PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy of Vitamin D3 supplementation on cancer mortality in the general population and the prognosis of cancer patients: Protocol of a systematic review and individual patient data meta-analysis of randomized controlled trials
AUTHORS	Schöttker, Ben; Kuznia, Sabine; Brenner, Hermann

VERSION 1 – REVIEW

REVIEWER	Philippe Autier International Prevention Research Institute, Lyon (France)
REVIEW RETURNED	03-Aug-2020

GENERAL COMMENTS	The paper of B Schöttker and colleagues is about a protocol for a systematic review and individual patient data (IPD) analysis of the effect of vitamin D supplementation (VDS) on cancer-specific mortality and survival. Eligible studies are randomised trials. Many meta-analyses of VDS and cancer-specific mortality have been carried out over the last ten years. The last of Yu Zhang et al was published in 2019 in the BMJ. PROSPERO contains 2-3 other on-going protocols on same topic. It is clear to this reviewer that VDS with ordinary doses of vitamin D3 is consistently associated with decreased cancer-specific mortality. Since cancer incidence is not affected by VDS with vitamin D3, cancer-specific survival must also be improved with VDS. A quick look at largest trials on VDS (Trivedi, RECORD, VITAL) clearly demonstrate that VDS with vitD3 influences cancer-specific mortality, and not deaths due to causes other than cancer. The main question is thus to understand reasons underlying the survival advantage conferred by VDS to cancer patients. This is the job to lab and clinical research. So, what this study is going to add to what is already known or on-going studies is not straightforward. Authors need to convincingly show it will not represent “research waste”. This reviewer is not convinced that IPD would unveil new key aspects of the VDS-cancer death relationship. Hence, B Schöttker and colleagues need to heavily insist on the likely new knowledge an IPD analysis will bring. Other comments: 1/ Meta-analyses of observational studies are irrelevant. Reverse causation is highly likely to be the factor at play in low serum 25OHD and virtually ALL diseases. 2/ References of WB Grant (ref. 8) are just vitamin D propaganda by cracked believers. 3/ Lines 146-147 are unclear (what is “eligible data”?) 4/ Why to exclude patients with a particular condition? There is must be a strong, very relevant reason for doing this.

	5/ Line 171: is the study going to exclude the numerous trials that had no placebo comparator? Why not to perform a stratified analysis instead. 6/Quality assessment section needs to go more over the drop-out issue. A few drop-out may represent bias on outcome (information bias), mainly if drop-out rates are unequal between randomised groups. 7/ Der Simonian Laird based on normal distribution should be replaced by methods based on Student t distribution. 8/ I did not at all understand lines 318 to 324 with curious adjustments on cancer stage. Advanced stages should include stage 2 (both power and clinical issues). 9/ Line 340: BMI less than 25? I doubt there will be many subjects in that category. 10/ Line 344: same as comment 8/ 11/ A lag analysis should look at outcomes with ignoring the first year or first 6 months of VDS.

REVIEWER	Michal Zmijewski Medical University of Gdansk
REVIEW RETURNED	02-Sep-2020

GENERAL COMMENTS	Thank you for an opportunity to review the manuscript by Ben Schöttker et al. Generally, the idea of writing protocol for meta-analysis focused on efficacy of vitamin D3 supplementation on cancer mortality is important and could be of the general interest to BMJ Open readers, especially that this is going to be published not as an article but as protocol for further studies. However, the design and criteria suggested by authors are not clear and/or need some rethinking and redesign. Please, note that I am currently not able to review statistical approach suggested by authors. Major suggestions: 1. Overall, I am not sure whether in proposed protocol main criterion (variable) will be supplementation (dose and time) or the 25(OH)D3 level (before and after intervention). Many recent and older studies have failed to show any effect of vitamin D supplementation only due to too low dose of vitamin D supplementation (400-800 IU daily) or were based on population where majority of individuals were vitamin D sufficient even before supplementation (with 25(OH)D3 level above 30 ng/mL, see N Engl J Med 2019; 380:33-44). I would suggest to use 25(OH)D3 level (or its change after supplementation) rather than supplementation dose as criterion. 2. I understand that this is just a protocol, but authors should at least try to estimate potential size of expected group, which is required for proper (valid) statistical analyses. Also, some preliminary data can be presented. How many studies should be included (expected), how many individuals? 3. I not sure why you should “exclude those (studies) limited to particular diseases or conditions” – majority of publish studies on vitamin D supplementation are planned to investigate association of vitamin D level with some disease or condition. I would rephrase (simplify) this part and state that only studies providing data concerning cancer patients will be taken under consideration. 3. What is the rationale for excluding NMSC and benign tumors? Maybe you could show that high levels of vitamin D prevents NMSC and malignancy? And in next sentence in contrary to this

	restriction, you are stating that, no restrictions will be made regarding cancer stage or tumor site, as the anti-proliferative effects of vitamin D3 are not assumed to be specific for cancer site or stage." 4. Authors should take under consideration that supplementation with 25(OH)D3, 1(OH)D3 or 1,25(OH)D3 have different effects on 25(OH)D3 levels (which is actually used as a criterion to define deficiency or sufficiency), these compound show also different pharmacokinetics. Please note, that, here is ongoing and still not resolve debate concerning: what is better D3 or D2?; so please provide some more recent papers then 2011, to justify D2 exclusion form meta-analysis. 5. It would be extremely interesting to find out whether using your protocol you could actually show any effects of low, medium and high responders to vitamin D supplementation on cancer (see Front Endocrinol (Lausanne). 2018 May 23;9:250). 6. Finally, because, it is just a protocol, I am wondering if you can suggest how this protocol could be applied for other studies (can you design it as more universal protocol for meta-analysis). Minor suggestions: 1. Title is too long, please make is shorter. 2. Line 106. I am not sure you can treat "subjects with optimal 25(OH)D levels". Please rephrase. 3. Line 164 - calcitriol, cannot be metabolized actually to the active vitamin D hormone 1,25(OH)2D, calcitriol is 1,25(OH)2D3. 4. Line 415 – please define "low dose"

REVIEWER	LUIS Collado Yurrita Medicine Dpt Complutense University of Madrid Spain
REVIEW RETURNED	06-Sep-2020

GENERAL COMMENTS	The paper addresses an interesting topic. From a methodological point of view it is correct and its results are conclusive It is a good job

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1 (Autier, Philippe)

General reply to the reviewer

Thank you very much for your meticulous review of our manuscript and for the reflective comments, which helped to improve the quality of the protocol. All changes in the manuscript are highlighted via track-change and our point-to-point response to your comments can be found below.

Comments to the author

The paper of B Schöttker and colleagues is about a protocol for a systematic review and individual patient data (IPD) analysis of the effect of vitamin D supplementation (VDS) on cancer-specific mortality and survival. Eligible studies are randomised trials.

Many meta-analyses of VDS and cancer-specific mortality have been carried out over the last ten years. The last of Yu Zhang et al was published in 2019 in the BMJ. PROSPERO contains 2-3 other on-going protocols on same topic. It is clear to this reviewer that VDS with ordinary doses of vitamin D3 is consistently associated with decreased cancer-specific mortality. Since cancer incidence is not affected by VDS with vitamin D3, cancer-specific survival must also be improved with VDS. A quick look at largest trials on VDS (Trivedi, RECORD, VITAL) clearly demonstrate that VDS with vitD3 influences cancer-specific mortality, and not deaths due to causes other than cancer. The main question is thus to understand reasons underlying the survival advantage conferred by VDS to cancer patients. This is the job to lab and clinical research.

So, what this study is going to add to what is already known or on-going studies is not straightforward. Authors need to convincingly show it will not represent “research waste”. This reviewer is not convinced that IPD would unveil new key aspects of the VDS-cancer death relationship. Hence, B Schöttker and colleagues need to heavily insist on the likely new knowledge an IPD analysis will bring.

Author Response

In the process of developing the protocol and before submission to BMJ Open, we have verified PROSPERO and checked regularly for similar ongoing protocols on the same topic. Initiated by this comment, we have searched it again on 21 September 2020 using the keywords “vitamin D” and “mortality”. At first glance, it seems to be true, that many researchers are working on the same topic but a close look reveals the differences:

- Instead of RCTs, another study type is included (prospective studies (CRD42020161582), observational studies (CRD42015019395, CRD42016052007)
- Umbrella review as type of review: CRD42015010571, CRD42019129540
- Different population: children (CRD42016026617), >65 YO (CRD42020153856, CRD42020168802), ICU patients (CRD42020179195, CRD42020170618, CRD42020163692), patients exclusively with cardiovascular outcomes (CRD42019120689)
- Similar, but different inclusion criteria (exclusion of the non-cancer population and restriction of the search with dates from 2014 to current (CRD42019127295), exclusion of the cancer population (CRD42019078090), inclusion of trials that seek to prevent disease before it occurs (CRD42014014801)).
- Systematic reviews that are supposedly on-going are indeed already published with results (e.g. by reviewer cited Zhang et al. (CRD42018117823), McNally et al. (CRD42016026617)).

In summary, none of the planned reviews is using even close to all our inclusion and exclusion criteria for studies and particularly not our approach to conducting the IPD meta-analysis. Furthermore, previous systematic reviews considered only published information about cancer mortality but a selective reporting bias may have occurred due to the lack of reporting this outcome.(1) One of the main highlights of our work is that we do not only include published but also unpublished data on cancer mortality.

Nevertheless, the first IPD meta-analysis on RCTs to approach the benefit of vitamin D on cancer-specific and overall survival of cancer patients is the most important novel feature. With the help of the IPD, we will spotlight various subgroup analyses for instance regarding initial vitamin D insufficiency, obesity, and compliance. It has not been investigated to date, which population characteristics are important to consider to predict who benefits from vitamin D supplementation regarding cancer mortality. Thus, the results of this systematic review and IPD meta-analysis are indispensable for clinical practice in the light of the emerging personalized medicine approach in cancer care. A therapy tailored to the needs of the individual patient enhances the likelihood of an improved outcome to ensure the best possible patient care.

For example, without the separation between vitamin D deficiency and sufficiency, the revelation of vitamin D’s true benefit remains very limited.(1) An IPD meta-analysis on cohort studies indicated an association between low 25(OH)D and all-cause mortality but also mentioned that “the observational nature of their [our] work precludes final conclusion regarding causality”.(2) Here come our IPD meta-analyses into play, as we will obtain IPD on baseline 25(OH)D from previous RCTs, finally separating subjects with sufficient and insufficient vitamin D levels.

Moreover, our systematic review will fundamentally guide future trials by spotlighting which populations to address and by identifying gaps where more research is needed to draw conclusions. The novelty aspects of the planned systematic review and IPD meta-analysis have now been extended in the manuscript (line 71-73).

Point 1

Meta-analyses of observational studies are irrelevant. Reverse causation is highly likely to be the factor at play in low serum 25OHD and virtually ALL diseases.

Response
We agree that reverse causation can confound the results of observational studies. We exclude observational studies from our review.

Point 2
References of WB Grant (ref. 8) are just vitamin D propaganda by cracked believers.
Response
The reference was exchanged as requested.

Point 3
Lines 146-147 are unclear (what is “eligible data”?)
Response
Thanks for your comment which we like to clarify: Studies can only be included in our meta-analyses if the risk ratio and 95% confidence interval for at least one outcome of interest (cancer mortality in the general population, cancer-specific survival of cancer patients, or overall survival of cancer patients) are either reported in the publication or could be obtained from authors or individual participant data.
Action
The sentences were rephrased:
Line 147-148: In the second step, only trials with eligible data for a meta-analysis will be included.
Line 184-190: Step 2: Inclusion criteria for pooling in meta-analysis
Studies will be included for pooling in the meta-analysis, if the risk ratio and 95% confidence interval for at least one outcome of interest (cancer mortality in the general population, cancer-specific survival of cancer patients, or overall survival of cancer patients) were either reported in the publication or could be obtained from authors or individual participant data.
In the case of double publication from the same trial, only the publication with the largest amount of information, e.g. the longest follow-up, will be included in the meta-analysis.

Point 4
Why to exclude patients with a particular condition? There is must be a strong, very relevant reason for doing this.
Response
Thanks for pointing out this important matter. We now removed this exclusion criterion and add the subgroup analysis “general healthy population vs. diseased population (e.g. cancer, diabetes mellitus, ...)” instead.
Action
The sentences were rephrased and the described change included:
Line 150-152: Participants: We will include studies investigating the adult population (18 years or older). We will also include studies conducted solely with cancer populations or patients with other conditions (e.g. studies that recruited only patients with type 2 diabetes).
Line 307-313: The model for the outcome cancer mortality among general population studies will contain …. health status (general healthy population vs. diseased population)…
Line 330: - Health status (general population vs. diseased population)

Point 5
Line 171: is the study going to exclude the numerous trials that had no placebo comparator? Why not to perform a stratified analysis instead.
Response

Thank you for the comment. The very large WHI trial (Wactawski-Wende, et al) tested 400 IU/d and had an HR of 0.89 (95% CI, 0.77-1.03) for cancer mortality, which contributed to the significant reduction overall.(3) Given that 400 IU/d has been defined as the estimated average requirement for vitamin D (by the IOM/NAM), we decided to choose a true placebo group (no vitamin D supplementation) for the primary analysis.(4) As a secondary aim, we consider various regimen by performing subgroup analysis investigating low (<1,000 IU), moderate (1,000 – 2,000 IU), and high (>2,000 IU) vitamin D dosing.

Point 6
Quality assessment section needs to go more over the drop-out issue. A few drop-out may represent bias on outcome (information bias), mainly if drop-out rates are unequal between randomised groups.
Response
We are very thankful for this comment. First of all, it is important to mention that our main outcome is mortality which is ascertained by registries that are usually almost 100% complete. Yet, to account for any attrition bias of included studies, we repeat the analysis by excluding those studies with a high or unknown risk of bias as the Cochrane risk-of-bias tool for randomized trials covers bias due to differential rates of dropout in the 'Bias due to missing outcome data' domain. We will consider each domain independently without an attempt to compile and award a total score to see whether drop-out rates are generally high or low or unequal between the randomized groups. In case risk attrition bias is present, the body of evidence will be additionally down-rated according to the GRADE approach. Important to note, if the issue of attrition bias, including unequal drop-out rates among the randomized groups, clearly arises during the review process, we will follow the recommendations of the Cochrane's Handbook Chapter 10.12.3 and choose an appropriate method accordingly.(5) As stated in chapter 10.14 we have to always keep in mind, that "many issues suitable for sensitivity analysis are only identified during the review process where the individual peculiarities of the studies under investigation are identified.".(5)

Point 7
Der Simonian Laird based on normal distribution should be replaced by methods based on Student t distribution.
Response
Thanks for emphasizing this important point. We consulted Dr. Calderazzo, a statistician of the biostatistics division of the German Cancer Research Center, on this topic. The DerSimonian-Laird method counts as standard to estimate the heterogeneity parameter using the random-effects model. The usual confidence interval is based on the normal distribution, while the Hartung, Knapp, Sidik and Jonkman confidence interval (HKSJ-CI) is based on the (Student) t-distribution and a different estimation of the variance of the summary effect size. At first glance, the HKSJ tends to perform better in terms of nominal type I error rates, but there are residual concerns to consider.(6) The paper of IntHout et al. compares the performance of both by investigating a small number of studies, i.e. less than 20, with various sizes.(7) It is important to bear in mind that the degrees of freedom increase with the number of trials, i.e. the rule of thumb says that above 20 trials, the t-distribution tends to approximate the normal distribution. Thus, the difference between the two methods is likely to be negligible when many studies are available. At the time of the analysis when the number of trials and the distribution of their results is known, we will re-evaluate the appropriateness of the DerSimonian-Laird method, and if necessary use the Hartung, Knapp, Sidik and Jonkman confidence interval (HKSJ-CI), which is based on the (Student) t-distribution, instead.

Point 8
I did not at all understand lines 318 to 324 with curious adjustments on cancer stage. Advanced stages should include stage 2 (both power and clinical issues).

Response
From a piloting search strategy, we know that the vitamin D supplementation trials with cancer populations either focus on stage III, stage IV, or stage III+IV cancer.(8, 9) Thus the cut-off between stages 2 and 3 is pragmatic. If we find any study that also included stage II patients, we will amend the subgroup analysis accordingly, combining stage II-IV cancer trials.

Point 9
Line 340: BMI less than 25? I doubt there will be many subjects in that category.
Response
Thanks a lot for your comment which we like to address first in the general sense and then applied to our collaborating trials.
According to an overview from the NCD Risk Factor Collaboration in 2016, the prevalence of the BMI category <25 kg/m² for both, men and women worldwide was approximately 63%.(10) For instance, regarding Japan, around 74% of men and 80% of women had a BMI of <25 kg/m² which complies with the results of the AMATERASU trial, where approximately 75% of the participants fell in this BMI category.(10-12) In 2016 in the US around 30% of men and 38% of women had a BMI of <25 kg/m² which complies with the VITAL study as well, since around 31% of the participants had a BMI <25 kg/m², 40% 25-30 kg/m² and 29% >30 kg/m².(10, 11, 13)
At our discretion, there are enough subjects in this category for the analysis. Not least, it is generally important to include the normal BMI category into the analyses as effects of vitamin D supplementation vary compared to overweight or obese.(14)

Point 10
Line 344: same as comment 8/
Action
According to point 8, the sentence has been rephrased:
Line 340: Cancer stage (only advanced stages III and/or IV vs. unknown)

Point 11
A lag analysis should look at outcomes with ignoring the first year or first 6 months of VDS.
Response
Thank you very much for your comment. A sensitivity analysis excluding events in the first year of follow-up was added (line 356).

Reviewer: 2 (Zmijewski, Michal)
General reply to the reviewer
Thank you very much for your profound review of our manuscript and for the valuable comments, which helped to improve the quality of the protocol. All changes in the manuscript are highlighted via track-change and our point-to-point response to your comments can be found below.

Comments to the author
Dear Editor,
Thank you for an opportunity to review the manuscript by Ben Schöttker et al. Generally, the idea of writing protocol for meta-analysis focused on efficacy of vitamin D3 supplementation on cancer mortality is important and could be of the general interest to BMJ Open readers, especially that this is going to be published not as an article but as protocol for further studies. However, the design and criteria suggested by authors are not clear and/or need some rethinking and redesign.

Please, note that I am currently not able to review statistical approach suggested by authors.

Major suggestions
Point 1
Overall, I am not sure whether in proposed protocol main criterion (variable) will be supplementation (dose and time) or the 25(OH)D3 level (before and after intervention). Many recent and older studies have failed to show any effect of vitamin D supplementation only due to too low dose of vitamin D supplementation (400-800 IU daily) or were based on population where majority of individuals were vitamin D sufficient even before supplementation (with 25(OH)D3 level above 30 ng/mL, see N Engl J Med 2019; 380:33-44). I would suggest to use 25(OH)D3 level (or its change after supplementation) rather than supplementation dose as criterion.
Response
Thank you for your comment which we like to address in the following. The main exposure of interest is vitamin D supplementation and not the 25(OH)D3 level (or its change after supplementation) because few trials measured the 25(OH)D level or did this only in small subsets of the total study population.
However, we agree that the potential of vitamin D might have been substantially underestimated because patients were not restricted to hypovitaminosis D at inclusion.(15, 16) Hence, in a subgroup analysis, we will re-analyze IPD from previous trials restricted to subjects with vitamin D insufficiency or deficiency. Furthermore, we will conduct subgroup analysis according to vitamin D3 dose (< 1,000 IU vs. 1,000 – 2,000 IU vs. > 2,000 IU per day or equivalent weekly or monthly taken dose).

Point 2
I understand that this is just a protocol, but authors should at least try to estimate potential size of expected group, which is required for proper (valid) statistical analyses. Also, some preliminary data can be presented. How many studies should be included (expected), how many individuals?
Response
Based on the latest previous systematic reviews of Keum et al, and Zhang et al. we estimate that more than 30 trials could be included.(1, 17, 18) As these two previous systematic reviews provided statistically significant results for the main analysis on vitamin D supplementation and cancer mortality, it is highly likely that we will replicate this finding. The meta-analysis is highly driven by the results from 5 large trials of which we list the sample sizes here:
Trivedi: 2,686
RECORD: 5,292
ViDA: 5,108
VITAL: 25,871
WHI: 36,282
Accordingly, we expect a total of at least 75,239 individuals to be included in the main meta-analysis. It is important to note that this is only an estimate to address the reviewer's question. This sample size estimation has not been added to the protocol because we do not want to create bias through a pre-selection and screening of studies.(19, 20) At a later stage of the review, the calculation of the optimal information size will be carried out within the domain "precision" in the framework of the GRADE assessment according to the chapter 5.2.4 in the GRADE handbook and chapter 14 in the Cochrane handbook.(21, 22)
Point 3
I not sure why you should "exclude those (studies) limited to particular diseases or conditions" – majority of publish studies on vitamin D supplementation are planned to investigate association of vitamin D level with some disease or condition. I would rephrase (simplify) this part and state that only studies providing data concerning cancer patients will be taken under consideration.
Response

Thanks for pointing out this important matter. We now removed this exclusion criterion and add the subgroup analysis “general healthy population vs. diseased population (e.g. cancer, diabetes mellitus, ...)” instead.
Action
The sentences were rephrased and the described change included:
Line 150-152: Participants: We will include studies investigating the adult population (18 years or older). We will also include studies conducted solely with cancer populations or patients with other conditions (e.g. studies that recruited only patients with type 2 diabetes).
Line 307-313: The model for the outcome cancer mortality among general population studies will contain …. health status (general healthy population vs. diseased population)…
Line 330: - Health status (general population vs. diseased population)

Point 4
What is the rationale for excluding NMSC and benign tumors? Maybe you could show that high levels of vitamin D prevents NMSC and malignancy? And in next sentence in contrary to this restriction, you are stating that, no restrictions will be made regarding cancer stage or tumor site, as the anti-proliferative effects of vitamin D3 are not assumed to be specific for cancer site or stage.”
Response
Thank you for emphasizing this point. We used a common cancer definition that reflects the assessment of cancer in most trials with cancer registry-based follow-up. Cancer registries have different practices for recording NMSC and benign tumors: Some registries do not track or identify them at all.(23-25) Therefore, we need to adhere to our exclusion criteria to the current practice in order to have a homogenous set of trial data for the meta-analysis.

Point 5
Authors should take under consideration that supplementation with 25(OH)D3, 1(OH)D3 or 1,25(OH)D3 have different effects on 25(OH)D3 levels (which is actually used as a criterion to define deficiency or sufficiency), these compound show also different pharmacokinetics. Please note, that, here is ongoing and still not resolve debate concerning: what is better D3 or D2?; so please provide some more recent papers then 2011, to justify D2 exclusion form meta-analysis.
Response
Thank you for your valuable comment which we like to answer in the following:
Regarding the pharmacokinetics of the vitamin D analogs: Indeed, Calcifediol (25(OH)D), Alfacalcidol (1(OH)D), and Calcitriol (1,25(OH)D) are indicated in different patients as they differ in their metabolic properties directly related to their ability to increase 25(OH)D levels.(26) However, it is not primarily our goal to examine to what extent the level was increased after which intervention. Not least, the studies we plan to include are not designed to investigate this topic. This can be observed from the fact that 25(OH)D was measured only in small subgroups after the intervention in some trials (further described in point 6).
Regarding the use of vitamin D3 vs. vitamin D2: After 2011, systematic reviews and meta-analysis on randomized controlled trials showed vitamin D3 to be more efficient in raising and maintaining the 25-hydroxyvitamin D status compared to vitamin D2.(27, 28) These results were further confirmed by later RCTs.(29-33) Interestingly, a study found that vitamin D3 increases particularly the total and free 25(OH)D levels to a greater extent than vitamin D2.(34)
In 2017, the comprehensive umbrella review of Rejnmark et al. summarized several systematic reviews by that time and found no beneficial effect on mortality in response to vitamin D2.(15, 35-39) In the systematic review and meta-analysis of Chowdhury et al. (2014) for example, vitamin D3 was associated with reduced all-cause mortality by 11% while vitamin D2 had no overall effect on mortality.(36) Finally, Zhang et al. found no reduced cancer mortality with vitamin D2.(1)
Action

We have added more recent references to the manuscript:
Line 162-163: We will exclude studies with vitamin D2 supplementation since the Cochrane review of Bjelakovic et al. and recent data showed clearly no efficacy on mortality.(10, 11, 15, 21)

Point 6
It would be extremely interesting to find out whether using your protocol you could actually show any effects of low, medium and high responders to vitamin D supplementation on cancer (see Front Endocrinol (Lausanne). 2018 May 23;9:250).
Response
Thanks for sharing this interesting idea with us. We cannot show such effects as the sample size of our studies would not be sufficient to investigate this matter: vitamin D was only measured in a small subset compared to the total number of participants, e.g. the mean 25-hydroxyvitamin D levels were measured only in 1,644 of 15,787 participants in the VITAL study and in 438 of 5108 participants in the ViDA study.(13, 40) Most trials did not measure 25(OH)D levels during follow-up at all.

Point 7
Finally, because, it is just a protocol, I am wondering if you can suggest how this protocol could be applied for other studies (can you design it as more universal protocol for meta-analysis).
Response
Thank you for your comment. We did not intend to design a more universal protocol, because each protocol has its nuances depending on the research question, which cannot be universally established in advance, particularly in today's rapidly changing sciences. Using the EQUATOR Network, one can readily find appropriate guidelines to plan their study and develop a corresponding protocol. In particular, PRISMA (http://www.prisma-statement.org/), Cochrane Collaboration (https://training.cochrane.org/handbook/current), and the Institute of Medicine (https://www.nap.edu/catalog/13059/finding-what-works-in-health-care-standards-for-systematic-reviews) provide fundamental resources to create systematic reviews and (individual patient data) meta-analyses.

Minor suggestions
Point 1
Title is too long, please make is shorter.
Response
Thank you for your comment but we did not see any chance to shorten the title without losing important aspects to report already in the title.

Point 2
Line 106. I am not sure you can treat "subjects with optimal 25(OH)D levels". Please rephrase.
Response
Thank you for your comment which we have implemented for better understanding.
Action
The sentence was rephrased:
Line 107-109: Neglecting this dose-response relationship by treating subjects without hypovitaminosis D is expected to have led to a substantial underestimation of the potential efficacy of vitamin D supplementation in previous clinical trials.

Point 3

Line 164 - calcitriol, cannot be metabolized actually to the active vitamin D hormone 1,25(OH)2D, calcitriol is 1,25(OH)2D3.
Response
Thank you very much for finding this error.
Action
The sentence was corrected:
Line 159-161: Besides, we will also include studies using vitamin D3 bioequivalent substances such as calcitriol, being the active vitamin D hormone 1,25(OH)2D, as well as alfacalcidol and calcifediol, which are both equally metabolized to 1,25(OH)2D.

Point 4
Line 415 – please define “low dose”
Response
The sentence was rephrased for better comprehension:
Line 415-418: In some clinics, cancer patients receive a uniform dose of vitamin D with a “one-dose-fits-all” approach, which does not take individual 25(OH)D levels or other patient characteristics into account. The optimal dose for one person may be utterly insufficient for another one to achieve beneficial vitamin D levels.

Reviewer: 3 (COLLADO YURRITA, LUIS)
Comments to the author
Please leave your comments for the authors below
The paper addresses an interesting topic. From a methodological point of view it is correct and its results are conclusive It is a good job
General reply to the reviewer
Thank you very much for your thorough review of our manuscript and the encouraging feedback.

References
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VERSION 2 – REVIEW

REVIEWER	Michal Zmijewski Medical University of Gdansk, Poland
REVIEW RETURNED	06-Nov-2020

GENERAL COMMENTS	Thank you for fruitful discussion and all correction.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2 (Zmijewski, Michal)

Comments to the Author

Thank you for fruitful discussion and all correction.

Author Response

Thank you very much for your in-depth review of our manuscript and the affirming feedback.