Peripheral Myeloid-Derived Suppressor Cells are good biomarkers of response and efficacy for Fingolimod treatment in multiple sclerosis

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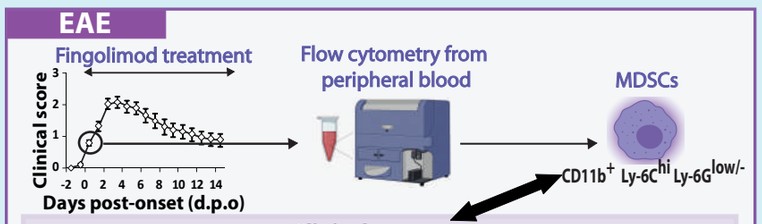
INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Around 85% of patients present the relapsing-remitting (RRMS) form of the disease, which is characterized initially by episodes of neurological dysfunction (relapses) followed by partial, or full recovery. The current knowledge on MS immunology has led to the approval of 17 treatments for RRMS providing a wide range of options available for neurologists and patients. This fact highlights the need to find biomarkers that help clinicians to determine what is the most effective treatment for each patient, avoiding side effects, reducing costs to the Health System pursuing personalized medicine. Fingolimod (FTY720), an oral disease-modifying drug approved for RRMS, acts as a sphingosine-1-phosphate receptor modulator (S1PR) and can bind to S1PR1-5. Fingolimod efficiently prevents lymphocyte egress from lymphoid tissues via internalization and degradation of S1PR1. Nevertheless, it has been described some other biological actions over other CNS or immune cells. Fingolimod promotes the immunosuppressive activity of Myeloid-Derived Suppressor Cells (also known as Ly-6C^{hi} cells), a heterogeneous population of the innate immune response whose monocytic fraction (M-MDSCs) can be used as biomarker of disease severity and tissue damage extent in MS. In the present work, we interrogate whether the abundance of Ly-6C^{hi} cells at the onset of the MS model, experimental autoimmune encephalomyelitis (EAE) or the abundance M-MDSCs prior to initiate the treatment in MS patients, can be related to a high efficacy to fingolimod.

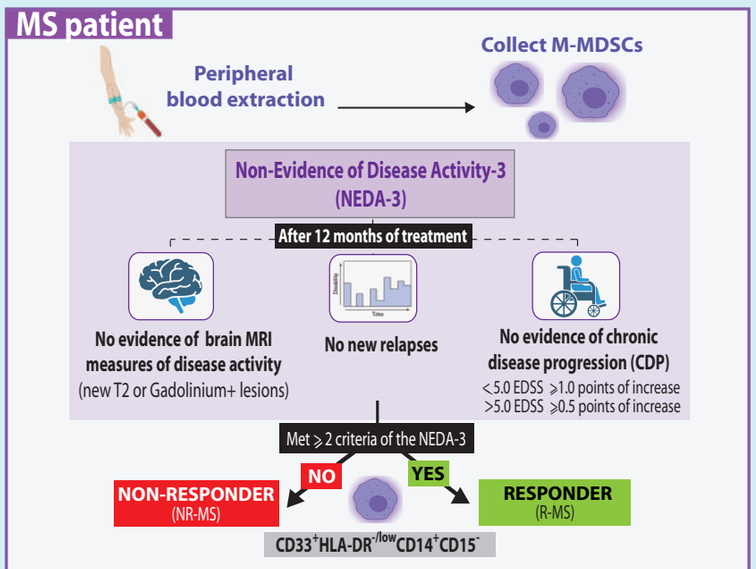
MATERIAL AND METHODS

EAE



Ly-6C^{hi} cells were analyzed in the peripheral blood during the onset (C.S. = 0.5 -1.5) of the disease course of the EAE mouse model. Animals were treated on a daily basis with 3 mg/Kg FTY720 (kindly provided by Novartis Pharma) or its respective vehicle by oral gavage. Animals were individually followed-up till the end of treatment (14 dpo). A correlation analysis was carried out between the peripheral blood Ly-6C^{hi} cells and the future clinical outcome. Vehicle group, N=19; Fingolimod group, N=18.

MS patient



All patients had an indication of fingolimod treatment with 0.5 mg/day according to the current recommendations of use for fingolimod. M-MDSCs were analyzed in the peripheral blood of MS patients before the start of fingolimod treatment. Patients were classified as responder (R-MS) or non-responder (NR-MS) according to Non-Evidence of Disease Activity-3 criteria (NEDA-3) in order to find differences in M-MDSC abundance between two subgroups. A patient was considered as R-MS to fingolimod treatment when met two or more of the criteria of NEDA-3 12 months after treatment. N=31.

RESULTS

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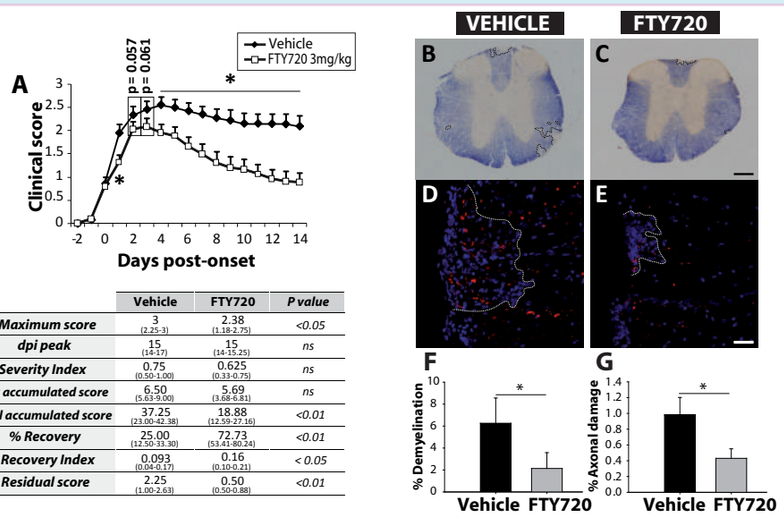

	Vehicle	FTY720	*P value*
Maximum score	3 (2.25-3)	2.38 (1.18-2.75)	*<0.05*
dpi peak	15 (14-17)	15 (14-15.25)	*ns*
Severity Index	0.75 (0.50-1.00)	0.625 (0.33-0.75)	*ns*
Peak accumulated score	6.50 (5.63-9.00)	5.69 (3.68-6.81)	*ns*
Total accumulated score	37.25 (23.00-42.38)	18.88 (12.59-27.16)	*<0.01*
% Recovery	25.00 (12.50-33.30)	72.73 (53.61-80.24)	*<0.01*
Recovery Index	0.093 (0.04-0.17)	0.16 (0.10-0.21)	*< 0.05*
Residual score	2.25 (1.00-2.63)	0.50 (0.50-0.88)	*<0.01*

Figure 1. Individualized fingolimod treatment accelerates disease recovery and decrease the histopathological damage in EAE. A: The individualized fingolimod treatment from disease onset significantly reduced the clinical course severity in EAE mice (EAE-Vehicle: n= 19; EAE-FTY720: n=18). **B-C:** Panoramic views of the spinal cord of one EAE-Vehicle (**B**) and one EAE-FTY720 (**C**) mouse stained with EC (myelin, blue). Dashed lines delimitate the demyelinated area. **D-E:** Detailed view of SMI-32 staining (axonal damage, red) in infiltrated areas (delimitated with dashed lines) of the spinal cord of one EAE-Vehicle (**D**) and one EAE-FTY720 (**E**) mouse (nuclei are stained with Hoechst). **F-G:** Quantification of the % of demyelination within the white matter or the % of axonal damage within the infiltrated area in the histological sub-cohort of EAE mice (EAE-Vehicle: n= 9; EAE-FTY720: n=9). Scale bar: B-C= 250 µm; D-E = 50 µm.

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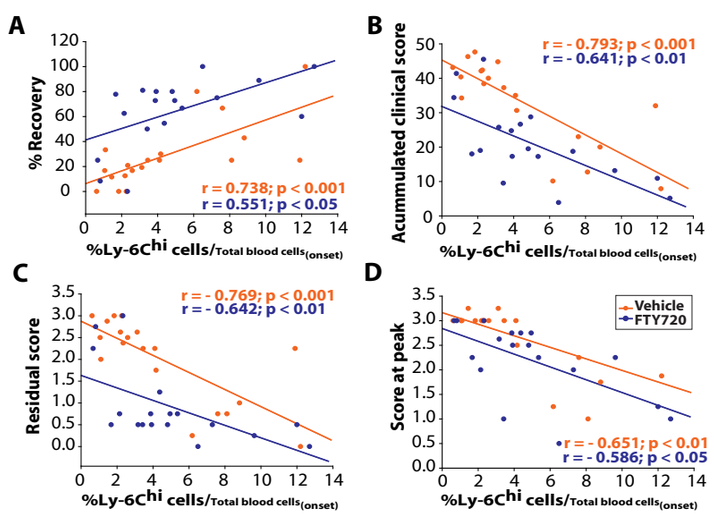

Figure 2. Ly-6C^{hi} cell abundance at the onset of symptoms are related to future milder disease courses. A-D: Circulating Ly-6C^{hi} cells at disease onset directly correlated with the % of recovery (**A**) and inversely correlated with the total accumulated clinical score (**B**), the residual score (**C**) and the score at peak (**D**) in both EAE-Vehicle and EAE-FTY720 mice. Spearman correlation test was carried out.

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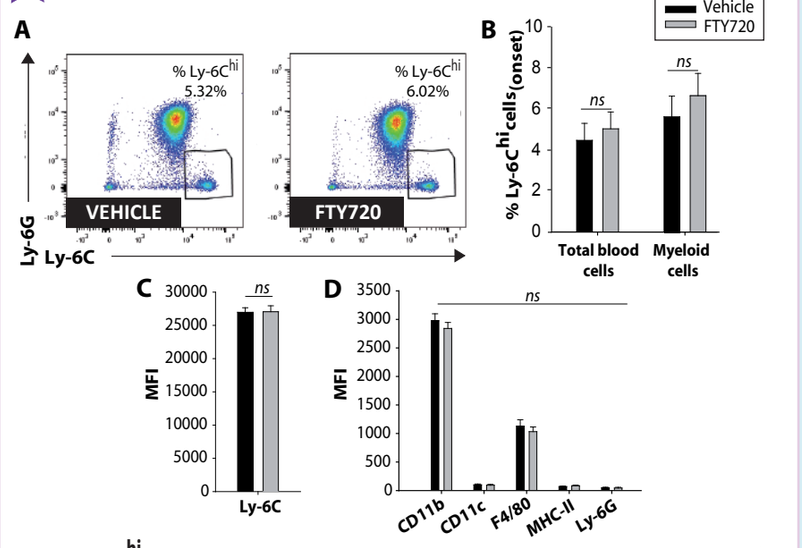

Figure 3. Ly-6C^{hi} cells are homogeneously represented in EAE-Vehicle and EAE-FTY720 mice before treatment. A: Representative plots of Ly-6C^{hi} cells from EAE mice that will be treated with vehicle (left panel) or fingolimod (right panel). **B**: The abundance of Ly-6C^{hi} cells relative to the total blood cells or to the myeloid component at disease onset was similar between both experimental study groups prior to treatment beginning. **C-D**: The MFI of the different markers of Ly-6C^{hi} cells did not show differences previous to treatment start. Student's t test was carried out.

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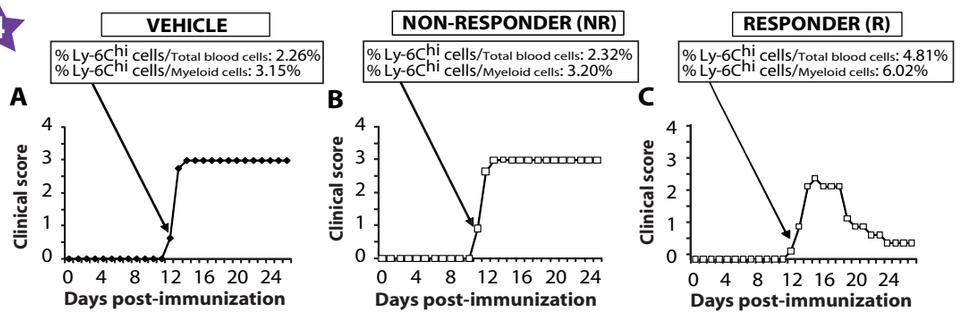

Figure 4. Circulating Ly-6C^{hi} cell levels are dramatically lower in fingolimod non-responder EAE mice. A-C: Representative clinical course graphs of one typical EAE-Vehicle (**A**), one NR (**B**) and one R EAE-FTY720 mouse (**C**). EAE-Vehicle animals developed a severe clinical course undistinguishable from NR EAE-FTY720 mice. **D-E**: NR EAE-FTY720 mice (n = 3) showed a much lower abundance of circulating Ly-6C^{hi} cells within the total blood cells (**D**) or within the myeloid component (**E**) than R EAE-FTY720 mice (n=15) prior to treatment initiation. Student's t test was carried out in **D-E**.

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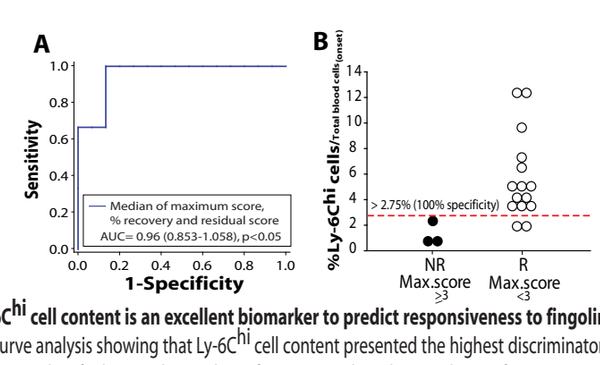

Figure 5. Ly-6C^{hi} cell content is an excellent biomarker to predict responsiveness to fingolimod in EAE mice. A: ROC curve analysis showing that Ly-6C^{hi} cell content presented the highest discriminatory ability for R and NR EAE mice classified using the median of maximum clinical score, the % of recovery or the residual score. **B**: Cut-off value for the Ly-6C^{hi} cells/Total blood cells to predict a good responsiveness to fingolimod. AUC: area under the curve. N= 18 mice.

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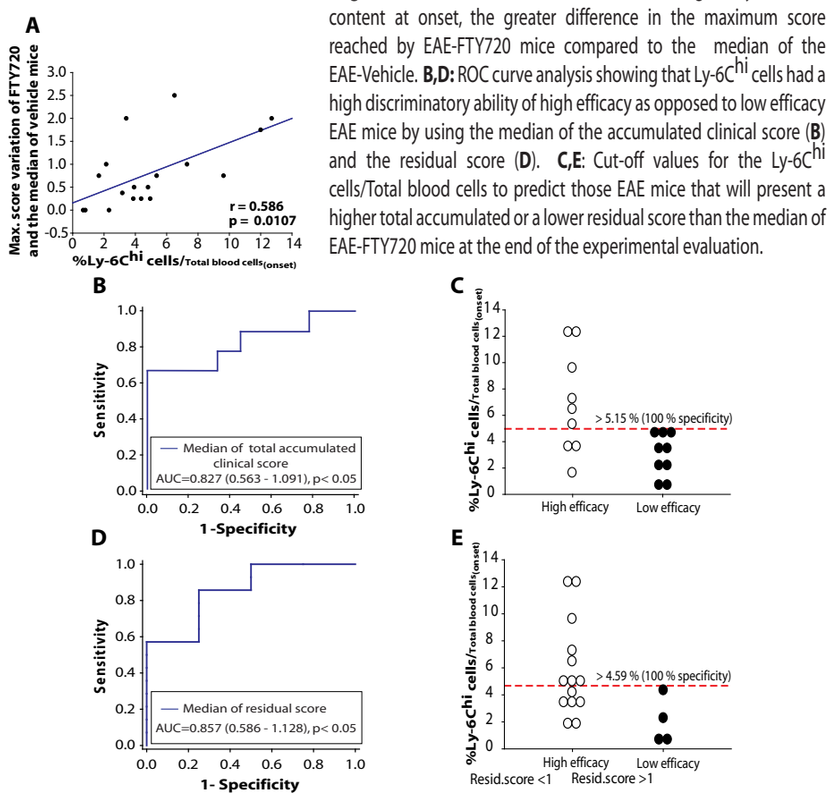

Figure 6. Ly-6C^{hi} cells are a good biomarker for the efficacy of fingolimod treatment in EAE mice. A: The higher Ly-6C^{hi} cell content at onset, the greater difference in the maximum score reached by EAE-FTY720 mice compared to the median of the EAE-Vehicle. **B,D:** ROC curve analysis showing that Ly-6C^{hi} cells had a high discriminatory ability of high efficacy as opposed to low efficacy EAE mice by using the median of the accumulated clinical score (**B**) and the residual score (**D**). **C,E**: Cut-off values for the Ly-6C^{hi} cells/Total blood cells to predict those EAE mice that will present a higher total accumulated or a lower residual score than the median of EAE-FTY720 mice at the end of the experimental evaluation.

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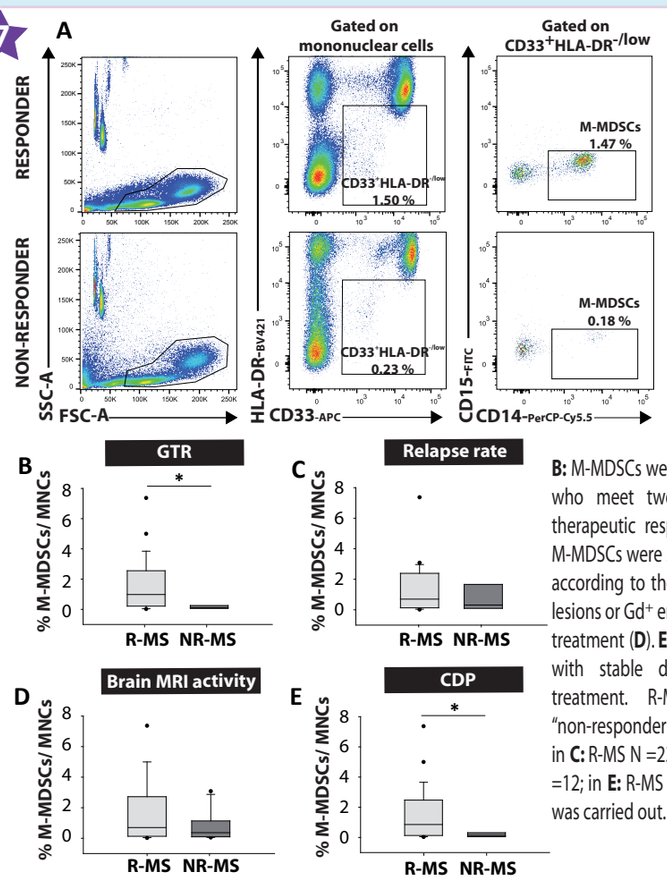

Figure 7. Circulating M-MDSCs are more abundant at baseline in patients with a good therapeutic response to fingolimod at 12 months. A: Flow cytometry strategy of analysis for M-MDSCs in human PBMCs samples. In both "responder" and "non-responder" MS patients, immature myeloid cells were gated from mononuclear fraction as CD33^{+}HLA-DR$^{-/low}$ populations. CD33^{+}HLA-DR$^{-/low}$ cells were assessed for CD14 and CD15 expression to identify the M-MDSC subset defined as CD14^{+}CD15^{-}. **B:** M-MDSCs were more abundant in R-MS patients (those who meet two or more criteria for NEDA-3: good therapeutic response –GTR) than NR-MS patients. **C-D:** M-MDSCs were similar between R-MS and NR-MS patients according to the absence of new relapses (**C**), or new T2 lesions or Gd^{+} enhancing lesions in MRI after 12 months of treatment (**D**). **E:** M-MDSCs were clearly higher in patients with stable disability 12 months after fingolimod treatment. R-MS: "Responder"-MS patients; NR-MS: "non-responder"-MS patients. **B:** R-MS N =25, NR-MS N =6; in **C:** R-MS N =23, NR-MS N =8; in **D:** R-MS N =19, NR-MS N =12; in **E:** R-MS N =26, NR-MS N =5. Mann-Whitney t-test was carried out.

CONCLUSIONS

1) The individualized treatment is a good alternative to study the efficacy of fingolimod.

2) A higher number of Ly-6Chi cells at disease onset correlates with less severe EAE clinical courses of EAE regardless of having received fingolimod or vehicle.

3) Non-responder animals to fingolimod showed lower levels of Ly-6Chi cells in the peripheral blood before initiation of treatment.

4) Ly-6Chi cells analyzed at disease onset are excellent biomarkers of responsiveness and efficacy of fingolimod treatment.

5) M-MDSCs are highly represented in MS patients who will exhibit a good therapeutic response to fingolimod.

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