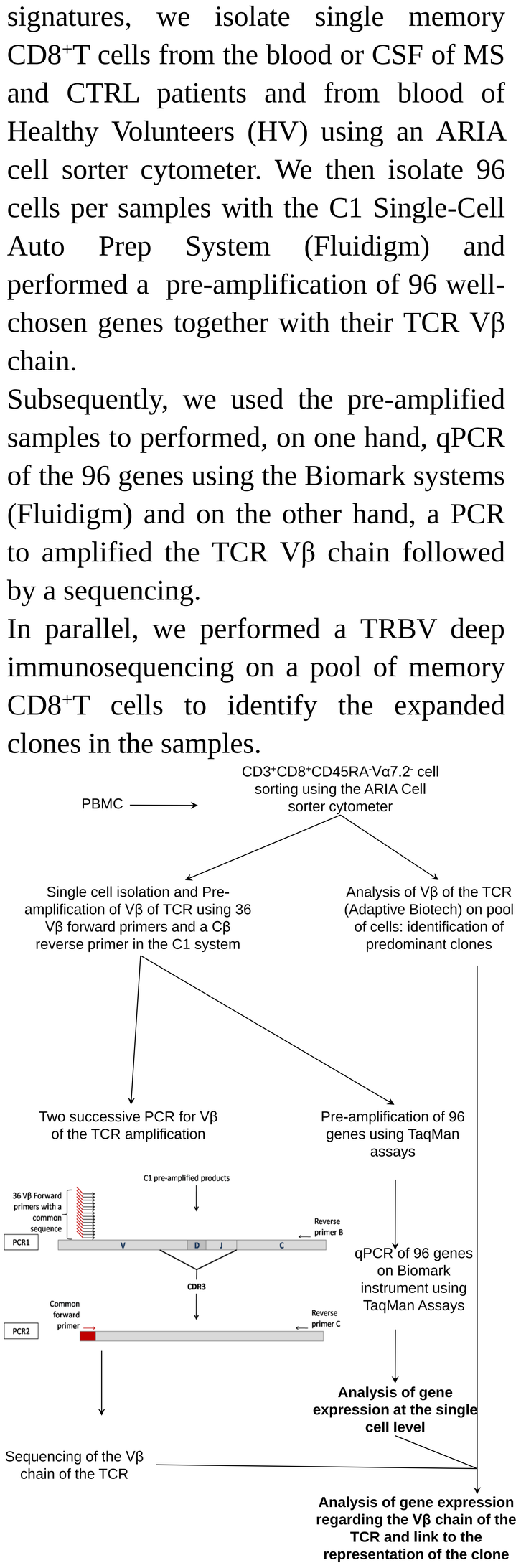



Figure 1: Workflow of the methods used in the study

MS patients are relapsing-remitting untreated patients. CTRL patients display optical neuritis or viral meningo-encephalitis. HV are sex and age matched with MS patients counterpart

## RESULTS

### 1- The transcriptional analysis of Blood memory CD8⁺T cells from MS patients delineates a specific pattern compared to CTRL or HV

Principal genes differentiating MS from HV are involved in T cell activation or in MS pathophysiology (CD69, VLA-4, CD107a, LFA-1…)

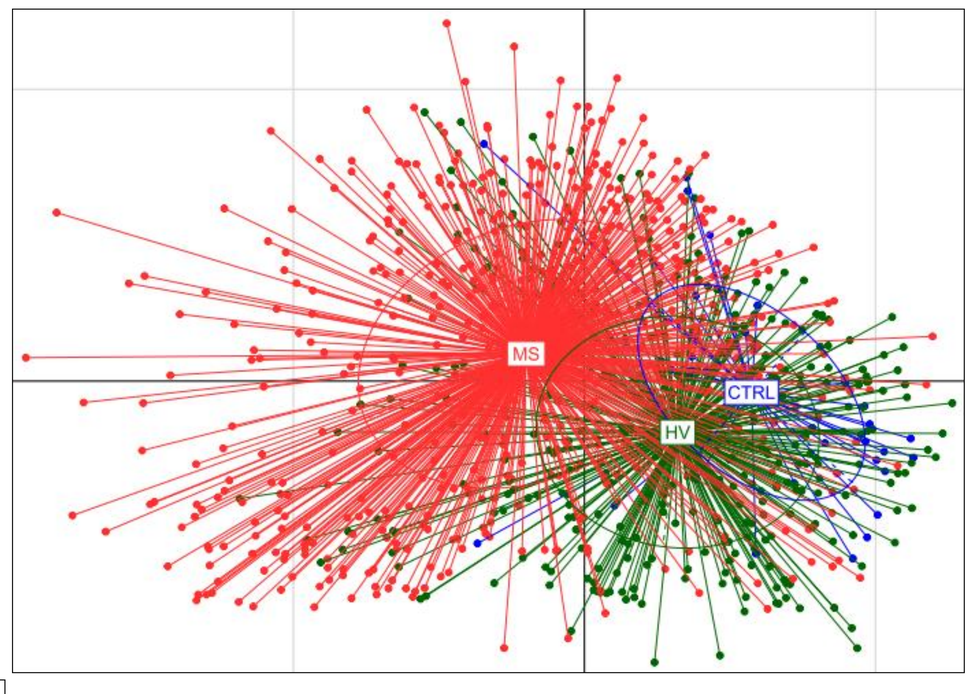
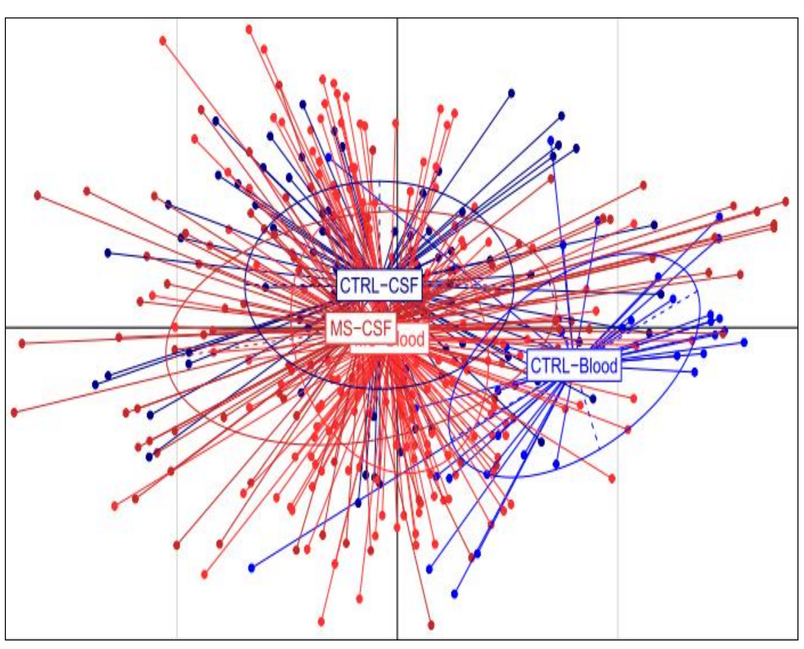



Figure 2: PCA representing the genes expression of Blood cells from MS patients, healthy volunteers and patients with other neurological diseases

### 2- The memory CD8⁺T cells from CSF and blood of MS patient harbor the same transcriptional profile whereas in patients with other neurological disease (CTRL) Blood and CSF memory CD8⁺T cells exhibit strong differences




Similar phenotype of Blood and CSF CD8⁺ T cells of MS patients suggest that the blood cells have already acquired the phenotype to reach the CSF.

Figure 3: PCA representing the genes expression of Blood cells and CSF cells from MS patients and patients with other neurological diseases

### 3- Expanded CD8⁺T cells from MS patients exhibit a specific pattern compared to their counterparts in HV

Oligoclonally expanded T cells from MS patients dramatically down regulates KLRG1 and CD62L but over-regulates STAT3, Tbet, CD58 and CD57 markers compared to the healthy control counterpart.

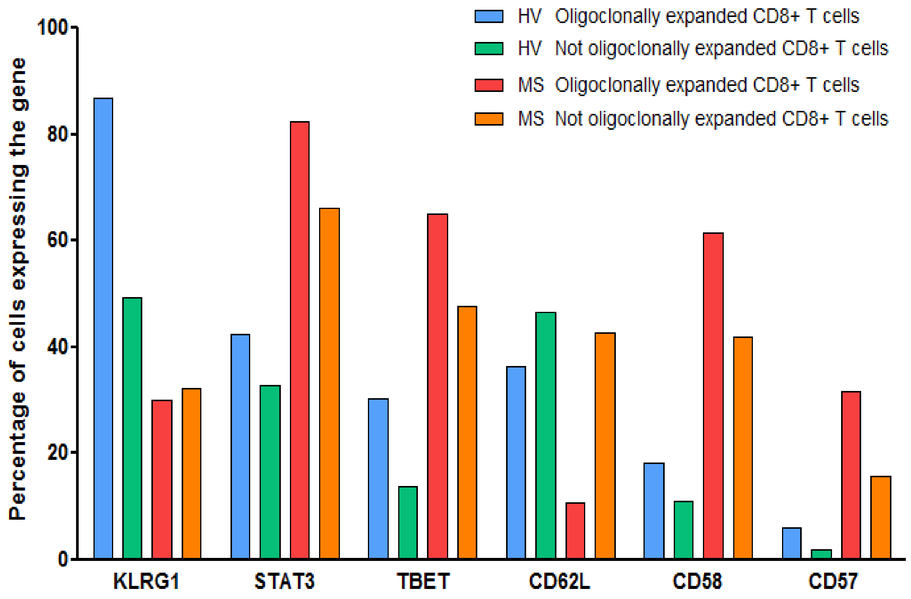



Figure 4 : Histograms representing the percentage of cells expressing the gene in oligoclonally expanded cells or not from MS patients or healthy control

## CONCLUSION

Our data are the first to describe a **specific molecular pattern** of memory CD8⁺T cells from blood and CSF in MS patients and to link this pattern to the cell clonality. We detect a specific signature of MS single cells strongly orienting the cells toward an **activated, effector and cytotoxic profile and allowing the cells to migrate to the CNS**. We also highlight a **specific profile of oligoclonally expanded memory CD8⁺T** cells of MS patients compared to those of healthy volunteers.

## DISCLOSURES

This project is support by ARSEP Foundation and Association ANTARES. DE, VI, GA, NB, MJ, JFM, JN, LFF, TK, NA, BL and GPA have nothing to disclose. WS, ML and LDA received honoraria for lectures from Biogen, Novartis, Sanofi-Gensyme and Merck.