# Effect of vitamin D on MS activity by disease-modifying therapy class

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## ABSTRACT

**Objective:** To determine whether vitamin D status predicts disease activity in patients with multiple sclerosis (MS) taking interferon-β (IFN), glatiramer acetate (GA), and fingolimod (FTY).

**Methods:** Participants (n = 324) with relapsing-remitting MS on IFN (96), GA (151), or FTY (77) were identified from the Comprehensive Longitudinal Investigation of MS at Brigham and Women's Hospital (CLIMB) Study at the Partners MS Center. FTY-treated participants were analyzed separately because of differences in selection. Serum vitamin 25(OH)D concentration was adjusted for season. We evaluated the relationship between 25(OH)D tertile and time to relapse or gadolinium-enhancing lesion using a Cox model adjusted for age, sex, and disease duration.

**Results:** Higher 25(OH)D was associated with longer time to the combined endpoint in the overall IFN/GA cohort ($p$ for trend = 0.042; hazard ratio [HR] = 0.77) and in the IFN subgroup ($HR_{IFN}$ = 0.58; $p_{IFN}$ = 0.012), but not in GA-treated participants ($p$ = 0.50; HR = 0.89). For gadolinium-enhancing lesions alone, there was a significant association observed in GA and IFN subgroups, although the effect was more pronounced on IFN ($HR_{GA}$ = 0.57; $p_{GA}$ = 0.039 vs $HR_{IFN}$ = 0.41; $p_{IFN}$ = 0.022). No significant associations were found for relapses. For FTY, higher 25(OH)D was associated with longer survival for the combined endpoint ($HR_{FTY}$ = 0.48; $p_{FTY}$ = 0.016) and for relapses ($HR_{FTY}$ = 0.50; $p_{FTY}$ = 0.046), but not for gadolinium-enhancing lesions.

**Conclusions:** Disease activity generally improved with higher 25(OH)D, but this study raises the question of effect modification by treatment class. *Neurol Neuroimmunol Neuroinflamm* 2015;2: e167; doi: 10.1212/NXI.0000000000000167

## GLOSSARY

**CLIMB** = Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital; **COV** = coefficient of variation; **DMT** = disease-modifying therapy; **EDSS** = Expanded Disability Status Scale; **FTY** = fingolimod; **GA** = glatiramer acetate; **Gd+** = gadolinium-enhancing; **HR** = hazard ratio; **IFN** = interferon; **MS** = multiple sclerosis; **RRMS** = relapsing-remitting multiple sclerosis.

Vitamin D deficiency has been associated with increased disease activity in established relapsing-remitting multiple sclerosis (RRMS).[1-7] Protective effects of vitamin D are thought to be related to its immunomodulatory properties, including inhibiting dendritic cell differentiation and $T_H1$ and $T_H17$ cell responses.[8,9] Disease-modifying therapies (DMTs) in multiple sclerosis (MS) have some of the same properties, although they vary with class of drug. To date, the effect of vitamin D has been studied in patients treated with interferon (IFN)–β[4,5,7,10-12] or where treatment was undifferentiated.[6,13] It is unknown whether increased vitamin D status may exert the same protective effect in the majority of patients with MS treated with DMTs other than interferons. We set out to determine the effect of vitamin D status on clinical and MRI outcomes in patients with MS treated with IFN, glatiramer acetate (GA), and fingolimod (FTY).

**METHODS** **Participants.** Three groups of participants contributed to the analysis; all were enrolled from the Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital (CLIMB), a prospective cohort study that began

enrolling participants in 2000.[14] Participants in CLIMB have a clinical visit with complete neurologic examination every 6 months, including the Expanded Disability Status Scale (EDSS), and brain MRI scan every 12 months. All participants were age 18–65 years, EDSS <5, with a diagnosis of RRMS based on McDonald 2005 criteria.[15] The first 2 groups consisted of participants treated with GA or IFN as their initial DMT. The latter included patients on IM IFN-β-1a weekly (Avonex; Biogen, Cambridge, MA), subcutaneous IFN-β-1a TIW (Rebif; Merck Serono, Darmstadt, German), and subcutaneous IFN-β-1b QOD (Betaseron; Bayer HealthCare Pharmaceuticals, Whippany, NJ). Each participant was required to be on monotherapy and have a blood draw within 18 months of treatment onset. The third group consisted of participants on FTY monotherapy. All patients initiating FTY and participating in research at our Center were asked to have a blood draw at the time of their first-dose observation. For participants in all 3 treatment groups, a second blood sample was analyzed where available on the same treatment within 3–18 months after the first. Demographic characteristics of the 3 groups are shown in table 1. We decided at the outset to analyze the FTY-treated cohort separately from the IFN- and GA-treated groups because most FTY participants had previously been treated with other agents and because of the differences in blood sample collection.

**Standard protocol approvals, registrations, and patient consents.** The CLIMB study was approved by the Institutional Review Board at the Brigham and Women's Hospital. All participants provided written informed consent.

**Vitamin D level measurement.** The 25-hydroxyvitamin D (25[OH]D) concentration was measured from serum samples using a chemiluminescent microparticle assay (ARCHITECT from Abbott Laboratories) at Harvard Catalyst Laboratory, Massachusetts General Hospital. The intra-assay coefficient of variation (COV) has been reported as 2.6%–4.6%. Interassay COV was not available. Serum samples were stored at $-80°C$ before use. Testing was conducted in 3 batches: 2 consisting of FTY participants and a final batch including all samples from GA- and IFN-treated participants. The 25(OH)D level was measured in ng/mL (1 ng/mL = 2.496 nmol/L).

The 25(OH)D was adjusted for seasonal variation using a standard model for day of the year (see appendix e-1 at Neurology.org/nn for further details).

**Clinical/radiologic outcome measures.** Our primary outcome measure was development of a relapse or new T1 gadolinium-enhancing (Gd+) lesion on MRI. Relapse was defined as the appearance of new symptoms lasting more than 24 hours in the absence of fever or intercurrent illness and was determined by the treating physician at the biannual clinical visit.[16] Appearance of new Gd+ MRI lesions was based on the neuroradiologist's report. Other secondary outcome measures were relapses alone and disability progression, as defined by an increase of at least 1 for participants with a baseline EDSS between 1 and 5, or at least 1.5 for participants with a baseline EDSS of 0. Progression was not required to be sustained because of limited duration of follow-up.

**Statistical analysis.** *IFN/GA group.* We used survival analysis to explore time from the first 25(OH)D level to the various outcome measures of interest. In the primary analysis, we investigated time to first inflammatory event, defined as time to first relapse or Gd+ lesion on brain MRI. In the secondary analyses, we investigated time to first relapse alone, first Gd+ lesion alone, or disability progression. Additional details are provided in appendix e-1.

*FTY group.* Baseline clinical and demographic characteristics of the FTY cohort are shown in table 1, but were not compared with the IFN/GA cohort because selection was not limited to those on their first DMT. We conducted the same analyses as in the IFN/GA cohort, except that events within 30 days after the first 25(OH)D measurement were excluded since all participants had their first 25(OH)D measurement on the day of FTY initiation, and FTY is known to take at least 30 days before its effects are observed.[17] Cox proportional hazards models were adjusted for age, disease duration, and sex as in the IFN/GA group; however, we did not adjust for the year of treatment initiation because FTY was only available during 2 of the years studied.

**Sensitivity analyses.** See appendix e-1.

**RESULTS** **IFN/GA cohort.** A total of 247 participants met inclusion criteria: 151 on GA and 96 on IFN

| Table 1 | Baseline clinical and demographic characteristics of all 3 treatment groups | | |
|---|---|---|---|
| | IFN | GA | FTY |
| No. | 96 | 151 | 77 |
| Female, n (%) | 66 (68.7) | 105 (69.5) | 60 (77.9) |
| White, n (%) | 91 (94.8) | 140 (93.3) | 67 (90.5) |
| Hispanic, n (%) | 4 (4.2) | 5 (3.3) | 7 (9.1) |
| Age at first vitamin D measurement, y, mean (SD) | 38.9 (9.5) | 39.2 (9.7) | 40.8 (9.8) |
| Disease duration at first vitamin D measurement, y, mean (SD) | 3.3 (3.6)[a] | 4.5 (4.6) | 9.2 (4.7) |
| EDSS at first vitamin D measurement, median (IQR) | 1 (0–2)[a] | 1 (0–1.75) | 1.5 (0.75–2.5) |
| Time from treatment initiation to first vitamin D measurement, y, mean (SD) | 0.724 (0.412) | 0.746 (0.449) | 0 (0) |
| Follow-up interval, y, mean (SD) | 4.5 (3.4) | 4.2 (3.0) | 1.6 (0.6) |
| 25(OH) D level, ng/mL | 24.9 (11.9) | 27.5 (13.0) | 30.8 (13.3) |
| Two 25(OH) D measurements, n (%) | 56 (58) | 94 (62) | 73 (95) |
| Supplementation at first measurement, n (%) | 31 (32.6)[a] | 83 (55.7) | 50 (64.9) |

Abbreviations: EDSS = Expanded Disability Status Scale; FTY = fingolimod; GA = glatiramer acetate; IFN = interferon.
[a] Significant difference from the GA group as indicated by $p < 0.05$.

(table 1). Baseline demographic characteristics were typical of early RRMS. Differences in disease duration ($p = 0.014$) and EDSS ($p = 0.046$) were observed, but the magnitude of these differences was minor. Duration of follow-up was similar in both groups: 4.2 years for GA and 4.5 years for IFN. The share with 2 25(OH)D measurements available was comparable, ranging from 58% to 62% ($p = 0.593$). There was no significant difference in absolute or seasonally adjusted 25(OH)D levels at baseline, although the rate of vitamin D supplementation was greater in the GA group (56% vs 33%; $p = 0.0007$).

The effects of vitamin D on the hazard of events in the overall cohort and individual treatment groups are provided in table 2, and the corresponding Kaplan-Meier curves in figure 1, figures e-1 and e-2. In the overall cohort, there was a decrease of approximately 25% in the hazard of relapse or Gd+ lesion with each tertile increase in 25(OH)D in both unadjusted and adjusted Cox models (table 2, figure 1A). In the IFN treatment group, there was a greater decrease of approximately 40% with each higher 25(OH)D tertile (table 2, figure e-1A). However, in the GA treatment group, there was no significant association between 25(OH)D level and event hazard (table 2, figure e-2A). In the GA group, there was evidence of a potential departure from proportional hazards for the 25(OH)D tertiles ($p = 0.067$ for test of Schoenfeld residuals in adjusted model). The lack of proportional hazards is also shown through the crossing survival curves. No significant interaction between 25(OH)D tertile and treatment was observed (table 2).

There was no statistically significant change in hazard of relapse with higher 25(OH)D levels in the combined cohort or either individual treatment group (figure 1B, figures e-1B and e-2B). A significant departure from proportional hazards was observed in the GA group ($p = 0.020$ for test of Schoenfeld residuals). Conversely, there was significant improvement in new Gd+ lesions with higher 25(OH)D levels in both treatment groups (figure 1C, figures e-1C, and e-2C). Hazard reduction was greater in the IFN group, where there was a 59% decrease in the hazard of new active MRI lesions per 25(OH)D tertile, compared to 43% in the GA group. In the combined cohort, there was a 53% decrease. There was no statistically significant change in disability progression with vitamin D status (figure 1D, figures e-1D and e-2D). No significant interaction between 25(OH)D tertile and treatment was observed for any outcome measure (table 2).

**Sensitivity analyses.** When analyses were repeated using only the first 25(OH)D measurement as the predictor, there was no appreciable change in results, although they became slightly less significant. For example, in the primary analysis examining time to first inflammatory event, the hazard ratio (HR) in the overall cohort was 0.82 ($p = 0.12$) and the HR in the IFN group was 0.66 ($p = 0.046$). Trends across the treatment groups remained the same, with the GA group demonstrating less of a benefit from vitamin D for each outcome measure. When vitamin D status was treated as a continuous variable, results were again consistent (table e-1).

The results likewise remained stable with addition of vitamin D supplementation as either a dichotomous covariate at baseline or time-varying covariate. In the primary analysis, the first approach led to

| Table 2 | Association between 25(OH)D tertiles and time to event in the IFN/GA cohort | | | |
|---|---|---|---|---|
| | IFN/GA | IFN | GA | Interaction p Value |
| **Time to first inflammatory event** | | | | |
| Unadjusted HR (95% CI) | 0.73 (0.57-0.93)[a] | 0.62 (0.40-0.95)[a] | 0.81 (0.60-1.10) | 0.30 |
| Adjusted HR (95% CI) | 0.77 (0.59-0.99)[a] | 0.58 (0.38-0.89)[a] | 0.89 (0.63-1.25) | 0.14 |
| **Time to first relapse** | | | | |
| Unadjusted HR (95% CI) | 0.81 (0.61-1.08) | 0.73 (0.45-1.18) | 0.89 (0.62-1.27) | 0.53 |
| Adjusted HR (95% CI) | 0.86 (0.64-1.16) | 0.66 (0.41-1.09) | 0.92 (0.61-1.38) | 0.31 |
| **Time to first Gd+ lesion** | | | | |
| Unadjusted HR (95% CI) | 0.49 (0.33-0.75)[a] | 0.42 (0.20-0.88)[a] | 0.55 (0.34-0.91)[a] | 0.52 |
| Adjusted HR (95% CI) | 0.47 (0.31-0.73)[a] | 0.41 (0.19-0.88)[a] | 0.57 (0.34-0.97)[a] | 0.40 |
| **Time to first EDSS progression** | | | | |
| Unadjusted HR (95% CI) | 1.17 (0.87-1.57) | 0.88 (0.53-1.45) | 1.39 (0.96-2.02) | 0.19 |
| Adjusted HR (95% CI) | 1.24 (0.90-1.72) | 0.99 (0.58-1.69) | 1.50 (0.97-2.32) | 0.23 |

Abbreviations: CI = confidence interval; EDSS = Expanded Disability Status Scale; GA = glatiramer acetate; Gd+ = gadolinium-enhancing; HR = hazard ratio; IFN = interferon.
[a] Significant effect as indicated by $p < 0.05$.

**Figure 1** Kaplan-Meier survival curves for the interferon/glatiramer acetate cohort
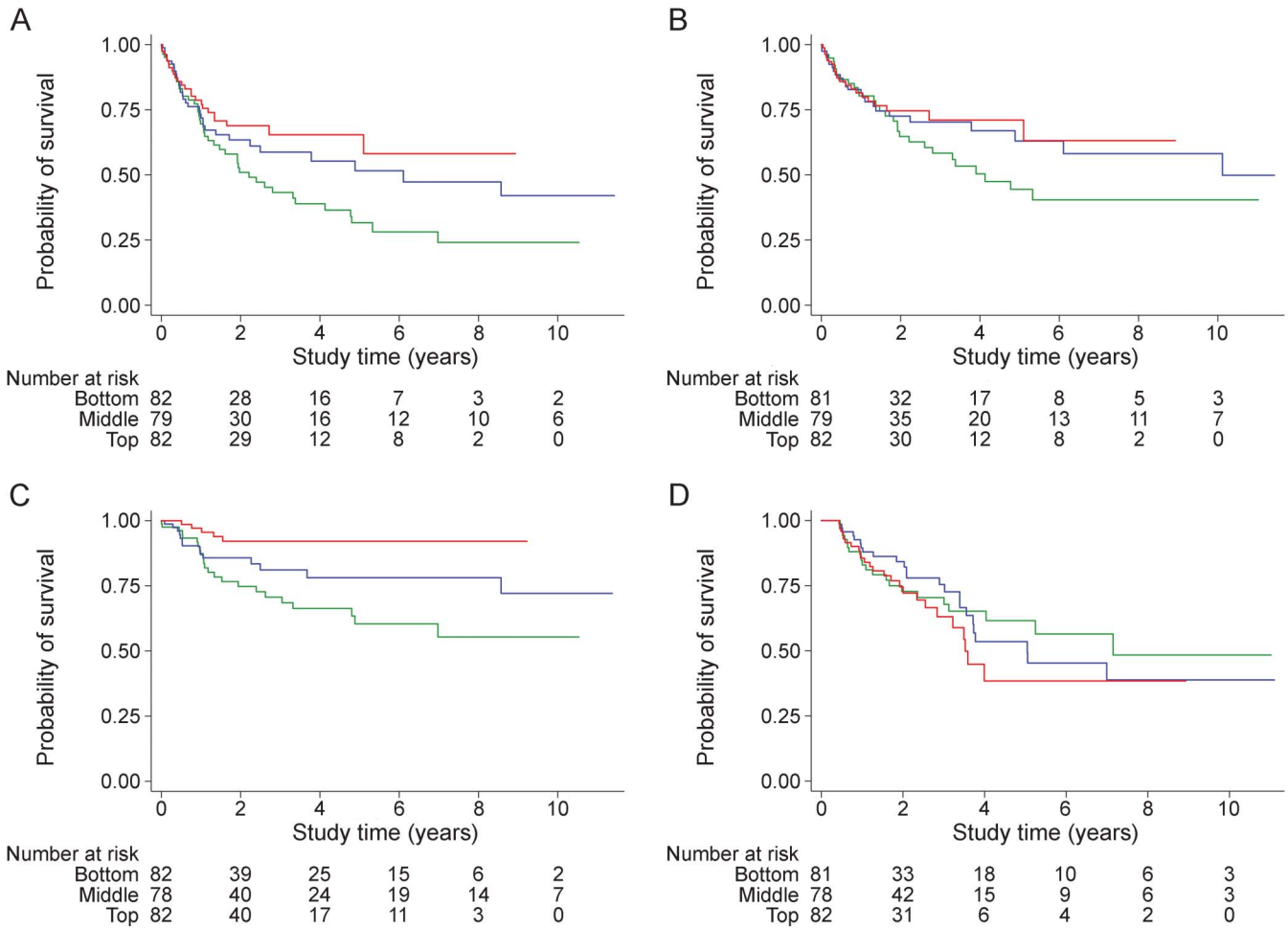
In each graph, bottom tertile: solid line; middle tertile: long dashed line; top tertile: short dashed line. (A) Time to first inflammatory event (relapse or gadolinium-enhancing lesion). (B) Time to first relapse. (C) Time to first gadolinium-enhancing lesion. (D) Time to first Expanded Disability Status Scale progression.

HR = 0.76 ($p$ = 0.044) for the overall cohort. Using the second approach of treating vitamin D supplementation as a time-varying covariate, HR was 0.75 ($p$ = 0.036).

**FTY cohort.** A total of 77 participants initiating FTY were included with mean follow-up of 1.6 years (table 1). FTY participants were older, with longer disease duration and higher baseline EDSS; these findings reflect use of FTY as a second-line therapy. A second blood sample was available for 73 (95%).

Effects of 25(OH)D on the hazard of event are provided in table 3, and the corresponding Kaplan-Meier curves in figure 2. A significant association was observed between 25(OH)D tertile and time to first inflammatory event in both unadjusted and adjusted analyses. For time to next relapse, a significant association was observed in the adjusted analysis. For time to first Gd+ lesion or EDSS progression, there was no significant association between 25(OH)D level and time to event.

**Sensitivity analyses.** When analyses were repeated using only first 25(OH)D measurement as the predictor, the impact of 25(OH)D tertile was smaller, and there was no significant effect. When 25(OH)D level was treated as a continuous variable, results were similar to the primary analysis. Finally, when vitamin D supplementation was included in the model, results remained the same as above.

**DISCUSSION** In this study, we took the approach of examining the effect of vitamin D in MS on DMTs other than IFN. We found that higher 25(OH)D levels conferred a significant protective benefit against new inflammatory events (relapses or Gd+ lesions) in participants treated with IFN, with an effect magnitude similar to what has been reported in previous studies of a 30%–50% reduction in event rate per 10 ng/mL increase in 25(OH)D level.[4,5,10] There was no significant benefit of higher 25(OH)D levels with respect to inflammatory events, relapses, or disability progression in the GA group; there was a significant,

**Table 3** Association between vitamin D tertiles and time to event in the fingolimod cohort

| | HR (95% CI) |
|---|---|
| **Time to first inflammatory event** | |
| Unadjusted | 0.54 (0.30–0.97)[a] |
| Adjusted | 0.48 (0.26–0.87)[a] |
| **Time to first relapse** | |
| Unadjusted | 0.52 (0.27–1.02) |
| Adjusted | 0.50 (0.25–0.99)[a] |
| **Time to first Gd+ lesion** | |
| Unadjusted | 0.77 (0.38–1.57) |
| Adjusted | 0.62 (0.30–1.28) |
| **Time to first EDSS progression** | |
| Unadjusted | 0.82 (0.39–1.74) |
| Adjusted | 0.80 (0.36–1.76) |

Abbreviations: CI = confidence interval; EDSS = Expanded Disability Status Scale; Gd+ = gadolinium-enhancing; HR = hazard ratio.
[a] Significant effect as indicated by $p < 0.05$.

but lesser, protective effect against active MRI lesions alone in the GA group compared to the IFN group. While there were differences in several baseline characteristics between the GA and IFN treatment groups, multivariate adjustment accentuated the gap between the HRs associated with each. However, we should still interpret comparison of the treatment groups with caution because there may have been unmeasured confounders of patient selection for treatment. In the FTY group, which was analyzed separately, we observed an approximately 50% reduction in new inflammatory events and in relapses alone per 25(OH)D tertile increase. Controlling for vitamin D supplementation did not meaningfully alter results in any of the treatment groups.

At the same time, we cannot conclude that the effect of vitamin D in MS is modified by DMT class because there was no statistically significant ($p < 0.05$) interaction when a 25(OH)D* treatment term was included in our models. Moreover, there was evidence of a departure from the proportional hazards assumption in the GA group, which can lead to inaccurate estimation of the effect size based on the Cox model. In particular, assessment of Kaplan-Meier curves in the GA group showed that the hazard in the high 25(OH)D tertile was the highest initially, but then it became the lowest after the initial phase. Thus the lack of significant findings in some of the analyses in the GA group does not necessarily indicate that vitamin D has no effect. The question of whether the protective effect of vitamin D on MS disease activity may be weaker in participants treated with GA as compared to IFN needs to be explored in other

cohorts before leading to any change in recommendations around vitamin D supplementation. An earlier cohort study reported that higher 25(OH)D was protective in participants on IFN but not in controls on GA, mitoxantrone, or no therapy, but the control group was small (n = 46).[10] There is an ongoing randomized controlled trial of vitamin D supplementation on GA, Vitamin D to Ameliorate Multiple Sclerosis (VIDAMS), which should assist in clarifying whether improved vitamin D status may indeed ameliorate MS disease activity on GA.[18] On the other hand, given the lack of a second DMT comparator group, this study will provide no direct information about potential effect modification.

25(OH)D levels were higher in the FTY compared to the IFN/GA cohort, which may be due to greater prevalence of supplementation and higher recommended doses in recent years. In the FTY group, we observed a 50% reduction in the event hazard with higher 25(OH)D levels, despite FTY's independent profound immunomodulatory properties and superior suppression of disease activity rates, at least relative to IM IFN-β-1a.[18] We cannot directly compare this result to event reduction in the IFN/GA group due to restriction of the IFN/GA cohort to participants on first-line therapy and the difference in blood sample collection times relative to treatment initiation. However, it is reasonable to conclude from this effect size that the influence of vitamin D on FTY is relatively comparable to what has been observed in previously untreated or IFN-treated patients with MS.

One possible explanation for differences in efficacy across treatment groups may be related to immunologic mechanisms of each of the treatments and vitamin D. Vitamin D's diverse immunologic functions include inhibiting monocytes, activating T-regulatory cell differentiation, and shifting $T_H1$ to $T_H2$ cellular responses.[8,9,19–21] FTY's mechanism of action is distinct, as it acts on the peripheral immune system to sequester T-lymphocytes in the lymph nodes, thus reducing T-cell trafficking into the CNS.[22] IFN, like vitamin D, promotes $T_H2$ over $T_H1$ responses, but does not affect monocytes or T-regulatory cells to a meaningful extent.[23,24] In contrast, GA shares each of these immune mechanisms with vitamin D. Thus, the immunomodulatory actions of FTY and IFN relative to vitamin D may harmonize to a greater extent, whereas those of GA and vitamin D may be more redundant. Indeed, if this theory were proven true, the observation that GA exerts a weaker effect on Gd+ MRI activity than IFN[22,23] may explain why vitamin D had a greater relative impact on MRI vs clinical metrics in the GA-treated group. Another explanation for differences in efficacy across treatment groups may be that specific genetic alleles

**Figure 2** Kaplan-Meier survival curves for the fingolimod cohort
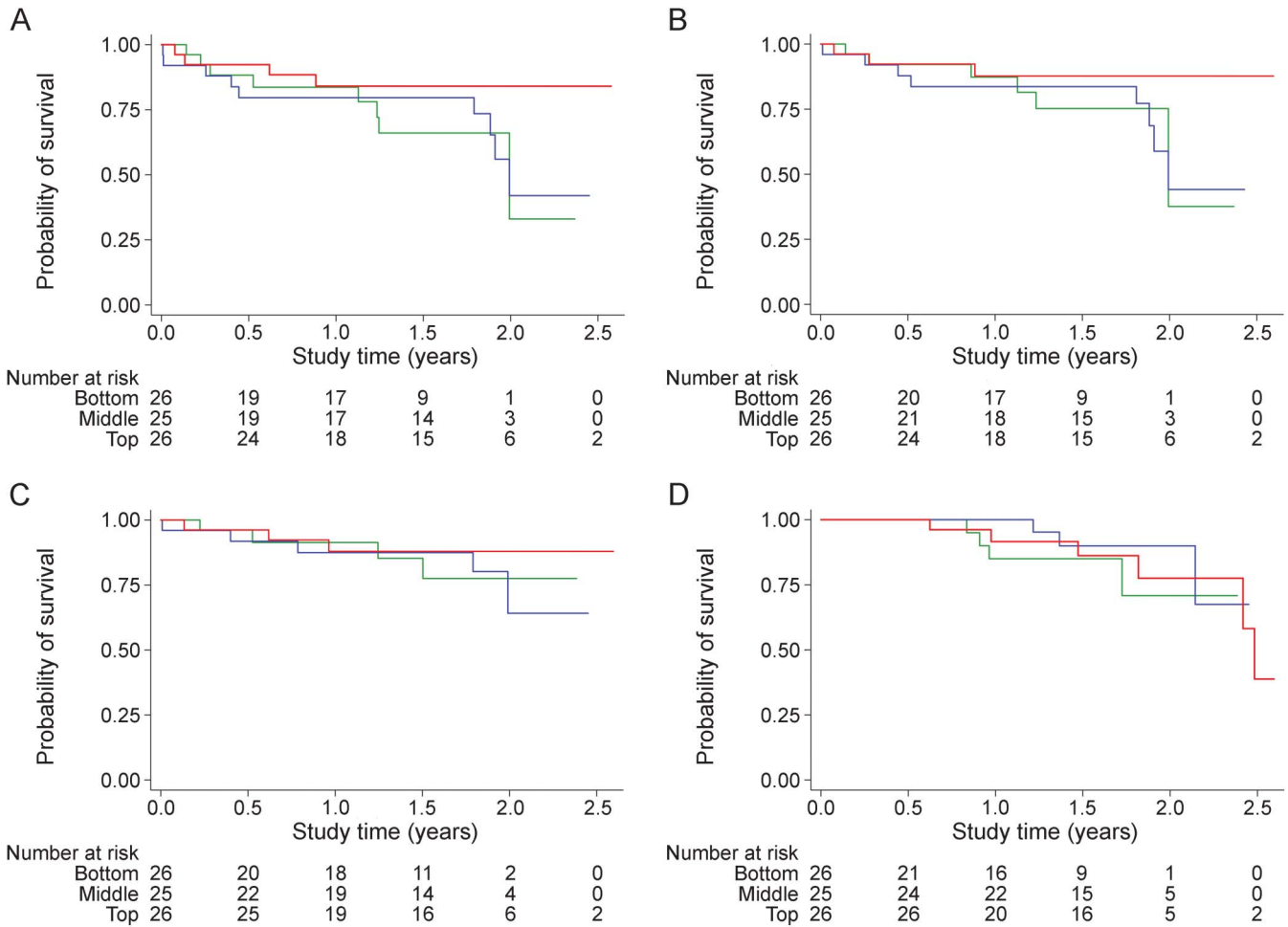
In each graph, bottom tertile: solid line; middle tertile: long dashed line; top tertile: short dashed line. (A) Time to first inflammatory event (relapse or gadolinium-enhancing lesion). (B) Time to first relapse. (C) Time to first gadolinium-enhancing lesion. (D) Time to first Expanded Disability Status Scale progression.

modulate an interaction between vitamin D and some or all of the DMTs. These hypotheses about potential immunologic interactions between vitamin D and the DMTs warrant further investigation.

This study utilized a large, prospective cohort to explore the relationship between vitamin D status and disease activity. Other strengths of this study include the availability of 2 25(OH)D measurements for the majority of participants and the prolonged follow-up times, particularly in the IFN/GA cohort. However, it would have been ideal to access regular 25(OH)D measurements throughout follow-up. Using the average of 2 vitamin D measurements allows for a better estimate of the underlying vitamin D level, although in a minority of cases the second 25(OH)D measurement followed the outcome, which is a limitation. Other limitations of this work emerged from difficulty in timing the initial investigations with DMT initiation in the first-line treatment cohort. The FTY cohort allowed for a unique opportunity to obtain blood samples on the day of

treatment start because of the mandated first-dose observation. Practically, we could not consistently acquire blood draws on the day of GA or IFN treatment start; we thus counted the first blood draw after treatment start as our baseline time point, but there was variability in when this occurred relative to treatment onset, although all baseline samples were obtained within 18 months. Likewise, it was difficult to investigate T2 lesions as an outcome measure because we could not determine whether a new T2 lesion on the first MRI study after the initial blood draw had occurred prior to treatment start as well. In contrast, Gd+ lesions generally develop within 30 days of the study date in question, providing increased assurance that the outcome followed the baseline vitamin D measurement. Finally, a linear impact of 25(OH)D levels was observed when the 3 participants with the highest 25(OH)D levels were removed. Future studies with a larger number of participants must investigate the potential nonlinear impact of very high 25(OH)D levels.

We attempted to control for evolution in vitamin D supplementation rates during the study period, although this analysis must be interpreted with caution because these data were not collected prospectively and compliance was not routinely elicited by clinicians. Nevertheless, by utilizing 25(OH)D level as the exposure measure, the overall analysis already takes the effect of vitamin D supplementation into account. The results for progression should also be interpreted with caution; we used nonsustained progression given the limited follow-up times, but this may be a surrogate for relapses. A final limitation of this analysis is that our findings cannot be generalized to patients of diverse ethnic backgrounds, given the preponderance of Caucasians in this cohort.

This study raises the possibility of whether DMT class may modify the effect of vitamin D on disease activity in MS. These findings need to be replicated in other cohorts, ideally in the setting of randomized controlled trials, and the effect of vitamin D needs to be explored with respect to other DMTs, such as natalizumab and dimethyl fumarate. Evidence of synergy between vitamin D and certain DMTs could be important in developing personalized approaches to MS therapy. The immunologic and genetic underpinnings of these relationships should be explored, as they will become of increasing relevance as we move towards therapeutic strategies targeting multiple mechanisms of action. Vitamin D supplementation is a low-cost, low-risk intervention that may potentiate the efficacy of certain DMTs against MS, without the risk of abetting serious adverse events as posed by other combination therapies.

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