



# Immune response to the third COVID-19 vaccine dose is related to lymphocyte count in multiple sclerosis patients treated with fingolimod

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## Abstract

**Background** The majority of multiple sclerosis [MS] patients treated with fingolimod fail to develop a protective level of IgG humoral and adaptive cellular immune responses following full BNT162b2 SARS-CoV-2 vaccination.

**Objective** To compare the efficacy of the third COVID-19 vaccine dose in vaccine non-responders fingolimod-treated MS patients.

**Study design** This is a prospective 3-month, single-center, randomized clinical trial.

**Methods** Twenty relapsing MS patients who had been on fingolimod therapy  $\geq 12$  months and failed to develop humoral IgG immune response to 2-dose Pfizer BNT162b2 COVID-19 vaccination were randomized into two groups: fingolimod-continuation group and fingolimod-discontinuation group. Humoral and memory cellular immune responses were assessed within 1 and 3 months following the third Pfizer BNT162b2 vaccine dose and compared between the groups.

**Results** A higher rate of patients in the fingolimod-discontinuation group [ $n = 8/10$ ] compared to fingolimod-continuation group [ $n = 2/10$ ] developed positive SARS-COV-2 IgG. Median IgG titer 1 month following the third dose was 202.3 BAU/ml vs. 26.4 BAU/ml, respectively,  $p = 0.022$ . The development of IgG humoral response correlated with absolute lymphocyte count. Specific SARS-COV-2 memory B cell and T cell immune responses were not detected in both groups, either at 1 month or 3 months following the third COVID-19 vaccine dose.

**Conclusions** Short period of fingolimod treatment discontinuation was associated with the development of humoral protection but not with adaptive cellular immunity.

**Keywords** COVID-19 · Multiple sclerosis · Fingolimod · Third BNT162b2 SARS-CoV-2 vaccine dose · IgG antibody · Humoral immunity · Cellular immunity

## Introduction

It is well known that high-efficacy disease modifying therapies (DMTs) induce immunomodulation associated with lymphocyte depletion involving T cells, B cells, or both [1, 2]. Fingolimod acts as an antagonist of sphingosine-1-phosphate receptor, and therefore, prevents lymphocyte egression from secondary lymphoid tissues and marked

peripheral blood lymphopenia [3, 4]. There are cumulative data reporting that MS patients treated with fingolimod failed to develop humoral as well as cellular immune responses due to low lymphocyte counts and thus are not protected from COVID-19 infection [5–7]. A significant decrease in the antibody level was associated with the presence and degree of lymphopenia [8, 9]. Inability to achieve immune protection can have deleterious effects for patients as they are at a greater risk of COVID-19 infection, need to preserve social distance and avoid social interactions that can lead to increased psychological stress [10, 11]. Moreover, these patients may risk the community for a greater viral transmission, and therefore, it is mandatory to assess possible benefits from additional SARS-CoV-2 vaccination.

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In the current study, our primary objective was to weigh the effect of the third vaccine dose to induce protective immunity in vaccination-failed MS patients treated with fingolimod. The fingolimod-discontinuation protocol was planned to enable patients to increase peripheral blood lymphocyte count and achieve immune protection benefits following the third COVID-19 vaccine dose.

## Methods

### Study design and participants

This is a prospective, open-label, single-center, randomized clinical trial. MS patients followed at the Sheba Multiple Sclerosis Center, Tel-Hashomer, Israel, were enrolled in this study. Inclusion criteria were (1) relapsing–remitting MS according to McDonald criteria [12]; (2) age  $\geq$  18 years; (3) under treatment with fingolimod for at least 12 months; (4) received full 2-dose Pfizer BNT162b2 COVID-19 vaccination; (5) failed to develop COVID-19 IgG antibody response; (6) signed written informed consent. Exclusion criteria were: (1) cognitive decline that prevented understanding of the study design; (2) active MS during the 12 months prior to the study manifested as the occurrence of an acute MS relapse or the appearing of active gadolinium enhancing or new T2 lesions on brain MRI. Participants were randomized into either fingolimod-continuation group or to fingolimod-discontinuation group. Fingolimod-discontinuation patients stopped the medication before receiving the third COVID-19 vaccine dose in attempt to increase peripheral blood lymphocyte count above 1000 cells/mm<sup>3</sup>. Fingolimod-continuation patients continued treatment with oral fingolimod at a dose of 0.5 mg daily. All patients received the third dose of Pfizer BNT162b2 COVID-19 vaccination within 6 months after the second dose. Humoral IgG and memory cellular immune responses were assessed within 1 and 3 months following the third Pfizer BNT162b2 vaccine dose and compared between groups.

### COVID-19 third vaccine dose

Patients received the third Pfizer BNT162b2 COVID-19 vaccine dose by an intramuscular injection delivered in the deltoid muscle. The injection contained 30  $\mu$ g of BNT162b2 (0.3 ml volume per dose).

### Lymphocyte count

Peripheral blood absolute lymphocyte counts (ALC, cells/mm<sup>3</sup>) were determined on a DxI hematology analyzer (Beckman Coulter USA), at the time of the third COVID-19 vaccine dose.

### SARS-CoV-2 IgG antibodies detection of SARS-CoV-2 IgG antibodies

Immunoassay for the detection of SARS-CoV-2 IgG antibodies in blood samples was performed using anti-SARS-CoV-2 QuantiVac ELISA IgG (Euroimmun, Lubeck, Germany), based on the S1 domain of the spike protein. Cutoff for positive IgG level was determined as  $> 35.2$  binding antibody units (BAU)/ml. The test has a sensitivity of 93.2% and a specificity of 100%. Correlation with neutralization tests was reported to be 98.2% (<https://www.euroimmun.de/en/>).

### Post-COVID-19 vaccination immune responses: memory B cells specific for SARS-CoV-2 RBD and memory T cells secreting IFN $\gamma$ and IL2 in response to SARS-CoV-2 peptides

Peripheral blood mononuclear cells (PBMCs) were isolated from EDTA-K2 anticoagulant whole blood using Ficoll-Hypaque (GE Healthcare, USA) gradient centrifugation.

#### Memory B cells

Analysis of SARS-CoV-2-specific memory B cells was performed by FluoroSpot assay detecting receptor-binding domain (RBD)-specific memory B cell following polyclonal B cell stimulation. We used reversed antigen human IgG SARS-CoV-2 RBD ELISpotPLUS (Mabtech, Sweden) according to manufacturer instructions. Briefly, PBMCs were incubated (250,000 cells/well) on an anti-IgG FluoroSpot plate after stimulation with a mixture of R848 (1 mg/ml) and IL2 (10 ng/ml) B-Cell stimpack, Mabtech, Sweden. The number of SARS-CoV-2-specific IgG secreting B cells was measured as Spot-Forming Units (SFU) using Mabtech IRIS<sup>TM</sup> reader. The results were expressed as the number SFU per 250,000 seeded cells after subtracting the background of unstimulated cells. Positive cutoff value was set above the 90% confidence interval in healthy non-vaccinated subjects ( $n = 10$ , cutoff = 5.0 SFU).

#### Memory T cells

IFN $\gamma$ - and IL2-secreting memory T cells were detected using SARS-CoV-2 FluoroSpot<sup>PLUS</sup> kit according to the manufacturer's protocol (Mabtech AB, Sweden). We used pre-coated plates with captured monoclonal anti-IFN $\gamma$  and anti-IL2 incubated overnight in RPMI-1640 medium containing 10% FCS supplemented with SARS-CoV-2 peptide pools (Mabtech AB, Sweden), covering the S1 domain (166 peptides), [13]. Anti-CD3 response served as positive control for

polyclonal T cell activation. Results for any memory T cell memory response to any S1 spike peptide were expressed as the number of SFU per 250,000 seeded cells after subtracting the background spots of healthy non-vaccinated subjects ( $n = 10$ , cutoff for S1 = 3.0 SFU). Results of ELISpot and Fluorospot assays were evaluated using IRIS-reader and analyzed by IRIS software version 1.1.9 (Mabtech AB).

### Peripheral blood lymphocyte sub-populations

Lymphocyte sub-populations including CD3, CD4, and CD8 T cells, CD19 B cells, IgD-CD27 + CD19 + class switch and IgD + CD27 + CD19 + non-class switch memory B cells, were analyzed using flow cytometry in three MS patients; one patient who stopped fingolimod treatment and developed humoral SARS-CoV-2 IgG response following the third vaccine dose, and two patients who continued fingolimod treatment and did not develop SARS-CoV-2 antibody response. Results are expressed as counts of cells/mm<sup>3</sup>.

### Statistical analysis

Categorical variables are described giving sample size, frequency, and percentage by study group. Continuous variables are reported by sample size, median and 25–75 interquartile (IQR) range values. The differences between qualitative variables were tested with the Chi-square test. The differences between the quantitative variables were tested with the parametric independent sample *t* test and non-parametric Mann–Whitney test for the comparison between the two groups. Correlation analysis was performed using the Pearson correlation test. A *p* value < 0.05 was considered statistically significant. Data were analyzed using the SAS® version 9.4 (SAS Institute, Cary North Carolina) and Python software (version 3.0).

## Results

Twenty MS patients were included in the study, 10 were randomized into the fingolimod-continuation group and 10 into the fingolimod-discontinuation group.

Patients in the study were followed for a period of up to 8 months. One patient in the fingolimod-continuation group had an acute relapse within 4.6 months, and one patient in the fingolimod-discontinuation group had an acute relapse within 5.2 months. Clinical and immune-related variables are presented in Table 1.

### Fingolimod-continuation group

On the day of administration of the third COVID-19 vaccine dose, the median ALC was 775 cells/mm<sup>3</sup> (25–75 IQR

572–817 cells/mm<sup>3</sup>). One month following the third dose only two patients (20%) developed positive SARS-CoV-IgG. SARS-CoV-2 IgG antibody titer 1 month following the third vaccine dose was median 26.4 BAU/ml (25–75 IQR 3.5–29.7 BAU/ml), and decreased in 3 months by 46.2%.

### Fingolimod-discontinuation group

On the day of the third vaccine dose, the median ALC was 1510 cells/mm<sup>3</sup> (25–75 IQR 1190–1668 cells/mm<sup>3</sup>); one month following the third dose, 8 patients (80%) developed positive SARS-CoV-2 IgG. SARS-CoV-2 IgG antibody titer 1 month following the third vaccine dose was median 202.3 BAU/ml (25–75 IQR 49.3–580.4 BAU/ml), decreased in 3 months by 34.9%, and two patients became seronegative.

Figure 1 demonstrates the correlation between SARS-CoV-2 IgG levels and lymphocyte counts in MS patients who continued and patients who stopped treatment with fingolimod at 1 and 3 months following the third vaccine dose, showing the significance of higher count with protective humoral immunity.

SARS-CoV-2-specific memory B cells and IFN $\gamma$  and IL2-secreting memory T cells were not detected in any of the patients either in the fingolimod-continuation group or the fingolimod-discontinuation group, at 1 and 3 months following the third vaccine dose.

### Peripheral blood lymphocyte sub-populations

CD19 B cells in the patient who stopped fingolimod treatment and developed humoral SARS-CoV-2 IgG response following the third vaccine dose was 202 cells/mm<sup>3</sup> (normal range 50–300 cells/mm<sup>3</sup>); CD19 B cells in the two patients who continued fingolimod treatment and did not develop SARS-CoV-2 antibody response were much lower, 4 and 10 cells/mm<sup>3</sup> were detected.

IgD-CD27 + CD19 + class switch and IgD + CD27 + CD19 + class non-switch memory B cells were not detected in the patient who stopped fingolimod treatment and developed humoral SARS-CoV-2 IgG response following the third vaccine dose, and neither in the two patients who continued fingolimod treatment and did not develop SARS-CoV-2 antibody response.

CD4 T cells were 155 cells/mm<sup>3</sup> (normal range 436–1394 cells/mm<sup>3</sup>) and CD8 T cells were 715 cells/mm<sup>3</sup> (normal range 166–883 cells/mm<sup>3</sup>) in the patient who stopped fingolimod treatment following the third vaccine dose, while both CD4 and CD8 were decreased in the two patients who continued fingolimod treatment (CD4 T cells were 4 and 19 cells/mm<sup>3</sup> and CD8 were 35 and 104 cells/mm<sup>3</sup>) (Suppl Table 1).

**Table 1** Demographic, clinical, and COVID-19 third vaccine dose immune-related variables

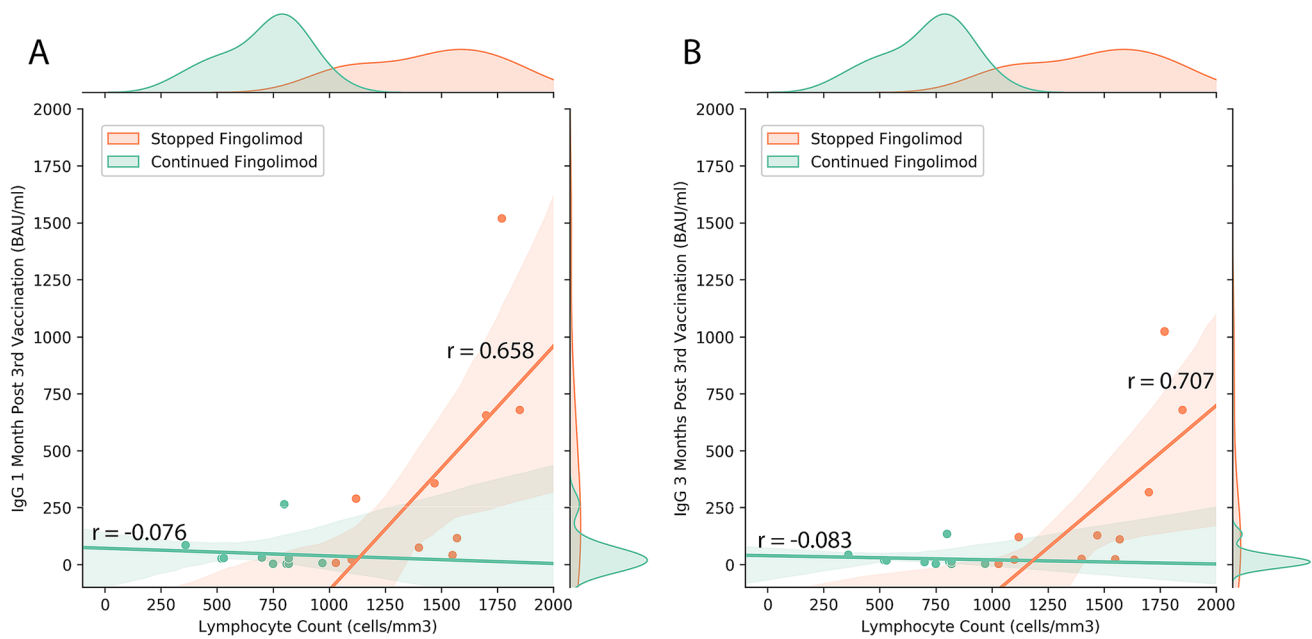
| Study population   | MS patients<br><i>N</i> = 20                     |   | <i>p</i> |
|--|--|---|----------|
|  | Group 1 fingolimod-continuation<br><i>N</i> = 10 | Group 2 fingolimod-discontinuation<br><i>N</i> = 10 |          |
| Gender, <i>n</i> (%)   |  |   |          |
| Females  | 8 (80.0)   | 4 (40.0)  |          |
| Males  | 2 (20.0)   | 6 (60.0)  |          |
| Age, years   |  |   |          |
| Median   | 51.6   | 47.5  | 0.69     |
| 25–75 IQR  | 46.9–58.1  | 43.0–54.9   |          |
| Disease duration, years  |  |   |          |
| Median   | 20.9   | 15.9  | 0.10     |
| 25–75 IQR  | 18.3–23.5  | 6.5–18.9  |          |
| Disability by EDSS   |  |   |          |
| Median   | 2.25   | 2.0   | 0.89     |
| 25–75 IQR  | 1.1–2.9  | 1.5–3.6   |          |
| Fingolimod Tx duration, years                                      |  |   |          |
| Median   | 7.25   | 4.8   | 0.15     |
| 25–75 IQR  | 5.6–8.3  | 2.5–8.4   |          |
| Time from 2nd to 3rd vaccine dose, months                          |  |   |          |
| Median   | 6.6  | 6.2   |          |
| 25–75 IQR  | 6.4–7.5  | 6.1–6.2   | NS       |
| Time without fingolimod treatment, months                          |  |   |          |
| Median   |  | 4.2   |          |
| 25–75 IQR  |  | 2.5–4.4   |          |
| ALC at 3rd vaccine dose cells/mm <sup>3</sup>                      |  |   |          |
| Median   | 775  | 1510  | < 0.0001 |
| 25–75 IQR  | 572–817  | 1190–1668   |          |
| Patients with positive SARS-COV-2 IgG, 1 month<br><i>N</i> , (%)   | 2 (20)   | 8 (80)  |          |
| SARS-COV-2 IgG titer, BAU/ml, 1 month                              |  |   |          |
| Median   | 26.4   | 202.3   | 0.022    |
| 25–75 IQR  | 3.5–29.7   | 49.3–580.4  |          |
| Patients with positive SARS-COV-19 IgG, 3 months<br><i>N</i> , (%) | 2 (20)   | 6 (60)  |          |
| SARS-COV-2 IgG titer, BAU/ml, 3 months                             |  |   |          |
| Median   | 12.1   | 115   | 0.0296   |
| 25–75 IQR  | 4.8–18.4   | 23.3–269.8  |          |
| % SARS-COV-2 IgG decay within 3 months                             | 46.2   | 34.9  |          |

## Discussion

In the current study, we evaluated whether third vaccine dose will be effective to induce immune response in fingolimod-treated patients who failed to develop SARS-COV-2 antibodies after full vaccination of two doses. As we suspected, the failure to develop this response was related to the low peripheral blood lymphocyte count; MS patients were offered the option to discontinue treatment for several weeks until the ALC would increase to above 1000 cells/mm<sup>3</sup>. This approach led to beneficial humoral immune protection

in the discontinuation group as eight of ten patients succeeded to develop IgG response following the third vaccine dose. Thus, the short period of fingolimod discontinuation led to raised ALC which was sufficient to generate humoral immune response. The re-appearing lymphocytes were effective to mount short-lived plasma cells and following antigenic stimulation by the third vaccine dose, naïve B cells underwent clonal expansion and produced antibodies.

These findings suggest that successful immune response can be developed and sustained once peripheral lymphocyte count increases. Moreover, re-starting fingolimod treatment



**Fig. 1** Correlation analysis between SARS-COV-2 IgG levels and ALC in MS patients who continued and patients who stopped treatment with fingolimod at 1 and 3 months following the third Pfizer

BNT162b2 COVID-19 vaccine dose, showing the significance of higher ALC with protective humoral immunity

will probably reduce again the number of lymphocytes but will not affect the existing anti-S1 immunoglobulin that block viral entry. The humoral response following the third vaccine dose persisted for 3 months and only two patients became seronegative.

Our suggestion to monitor ALC as a marker for successful humoral immune response is supported by the high correlation between the SARS-COV-2 IgG level and ALC. However, the expectation that discontinuation of fingolimod will result in adaptive cellular response was not materialized, as none of these patients recovered SARS-COV-2-specific memory B or T cells. These findings are consistent with the known effects of fingolimod treatment that cause a significant decrease by 9.6-fold in the fraction of memory B cell subsets as compared to a decrease of 3.3-fold in naive B cells, and a decrease by 2.5-fold in plasmablasts [14]. In addition, activated memory B cells and non-class switch and class switch memory B cells were preferentially reported to decrease in fingolimod-treated MS patients [15, 16], as seen in the peripheral lymphocyte sub-population analysis in our three patients. The normal number of CD19 B cells which was seen in the MS patient who discontinued fingolimod represents the ability to generate plasma cells producing IgG. The absence of CD27 memory B lymphocytes seen in all 3 MS patients is in concordance with the failure to develop SARS-CoV-2-specific memory B cells.

In relation to the undetected T cell memory response, it was reported that fingolimod also decreases T cells [16],

and therefore, the inability to develop spike-reactive memory T cells is expected. mRNA vaccines generate predominantly spike-specific memory CD4 T cells that are strongly associated with the development of spike-specific B cells [17–19]. However, peripheral lymphocyte reconstitution might be prolonged after fingolimod discontinuation, and therefore, the effect of short-treatment cessation was insufficient to produce adaptive cellular response. Real-life analysis showed that lymphocytes recover in most patients in average at 3 months post-cessation. This may explain the relative safety regarding disease activity in short-treatment discontinuation. However, fingolimod-induced lymphopenia was reported to persist in 22% of patients for at least up to 1 year after fingolimod stop, raising the possibility that in some patients despite treatment cessation, vaccination will not be effective [20, 21].

In summary, the current study analyzed the effect of a third vaccine dose for MS patients treated with fingolimod who failed to mount humoral immune response to COVID-19 vaccination. Our findings demonstrate that a short period of fingolimod discontinuation was associated with increased rate of patients who developed humoral protection but not adaptive memory cellular immunity.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00415-022-11030-0>.

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## Declarations

**Conflicts of interest** On behalf of all the authors, the corresponding author states that there is no conflict of interest.

**Ethical standard** The study was approved by Sheba Medical Center Institutional Review Board [Sheba.SMC-8182-02], and all participants signed written informed consent. Each patient record was coded anonymously to ensure confidentiality during statistical analyses.

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