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High-dose vitamin D supplementation does not improve outcome in a cutaneous melanoma population: results of a randomized double-blind placebo-controlled study (ViDMe trial)

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Abstract

Background Observational studies in cutaneous melanoma (CM) have indicated an inverse relationship between levels of 25-hydroxyvitamin D and Breslow thickness, in addition to a protective effect of high 25-hydroxyvitamin D levels on clinical outcome.

Objectives To evaluate whether high-dose vitamin D supplementation in curatively resected CM reduces melanoma relapse.

Methods In a prospective randomized double-blind placebo-controlled trial, 436 patients with resected CM stage IA to III (8th American Joint Committee on Cancer staging) were randomized. Among them, 218 received a placebo while 218 received monthly 100 000 IU cholecalciferol for a minimum of 6 months and a maximum of 42 months (treatment arm). Following randomization, patients were followed for a median of 52 months, with a maximum follow-up of 116 months. The primary endpoint was relapse-free survival. Secondary endpoints were melanoma-related mortality, overall survival, and the evolution of 25-hydroxyvitamin D serum levels over time.

Results In our population (mean age 55 years, 54% female sex) vitamin D supplementation increased 25-hydroxyvitamin D serum levels after 6 months of supplementation in the treatment arm by a median 17 ng mL^{-1} [95% confidence interval (CI) 9–26] compared with 0 ng mL^{-1} (95% CI 6–8) in the placebo arm ($P<0.001$, Wilcoxon test) and remained at a steady state during the whole treatment period. The estimated event rate for relapse-free survival at 72 months after inclusion was 26.51% in the vitamin D supplemented arm (95% CI 19.37–35.64) vs. 20.70% (95% CI 14.26–29.52) in the placebo arm (hazard ratio 1.27, 95% CI 0.79–2.03; $P=0.32$). After adjusting for confounding factors (including baseline stage, body mass index, age, sex and baseline season), the hazard ratio was 1.20 (95% CI 0.74–1.94, $P=0.46$). The number of deaths from progression of CM and nonmelanoma-related deaths was similar in both the vitamin D supplemented and placebo groups (deaths from progression of CM, $n=10$ and $n=11$, respectively; nonmelanoma-related deaths, $n=3$ and $n=2$, respectively). No major adverse events were observed during the study.

Conclusions In patients with CM, monthly high-dose vitamin D supplementation was safe, resulted in a sustained increase in 25-hydroxyvitamin D levels during the treatment period, but did not improve relapse-free survival, melanoma-related death or overall survival.

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Lay summary

Cutaneous melanoma (CM) is the most lethal form of skin cancer. Previous studies have shown that low vitamin D (VD) levels in the blood at the time of CM diagnosis are associated with thicker tumours, and a worse outcome.

The aim of this study was to examine whether monthly high-dose VD supplementation after diagnosis and surgical treatment of a primary melanoma could improve outcomes. We carried out a clinical trial that included 436 patients with CM who were randomly allocated into two groups. One group of 218 patients received a placebo (an inactive treatment) and another group of 218 patients received a monthly oral oil solution containing VD (known as the treatment group). We looked at relapse-free survival, levels of melanoma-related mortality, overall survival, and the evolution of VD levels over time, and compared the results for both groups. We found that monthly high-dose VD supplementation was safe, but did not protect against recurrence of CM or risk of death.

Therefore, based on our study findings, we do not recommend high-dose VD supplementation for people with CM to improve melanoma outcomes.

What is already known about this topic?

- Observational studies in cutaneous melanoma indicate an inverse relationship between 25-hydroxyvitamin D levels and Breslow thickness, in addition to a protective effect of higher vitamin D levels on melanoma outcome.

What does this study add?

- Compared with placebo, monthly high-dose vitamin D supplementation resulted in sustained increase in 25-hydroxyvitamin D levels during the treatment period.
- Safety profiles were similar between the vitamin D and the placebo arm.
- There was no significant difference in relapse-free survival, melanoma-related death or overall survival between the placebo and the vitamin D supplemented group.

Cutaneous melanoma (CM) represents the most lethal form of skin cancer, and its incidence has increased rapidly over the past decades. Earlier diagnosis has been established in recent years, possibly owing to a greater melanoma awareness as a result of public campaigns.[1] Several risk factors have been identified and associated with CM development, with emphasis on ultraviolet (UV) radiation exposure through tanning beds and sunlight.[2]

Vitamin D3 (VD3), the most beneficial isoform of VD for obtaining adequate VD levels, can be found in a limited number of animal dietary sources; however, the main source of VD3 is through production in the skin from 7-dehydrocholesterol via a solar UV-radiation-mediated photochemical reaction. VD has been implicated in exerting anticarcinogenic effects.[3]

Both *in vitro* and *in vivo* investigations have indicated that VD may protect against CM and other malignancies by virtue of its antiproliferative, prodifferentiating and proapoptotic properties. Moreover, VD influences tumour cell invasiveness and impacts tumour immunity.[4–25]

Epidemiological studies have explored the role of VD in the risk of CM development and its subsequent outcome as reviewed by Tsai *et al.*[26] and Shellenberger *et al.*[27] Prospective observational studies have demonstrated a negative correlation between VD levels and Breslow thickness,[28] alongside a protective effect of elevated VD levels on CM outcome, including disease-free survival, melanoma-related mortality and overall survival.[29–33]

As epidemiological observational studies indicated a protective role of VD in melanoma outcome, two randomized controlled trials (RCTs) were initiated – the MEL-D trial and MelaVid trial – to assess the impact of high-dose VD supplementation in patients with CM. The MEL-D trial has released safety outcomes, while the MelaVid trial was inadequately powered to achieve its primary endpoint of disease-free survival, consequently precluding definitive conclusions regarding its effect on CM outcome.[34,35]

Therefore, we conducted the ViDMe trial, a double-blind placebo-controlled study, aiming to investigate whether high-dose VD supplementation following staging and complete resection of the primary melanoma protects against CM relapse.[36] Furthermore, we explored a potential interaction of the vitamin D receptor (VDR) expression within the primary tumour tissue on outcome.

Patients and methods

Study participants

A total of 436 patients diagnosed with CM were enrolled in the ViDMe trial, a multicentre randomized placebo-controlled study.[36] For detailed information regarding the criteria for participant inclusion and baseline assessments we refer to our published protocol and previous publications.[36–38] Written informed consent was obtained from all participants.

Intervention and study procedures

Upon enrolment, patients diagnosed with CM were randomized in a 1 : 1 ratio to receive a monthly oral oil solution of cholecalciferol (100 000 IU) or placebo (containing only

oil), prepared by Laboratories SMB, stratified by the time interval since diagnosis (categorized into the following three strata: ≤3 months, 3 to ≤6 months, and 6 to ≤12 months ago). This treatment regimen was administered for a minimum duration of 6 months and a maximum duration of 42 months. As prespecified by the protocol,[36] patients were treated for maximum 42 months and study was stopped 6 months after the last patient was randomized allowing for a minimal follow-up duration of 6 months. Subsequent follow-up was done every 24 weeks, assessing relapse-free survival, until conclusion of the study. In order to be sure that every patient with CM entering the study would have sufficient 25(OH)D levels throughout the study, patients with 25(OH)levels below 20 ng mL^{-1} at baseline received an extra VD supplement of 25 000 IU per month (supplementary to study drug). This supplementary VD supplementation was administered every time 25(OH)D levels dropped below 20 ng mL^{-1} and until levels of 20 ng mL^{-1} were reached at follow-up blood measurements during the entire treatment period of the study. The process of randomization was managed by the Leuven Coordinating Centre situated in Leuven, Belgium, and has been described in detail in the published protocol.[36]

Any adverse events (AEs) and severe AEs (SAEs) were documented according to the protocol. The coding of these events, whether AEs or SAEs, was conducted in accordance with the Medical Dictionary for Regulatory Activities (version 5.0).

Laboratory methods

Baseline blood samples, including 25(OH)D at randomization and follow-up, pathology staging and VDR expression in the nucleus and cytoplasm of the primary tumour were determined as described.[36–38]

Statistical analyses

The sample size calculation conducted at the outset of the ViDMe trial was established based on the assumption of a comparable time to relapse as observed retrospectively at the University Hospital of Leuven. The trial included a total of 500 patients and aimed to achieve a statistical power of 90% in order to detect a hazard ratio (HR) of 0.40, favouring the arm receiving VD supplementation, as determined by employing a Cox proportional hazards model. This model was stratified by the time interval since diagnosis (categorized into the following three strata: ≤3 months, 3 to ≤6 months, or 6 to ≤12 months).

The chosen HR of 0.40 was in accordance with the intermediate effect size documented in the research by Newton-Bishop *et al.*[29] Additionally, this ratio accounted for a potential increase of 70 nmol mL^{-1} (equivalent to 28 ng mL^{-1}) in serum VD levels. This elevation corresponded to 80% of the observed effect size in a separate study involving patients with chronic obstructive pulmonary disease.[39] For data analysis, all randomized patients were evaluated according to the intention-to-treat (ITT) principle, which was considered the full analysis set. This approach entailed analysing patients based on the treatment group they were originally assigned to, regardless of whether they received the designated study drug or any study drug at all. A per protocol analysis was also employed as a secondary analysis method. Continuous variables were summarized by treatment group through the count of available data points, the mean, SD, median and interquartile range (IQR).

Categorical and ordinal variables were summarized by treatment group using observed frequencies and percentages relative to the total number of available responses. For comparison, tests for trend, the χ^2 test or the Fisher's exact test were employed, depending on the nature of the variables. All statistical tests were conducted as two-sided analyses.

For the primary endpoint, i.e. relapse-free survival, a comparison between the two treatment groups was conducted using a Cox proportional hazards model stratified by time since diagnosis (≤ 3 months, 3 to ≤6 months, and 6 to ≤12 months). Tied survival times were managed using Efron's method. To achieve reliable estimates, patients were censored at 72 months in case no event or end of follow-up had occurred. The resulting HR along with a 95% confidence interval (CI) and the corresponding *P*-value were reported. Different potential treatment effects in the different strata were verified. Subgroup analyses were conducted for baseline 25(OH)D levels, body mass index (BMI), inclusion season, age and birth sex. The interaction between different levels and treatment groups was examined.

Similarly to the primary endpoint, analyses were performed for melanoma-related death and overall survival using the same methodology. The secondary endpoint of melanoma-related death was analysed using competing risk methodology, considering nonmelanoma death as the competing risk.

The influence of VDR expression in the nucleus and cytoplasm (both in terms of percentage expressed and H-score) was investigated by adding the linear term to the model. Linearity was verified and, if necessary, VDR expression was included as a restricted cubic spline. For the nucleus, a categorized version (< 30%, 30% to <60%, 60–100%) was also analysed.

The trend of 25(OH)D serum levels over time was reported descriptively. The change at 6 months was compared between treatment groups by means of Wilcoxon rank sum test. The counts of patients experiencing AEs or SAEs, treatment-related AEs or fatal AEs were summarized according to their randomized treatment groups. This summary was categorized by system organ class. If a substantial difference was observed for a specific system organ class, a post hoc comparison was carried out using Fisher's exact test.

Throughout the analyses, a two-sided significance level of 0.05 was used, and no correction for multiple testing was implemented. All analyses were conducted using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Objectives

The primary objective of the study was to assess relapse-free survival in both arms. Relapse-free survival was defined as the period from randomization to any type of relapse or death, whichever came first. Secondary endpoints were melanoma-related mortality, overall survival, and the trajectory of 25(OH)D serum levels over time following the oral administration of 100 000 IU of cholecalciferol to patients with CM. Furthermore, the study also examined the incidence

and severity of AE as a safety endpoint. Additionally, we explored a potential interaction of the VDR expression within the primary tumour tissue on relapse-free survival.

Results

Descriptive data/study population

As stated in the published protocol,[36] the initial plan was to enrol patients between the fourth quarter of 2012 and the fourth quarter of 2016. However, owing to a lower recruitment rate than initially anticipated, the timeline of the study was extended until the third quarter of 2022. The flow of patients throughout the study is presented in Figure 1. In each treatment arm, a total of 218 patients were enrolled. These patients received their assigned treatments for a minimum duration of 6 months and a maximum of 42 months (with a median of 22 months). They were followed for a median duration of 50 months (IQR 31–70) in the VD supplemented group and 52 months (IQR 29–69) in the control group, with the maximum follow-up reaching 116 months.

Within the VD supplemented group, three patients were excluded from the per protocol set; one patient owing to a missing consent form and two patients owing to a violation of an inclusion criterion. One patient in the placebo group did not take any study medication, nor did two patients in the VD supplement group. In both treatment groups, 49 of the 218 patients discontinued before the end of the treatment period because of the following reasons: informed consent withdrawn, patient lost to follow-up, progression, AEs and other reasons (Table S1; see Supporting Information).

We exclusively present the outcomes of the ITT analysis. The results of the per protocol analysis were similar.

Patient baseline characteristics are shown in Table 1. The median age for both groups was 55 years, and there was a total of 237 female patients (54%). The mean BMI was 27 kg m^{-2} for both groups. The average baseline serum level of 25(OH)D was 23 ng mL^{-1} (SD 9) in the control group and 24 ng mL^{-1} (SD 9) in the VD group. At randomization, 29 of 218 patients in the control group (13%) and 35 of 217 patients in the VD group (16%) were taking VD supplements, with a maximum dosage of 25 000 IU per month. Therefore, 16% of patients received a maximum of 125 000 IU of cholecalciferol rather than 100 000 IU.

Most of the participants were enrolled in the trial within a period of 3–6 months following complete resection of primary CM (stratum 2). Superficial spreading melanoma was the most prevalent histological subtype in both groups, followed by nodular melanoma. Mean Breslow thickness was consistent at 2 mm (SD 2). In the control group, 18% of the CM tissue exhibited ulceration, and 86% showed mitosis. Similarly, in the VD group the percentage of ulceration and mitosis was 19% and 88%, respectively.

Regarding VDR immunoreactivity in the primary tumour tissue, the majority of patients in both the VD group and placebo group had a level of 2+. Lymph node metastasis was identified in 15% of patients within each group. According to the 8th American Joint Committee on Cancer staging, 62% of patients in the control group were classified as stage IB and IA, followed by 14% classified as stage IIA. In comparison, the VD group consisted of 58% patients classified as stage IB and IA, followed by 13% at stage IIA.

Primary endpoint: relapse-free survival

Of the 436 patients included in the study, a total of 73 experienced either relapse or death as the first event, with

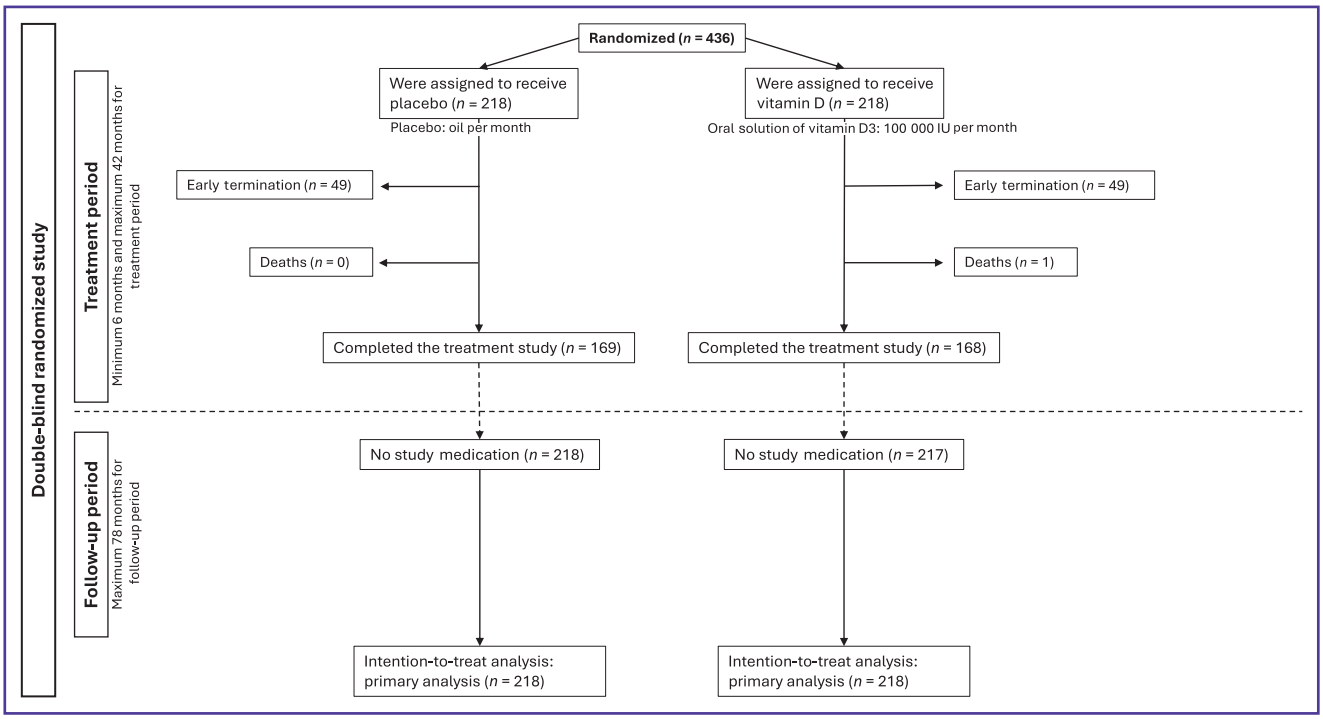

Figure 1 Patient flow through the ViDMe trial.

Table 1 Patient baseline characteristics

Patient characteristic	Control	Vitamin D
Demographic data: study population *N*	218	218
Sex		
Male *n*/*N* (%)	93/218 (42.66)	106/218 (48.62)
Female *n*/*N* (%)	125/218 (57.34)	112/218 (51.38)
Age (years), *N*	218	218
Mean	55.0	54.9
Median	55.0	55.0
SD	13.27	13.12
Q1–Q3	46.0–64.0	46.0–66.0
Physical examination		
BMI (kg m^{-2}), mean (SD)	27 (4) (*n* = 218)	27 (5) (*n* = 216)
Laboratory investigations		
Vitamin D (ng mL^{-1}), mean (SD)	23 (9) (*n* = 218)	24 (9) (*n* = 214)
Median (Q1–Q3)	21 (16–29) (*n* = 218)	24 (18–30) (*n* = 214)
Serum calcium (mg dL^{-1}), mean (SD)	10 (1) (*n* = 218)	9 (1) (*n* = 216)
Median (Q1–Q3)	10 (9–10) (*n* = 218)	10 (9–10) (*n* = 216)
Serum phosphate		
Not done	1/218 (0.46)	0/217 (0.00)
Within normal range	196/218 (89.91)	197/217 (90.78)
Outside normal range: not clinically significant	21/218 (9.63)	20/217 (9.22)
Supplements and concomitant medications		
Supplements		
Vitamin D supplements outside study	29/218 (13.30)	35/217 (16.13)
Calcium supplements	16/217 (7.37)	10/217 (4.61)
Pathology		
Time of diagnosis		
Newly diagnosed	53/218 (24.31)	53/218 (24.31)
Diagnosis ≤ 6 months previously	102/218 (46.79)	105/218 (48.17)
Diagnosis 6 to < 12 months previously	63/218 (28.90)	60/218 (27.52)
Histological subtype		
Nodular melanoma	29/218 (13.30)	38/217 (17.51)
Superficial spreading melanoma	142/218 (65.14)	115/217 (53.00)
Acrolentiginous melanoma	6/218 (2.75)	8/217 (3.69)
Lentigo maligna melanoma	5/218 (2.29)	7/217 (3.23)
Unknown	21/218 (9.63)	22/217 (10.14)
Other	15/218 (6.88)	27/217 (12.44)
Clark		
Level II	10/218 (4.59)	9/216 (4.17)
Level III	72/218 (33.03)	78/216 (36.11)
Level IV	114/218 (52.29)	107/216 (49.54)
Level V	12/218 (5.50)	15/216 (6.94)
Unknown	10/218 (4.59)	7/216 (3.24)
Breslow thickness, mm, mean (SD)	2 (2) (*n* = 217)	2 (2) (*n* = 216)
Median (Q1–Q3)	1 (1–2) (*n* = 217)	1 (1–2) (*n* = 216)
Ulceration	40/218 (18.35)	42/216 (19.44)
Mitoses	187/218 (85.78)	189/216 (87.50)
VDR immunoreactivity in the primary tumour		
0	3/215 (1.40)	2/217 (0.92)
1+	57/215 (26.51)	62/217 (28.57)
2+	73/215 (33.95)	75/217 (34.56)
3+	19/215 (8.84)	20/217 (9.22)
Failed	11/215 (5.12)	6/217 (2.76)
Not done	52/215 (24.19)	52/217 (23.96)
% Nucleus, mean (SD)	40 (30) (*n* = 152)	45 (31) (*n* = 158)
Median (Q1–Q3)	30 (10–70) (*n* = 152)	40 (17–70) (*n* = 158)
% Cytoplasm, mean (SD)	62 (35) (*n* = 152)	69 (34) (*n* = 158)
Median (Q1–Q3)	70 (30–100) (*n* = 152)	85 (40–100) (*n* = 158)
H-score, mean (SD)	84 (59) (*n* = 152)	98 (64) (*n* = 158)
Median (Q1–Q3)	85 (30–120) (*n* = 152)	100 (40–150) (*n* = 158)
Metastasis and staging		
Sentinel node biopsy	182/218 (83.49)	177/217 (81.57)
Metastasis in lymph nodes	33/218 (15.14)	34/217 (15.67)
Stage (8th AJCC) at baseline		
IA	26/217 (11.98)	24/216 (11.11)
IB	108/217 (49.77)	101/216 (46.76)
IIA	30/217 (13.82)	29/216 (13.43)
IIB	14/217 (6.45)	18/216 (8.33)
IIC	6/217 (2.76)	10/216 (4.63)
IIIA	10/217 (4.61)	14/216 (6.48)
IIIB	8/217 (3.69)	9/216 (4.17)
IIIC	14/217 (6.45)	10/216 (4.63)
IIID	1/217 (0.46)	0/216 (0.00)
IV	0/217 (0.00)	1/216 (0.46)

AJCC, American Joint Committee on Cancer; BMI, body mass index; VDR, vitamin D receptor. Data are presented as *n*/*N* (%) unless otherwise stated.

causes of death that included both melanoma-related and nonmelanoma-related factors. Among these, 32 patients were assigned to the control group and 41 patients were in the VD group.

Furthermore, 31 of these events occurred in the control group and 40 occurred in the VD group within 72 months after the inclusion of these patients in the study. During the initial 12 months, a numerically lower relapse rate of 4.68% (95% CI 5.54–8.52) was observed in the VD group, compared with a rate of 7.24% (95% CI 4.42–11.72) in the control group, although this finding did not achieve statistical significance. After 18 months, the difference between the groups diminished, and from 24 months onwards, a higher relapse rate (which was not statistically significant) was noticed in the VD group compared with the control group. Relapse rates at 24, 36 and 72 months, were 12.53%, 14.93% and 26.51% in the VD group vs. 9.34%, 11.76% and 20.70% in the control group, respectively. The HR was 1.27 (95% CI 0.79–2.03), and the corresponding *P*-value was 0.32.

When examining the distinct strata of inclusion within the ViDMe trial the differences in event rate between the VD and control group were higher in stratum 1, and then in stratum 2 and 3. However, no evidence for a significant interaction between treatment and the different strata was found ($P=0.9$) (Figure 2 and Table 2).

Upon adjusting for potential confounding factors including baseline stage, BMI, age, sex and baseline season, the HR was recalculated as 1.20 (95% CI 0.74–1.94) with a *P*-value of 0.46 (Table 3). HR while on treatment was 1.31 ($P=0.48$) (results not shown). A separate analysis was conducted on subgroups for baseline VD levels (< 20 ng mL^{-1} vs. ≥ 20 ng mL^{-1}), and for BMI (≤ 25 kg m^{-2} vs. > 25 kg m^{-2}), season, age and sex. However, no significant interaction was observed (Table S2; see Supporting Information).

A post hoc analysis that compared the event rate in the VD supplemented group for patients with CM who had very low baseline 25(OH)D levels (< 20 ng mL^{-1}) with patients with CM who had normal baseline 25(OH)D baseline levels (≥ 20 ng mL^{-1}) demonstrated a significant difference ($P=0.01$), with a higher event rate in the group with very low baseline 25(OH)D levels. In the placebo group, there was no difference in relapse/death between patients with low vs. normal 25(OH)D baseline levels ($P=0.30$) (results not shown).

Secondary endpoints: melanoma-related death and overall survival

A total of 21 patients (10 patients in the VD group and 11 in the control group) died owing to the progression of CM. In both groups, a total of 13 patients succumbed to all-cause deaths. Hence, five patients died owing to causes unrelated to CM (three in the VD group and two in the control group). The estimated event rate at various timepoints for the two groups and HR at 72 months are provided in Table 4.

Secondary endpoint: evolution of 25(OH)D3

The mean 25(OH)D level at baseline was 23 ng mL^{-1} (SD 9) in the control group and 24 ng mL^{-1} (SD 9) in the VD group. After 6 months, an increase in 25(OH)D level was observed in the VD supplemented arm, resulting in a mean 25(OH)D level of 43 ng mL^{-1} (SD 11), while in the placebo group the mean VD level was 23 ng mL^{-1} (SD 9) at the same timepoint. Subsequently, from 6 months until the end of the treatment period of the study, a more stable state in 25(OH)D levels was noted in the VD group. Additional data are shown in Figure 3 and Table S3 (see Supporting Information).

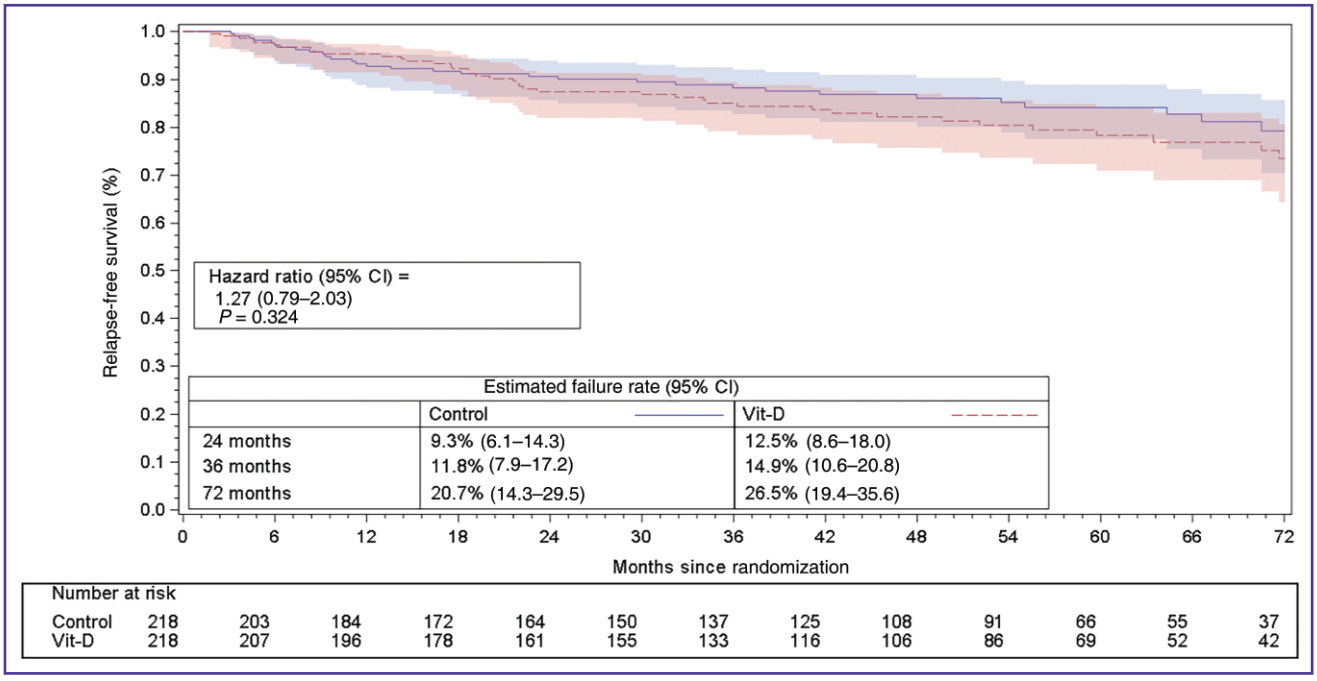

Figure 2 Primary endpoint: relapse-free survival: event (death or relapse) rate in vitamin D group vs. control.CI, confidence interval; Vit-D, vitamin D.

Table 2 Primary endpoint: relapse-free survival: event (death or relapse) rate in the vitamin D group vs. control

Randomized treatment				
	Control	**Vitamin D**	**Total**	***P*-value**
Total number of participants	218	218	436	
Death or relapse	32	41	73	
Death or relapse within 72 months	31	40	71	
Estimated event rates				
At 12 months	7.24 (4.42–11.72)	4.68 (2.54–8.52)	5.93 (4.05–8.66)	
At 24 months	9.34 (6.06–14.27)	12.53 (8.64–18.01)	10.94 (8.25–14.44)	
At 36 months	11.76 (7.95–17.21)	14.93 (10.60–20.83)	13.36 (10.32–17.19)	
At 72 months	20.70 (14.26–29.52)	26.51 (19.37–35.64)	23.67 (18.62–29.83)	
Event rate at 72 months	20.70 (14.26–29.52)	26.51 (19.37–35.64)	23.67 (18.62–29.83)	0.2910[a]
Hazard ratio		1.27 (0.79–2.03)		0.3238[b]
Interaction Trt*stratum				0.9218[b]
Stratum 1				
Event rate at month 72	15.26 (6.31–34.36)	28.01 (14.54–49.72)	21.28 (12.54–34.78)	
Hazard ratio		1.53 (0.54–4.30)		
Stratum 2				
Event rate at month 72	27.45 (17.51–41.43)	30.47 (20.78–43.27)	29.24 (21.80–38.51)	
Hazard ratio		1.20 (0.66–2.19)		
Stratum 3				
Event rate at month 72	12.56 (5.71–26.36)	14.05 (6.86–27.57)	13.34 (7.86–22.13)	
Hazard ratio		1.23 (0.41–3.67)		

CI, confidence interval; TrT*stratum, treatment stratum. [a]Log-rank test. [b]χ^2 test from stratified Cox regression. Only data up to month 72 were included in all calculations. Stratum 1, newly diagnosed; Stratum 2, diagnosed ≤ 6 months previously; Stratum 3, diagnosed 6 to < 12 months previously. Data are presented as *n* or percentage with 95% CI unless otherwise stated.

Vitamin D receptor expression and relapse-free survival

An exploratory analysis was conducted to assess the influence of VDR expression on relapse-free survival. VDR expression in the nucleus, analysed as a continuous covariate (spline), was found to be statistically significant when correlated with relapse-free survival, with a *P*-value of 0.04. No statistically significant effect on relapse-free survival was found for VDR expression in the cytoplasm, both in terms of percentage expressed and H-score (Figure S1 and Table S4; see Supporting Information).

Table 3 Relapse-free survival: hazard ratio upon adjusting for baseline, stage, body mass index, sex and baseline season

Predictor	**Category**	**Hazard ratio (95% CI)**	***P*-value**
Randomized treatment	Vitamin D	1.198 (0.741–1.938)	0.462
	Control[a]		
Staging at baseline	IIA/B	3.260 (1.644–6.467)	< 0.001
	IIC/III	8.171 (4.401–15.17)	
	IA/B[a]		
Body mass index (kg m^{-2})		1.019 (0.967–1.073)	0.484
Age (years)		1.052 (1.030–1.074)	< 0.001
Sex	Female	0.837 (0.517–1.356)	0.470
	Male[a]		
Season at baseline	Spring	0.896 (0.467–1.717)	0.447
	Summer	0.578 (0.289–1.159)	
	Autumn	0.840 (0.427–1.651)	
	Winter[a]		

CI, confidence interval. [a]Reference category for calculation of hazard ratio. For continuous variables, the hazard ratio reflects the change in hazards when the variable increases by 1 unit. Analyses were performed using a Cox regression, stratified for time since diagnosis.

Safety

Throughout the study, a total of 1083 AEs were recorded, with 536 events occurring in the control group and 547 events in the VD group. Among the participants, 178 patients (81.65%) in the control group and 180 patients (82.11%) in the VD group experienced at least one AE.

Overall, there were no significant differences in AEs between the two groups. SAEs occurred a total of 80 times, with 44 incidents in the VD group and 36 incidents in the control group. In the VD group, 28 patients experienced a SAE (12.84%) and in the control group, 33 patients experienced a SAE (15.14%). Additional information regarding safety (SAEs) can be found in Table S5 (see Supporting Information).

Discussion

In this randomized double-blind placebo-controlled trial (ViDMe trial), VD supplementation of 100 000 IU per month did not reduce the risk of CM relapse or mortality attributed to any cause. The estimated event rate for relapse-free survival was not significantly different between the placebo group and the treatment group, and remained not significantly different after correction for confounders or when limiting the analysis to the treatment period. Likewise, no statistically significant effects of VD supplementation were observed concerning melanoma-related death. Most CM in the VD group were stage IB ($n=209$). An analysis on the effect of VD on the number of recurrences per stage indicated that in stage IB, VD had no protective effect (Table S6; see Supporting Information).

These results are in concordance with two other RCTs. In patients with digestive tract cancer, the administration of VD supplementation at a daily dosage of 2000IU did not

Table 4 Secondary endpoint: melanoma-related death and overall survival

	Randomized treatment			
	Control	**Vit-D**	**Total**	***P*-value**
Total number of participants	218	218	436	
Melanoma-related death				
Melanoma-related death	11	10	21	
Nonmelanoma-related death	2	3	5	
Melanoma-related death within 72 months	11	10	21	
Nonmelanoma-related death within 72 months	1	3	4	
Estimated event rates (%)[a]				0.801
At 12 months	0.5 (0.0–2.5)	0.5 (0.0–2.5)	0.5 (0.1–1.6)	
At 24 months	2.1 (0.7–4.9)	2.6 (1.0–5.6)	2.3 (1.2–4.2)	
At 36 months	3.2 (1.3–6.6)	3.7 (1.6–7.2)	3.5 (1.9–5.7)	
At 72 months	8.1 (4.0–14.1)	7.0 (3.3–12.6)	7.5 (4.6–11.4)	
Hazard ratio vs. control[b]		0.874 (0.371–2.056)		0.757
Overall survival				
All-cause death	13	13	26	
All-cause death within 72 months	12	13	25	
Estimated event rates				
At 12 months	0.49 (0.07–3.44)	0.48 (0.07–3.35)	0.49 (0.12–1.93)	
At 24 months	2.08 (0.78–5.44)	2.59 (1.08–6.11)	2.34 (1.22–4.44)	
At 36 months	3.23 (1.46–7.06)	3.74 (1.80–7.70)	3.48 (2.04–5.93)	
At 72months	9.50 (5.19–17.04)	11.83 (6.54–20.88)	10.76 (7.02–16.30)	
Event rate at 72 months	9.50 (5.19–17.04)	11.83 (6.54–20.88)	10.76 (7.02–16.30)	0.879[c]
Hazard ratio		1.02 (0.47–2.24)		0.958[d]

CI, confidence interval. [a]Event rates were estimated as cumulative incidence functions, taking into account the competing risk of nonmelanoma-related death. [b]Obtained using a Fine & Gray model for competing risk data, stratified by time since diagnosis. [c]Log-rank test. [d]χ^2 test from stratified Cox regression. Only data up to month 72 were included in all calculations. Data are presented as *n* or percentage with 95% CI.

result in a lowered risk of relapse or all-cause mortality.[40] Similarly, in the nonsmall cell lung cancer population, a regimen of VD supplementation at a daily dose of 1200 IU also failed to improve either relapse-free survival or overall survival.[41]

While our ViDMe trial has not demonstrated a significant impact of high-dose VD supplementation (i.e. that supranormal VD levels had an effect on CM outcome), prior observational research unveiled a substantially higher mortality rate among patients with CM who had low VD levels.[26,27] Also, in patients with advanced CM who were undergoing anti-PD1 therapy, Galus *et al.* revealed that patients with normal 25(OH)D levels showed an increased progression-free survival[42] compared with patients who had low VD levels, underscoring the potential impact of normal vs. deficient VD levels on CM outcome.

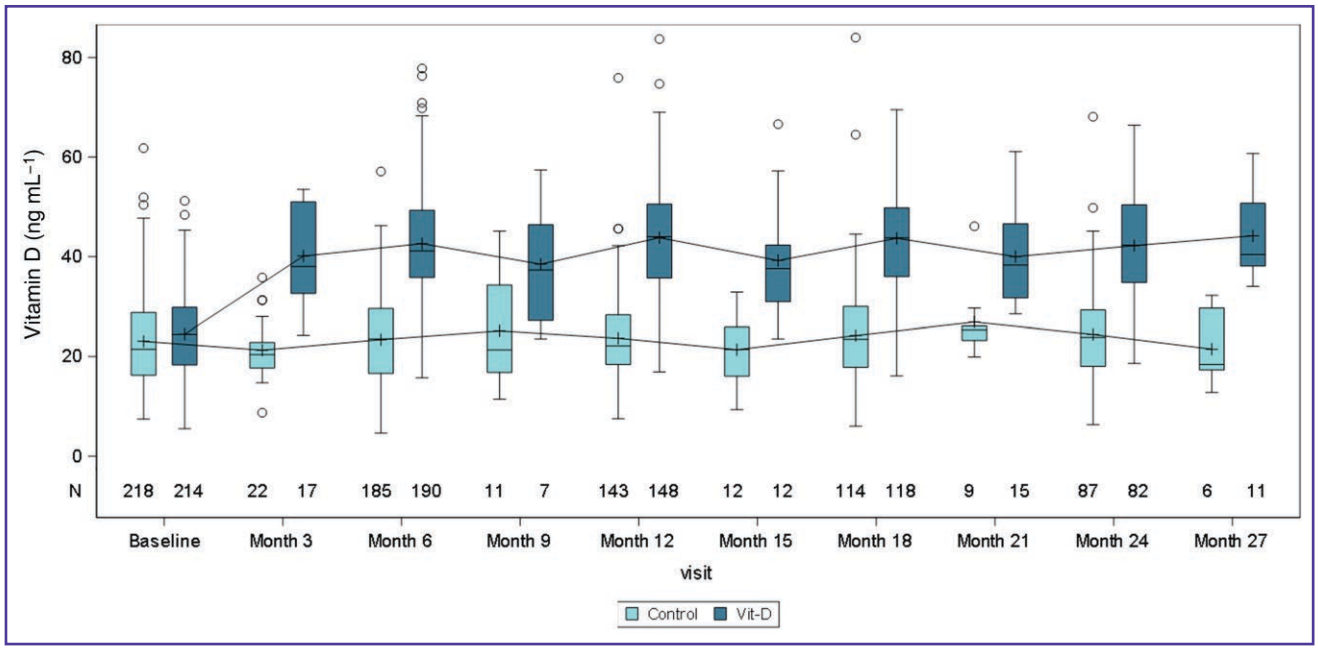

Figure 3 Evolution of 25(OH)D3. Box plot shows median and interquartile range (IQR). Whiskers are drawn at (Q3 + 1.5*IQR, Q1 – 1.5*IQR). Q1, Q3 = 1st and 3rd quartiles. IQR = Q3 – Q1. Plus sign indicates mean value. Circles indicate outlying values.

Other reports focusing on different cancer entities have consistently highlighted the correlation of normal 25(OH)D levels with more favourable prognostic outcomes compared with low VD levels.[43]

The ViDMe study revealed a significant relation between nuclear VDR immunoreactivity and relapse-free survival, but the nature of this relationship is not clear (Figure S1). We also observed in the same CM population an inverse correlation of nuclear VDR expression with mitosis and perineural invasion, which are two established histological prognostic markers.[37] Previous research showed that the presence of exclusive nuclear staining at the tumour base was strongly linked to the absence of metastasis.[44] This suggests that it might be worthwhile to further explore nuclear VDR immunoreactivity as a potential prognostic pathological parameter.

In our study, all enrolled patients, regardless of their randomization into the experimental or control group, received a low VD supplement of 25 000 IU per month throughout the treatment period when their VD levels were below 20 ng mL^{-1}. This strategy ensured that all participants in the placebo group achieved 25(OH)D levels in the vicinity of 20 ng mL^{-1}. In the control group, no significant change in event rates was observed across the study duration when comparing patients with baseline 25(OH)D levels below 20 ng mL^{-1} and those with levels $\geq$ 20 ng mL^{-1} ($P = 0.3$). However, in the high-dose VD supplemented group, a clear increase ($P = 0.01$) in event rates emerged in those with baseline 25(OH)D levels $<$ 20 ng mL^{-1} compared with those with baseline levels $\geq$ 20 ng mL^{-1}.

In our study, we did not observe higher rates of hypercalcaemia among patients who received 100 000 IU per month vs. those who received placebo. A small proportion of the patients received 125 000 IU per month, and even then no hypercalcaemia was registered. The study by Saw *et al.*[34] also demonstrated a safe high-dose VD supplementation without hypercalcaemia in patients with CM.

Newton-Bishop *et al.*[29] showed in a prospective study that higher 25(OH)D levels are associated with lower Breslow thickness and better relapse-free survival in CM. We also found an association between diminished 25(OH)D levels and heightened tumour stage in a previous analysis of a cohort of the ViDMe study.[38] However, our data indicate that aiming for very high VD levels with high-dose VD supplementation does not appear to be associated with improved outcome, as has been previously shown for other cancers,[40,41] and may even be counteractive in patients who are VD deficient. Consequently, we do not recommend high-dose VD supplementation, but rather we advocate routine assessment of baseline 25(OH)D serum levels upon CM diagnosis, in addition to proactive measures to ensure that patients maintain levels around 20 ng mL^{-1}. In terms of supplementation strategy, a gradual approach involving regular doses of 25 000 IU per month may be more beneficial than administering high doses of 100 000 IU per month in a short timeframe.

Our study had some limitations. The duration of patient treatment was a minimum of 6 months and a maximum of 3.5 years, with a median supplementation duration of 22 months. It is unclear at the moment what the impact of longer VD supplementation on outcome would be.

The initial plan was to enrol 500 patients with CM between the fourth quarter of 2012 and the fourth quarter of 2016. However, owing to the changing treatment landscape of resected malignant melanoma, patient recruitment proved to be more time-consuming. Therefore, the timeline of the study was extended until the third quarter of 2022, to increase the rate of events according to the statistical power calculation.

The rationale for the statistical power calculation was drawn from the work of Newton-Bishop *et al.*, who reported that an increase of 8 ng mL^{-1} in 25(OH)D levels was associated with an HR of 0.79 in favour of VD supplementation in patients with CM.[29] This effect size was extrapolated to the ViDMe study. A doubled increase in 25(OH)D levels was proposed and, consequently, an HR of 0.4 was calculated for the anticipated benefit of VD supplementation. We observed an HR of 1.31 (95% CI 0.62–2.77), with a *P*-value of 0.48. The most important reason for this discrepancy is the extrapolation to detect a doubled effect size in our study population compared with the findings of Newton-Bishop *et al.*[29]

In a double-blind, placebo-controlled study involving VD supplementation (at a dosage of 100 000 IU per month) within a CM population, spanning a maximum of 3.5 years, no significant differences were observed in relapse-free survival, melanoma-related mortality, or overall survival between the placebo group and the VD supplemented group. Based on our results, the administration of high-dose VD supplements to patients with CM to improve survival outcomes cannot be advocated at present. Rather, we propose to determine 25(OH)D levels upon diagnosis and to ensure that these levels remain between 20 and 30 ng mL^{-1} throughout the treatment and follow-up.

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Conflicts of interest

The authors declare they have no conflicts of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

The ViDMe trial obtained approval from the local ethics committees. The study adhered to the principles outlined in the

Declaration of Helsinki for medical research and received approval from the hospital medical ethics committees of all four participating centres. The studies and data accumulation were conducted with the approval of the Institutional Ethics Committee Research UZ/KU Leuven, Leuven, Belgium (S54288) and FAGG (EudraCT number: 2012-002125-30), ClinicalTrials.gov: NCT01748448.

Patient consent

Written informed consent was obtained from all participants.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

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