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Efficacy of Vitamin D_3 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

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ABSTRACT

Introduction

Vitamin D insufficiency is much more common among cancer patients than the general population. Previous meta-analyses of controlled trials showed an approx. 15% reduction of cancer mortality by vitamin D supplementation compared to placebo or no treatment in the general population.

On top of updating the latest systematic review on vitamin D supplementation and cancer mortality in the general population, we aim to conduct the first meta-analyses of trials on vitamin D_3 supplementation and cancer-specific and overall survival of cancer patients. In addition, none of the previous systematic reviews collected individual patient data. We will do this for the first time to be able to conduct subgroup analyses.

Methods and analysis

A systematic review and individual patient data meta-analysis will be performed on randomized placebo-controlled trials with a vitamin D_3 intervention. The addressed outcomes are cancer mortality in the general population as well as cancer-specific and overall survival of cancer patients. The quality appraisal of the studies will be evaluated by the Cochrane Collaboration's tool for assessing risk of bias. Trial results will be re-analyzed using adjusted and unadjusted Cox proportional hazard regression models and meta-analyses are planned. Cochran's Q-Test and the I^2 index will be used to statistically assess the level of heterogeneity, while sensitivity and subgroup analyses serve to identify potential causes of heterogeneity. Subgroup analyses will be conducted for vitamin D_3 dosing, follow-up time, co-supplementation of calcium, age, sex, obesity, vitamin D deficiency/insufficiency, history of cancer, and compliance. Publication bias will be assessed by funnel plots and Egger's test.

Ethics and dissemination

Ethical approval is not required since no human beings are involved in this systematic review. Results will be published in a peer-reviewed journal with open access. They will be presented at conferences and sent to patient advocacy groups and German oncologic rehabilitation centers.

Trial registration number

In accordance with the guidelines, our systematic review protocol was submitted to the International Prospective Register of Systematic Reviews (PROSPERO) on May 15th, 2020 [registration ID 185566].

Keywords

Vitamin D, cancer, mortality, systematic review, meta-analysis

Strengths and limitations of this study

- First meta-analysis on vitamin D_3 supplementation and cancer survival as well as first individual patient data meta-analysis on this research topic.
- Thorough assessment of study quality and sources of heterogeneity and bias in meta-analyses.
- Conduction of the systematic review according to this protocol minimizes the risk of bias and will gather reproducible results.
- Number of studies with eligible data for subgroup analyses may be limited.

INTRODUCTION

Background

The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018.(1) There is accumulating evidence from epidemiological studies that a low vitamin D status goes along with increased risks of several types of cancer. Meta-analyses of observational studies reported increased risks of lung cancer, colorectal cancer, breast cancer, bladder carcinoma, and lymphoma in subjects with low 25(OH)D serum concentrations.(2-5) Furthermore, epidemiological studies have shown that low serum levels of 25-hydroxyvitamin D (25(OH)D), the acknowledged best biomarker to measure vitamin D status, were strongly associated with substantially increased cancer mortality.(6) For example, in a German population-based cohort study of older adults, the risk to die of cancer was increased by 42% in study participants with vitamin D deficiency (defined as 25(OH)D < 30 nmol/L) compared to individuals with sufficient 25(OH)D levels > 50 nmol/L (hazard ratio [HR] and 95% confidence interval (95%CI): 1.42 [1.08; 1.87]).(7)

The molecular links between vitamin D and carcinogenesis and progression have been described in detail by Moukayed and Grant.(8) In brief, genomic mechanisms of the active hormone $1,25(OH)_2D$ impact signaling pathways that regulate cell proliferation, differentiation, and cell survival. $1,25(OH)_2D$ may primarily act as an anti-proliferative agent in many tissues and may slow down malignant cellular growth. Thus, there is biological plausibility that a sufficient vitamin D supply is especially essential for good cancer prognosis. A causal relationship of low 25(OH)D levels and cancer mortality was furthermore supported by a Mendelian randomization study conducted within three large cohorts from Denmark.(9)

Several randomized trials with vitamin D supplementation have been conducted with mostly the aim to improve skeletal outcomes at older ages. Cancer mortality was a secondary outcome in all trials and therefore the trials were not specifically designed for this outcome.(10) Despite strong heterogeneity in study populations, intervention schemes, and other important design aspects, three out of four meta-analyses demonstrated a statistically significant reduction in cancer mortality.(11-14)

However, most trials have not been restricted to patients that were vitamin D deficient.(10) The latter is important because the association of 25(OH)D levels and adverse health outcomes is not linear.(6) Neglecting this dose-response relationship by treating subjects with optimal 25(OH)D levels is expected to have led to a substantial underestimation of the potential efficacy of vitamin D supplementation in previous clinical trials.(15) Therefore, there is a need for a systematic review that re-analyses individual patient data (IPD) from previous trials restricted to subjects with vitamin D insufficiency (25(OH)D < 50 nmol/L) or deficiency (25(OH)D < 30 nmol/L).

Another important reason to re-analyze the previous trial data is that most studies were not restricted to cancer patients. Vitamin D deficiency or insufficiency are much more common among cancer patients than among the general population. In a study with 2,912 colorectal cancer patients, vitamin D deficiency (25(OH)D levels < 30 nmol/L) was found among 59% of colorectal cancer patients during or shortly after first-line treatment, and, in agreement with previous evidence, low 25(OH)D levels were strongly associated with poorer survival.(16, 17) Systematic reviews of observational studies on 25(OH)D levels and cancer prognosis concluded that sufficient 25(OH)D levels are associated with a better prognosis of breast and colorectal cancer, whereas there are too few studies for other cancer sites up to date to draw conclusions.(16, 18)

Further important potential effect modifiers of vitamin D effects on cancer survival that deserve close investigation are obesity and compliance. People with low compliance and/or obesity (who may need higher vitamin D doses because vitamin D is being stored in adipocytes) might have led to an attenuation of the overall treatment effect in the trials.(19)

Objective

The objective of our systematic review is to assess the efficacy of vitamin D_3 supplementation on cancer mortality in the general population and the prognosis of cancer patients with special attention to potential effect modifiers, including baseline 25(OH)D levels, cancer at baseline, BMI and compliance.

The main outcomes include “cancer mortality in the general population”, “cancer-specific survival of cancer patients” and “overall survival of cancer patients”. These outcomes are universally used in cancer studies and do not need further refinement during the review.

In a first step, we intend to update the previous systematic reviews on vitamin D supplementation and cancer mortality in the general population by including newly published trials and unpublished data from trials with outcome data on cancer incidence or all-cause mortality by asking the authors for data on cancer mortality. Second, we will obtain data for an IPD meta-analysis. Third, we will conduct IPD meta-analyses on vitamin D_3 supplementation and overall and cancer-specific survival among cancer patients. Forth, we will conduct subgroup analyses to explore sources of heterogeneity and to identify effect modifiers. The timetable for the review is shown in Table 1.

Table 1: Proposed timetable for conducting the review

Step	**Timeframe for completion**
Literature search, abstract and full-text selection	2.5 months
Data extraction & individual patient data acquisition	2.5 months
Quality appraisal	2 months
Data analysis & meta-analysis	3 months
Writing of manuscript	2 months
Total	**12 months**

METHODS AND ANALYSIS

Study selection criteria/ Eligibility criteria

We will follow a two-step approach for the study selection: First, all trials will be selected that could have potentially published or unpublished data on the research topic. All authors of trials with potentially unpublished data on cancer mortality/survival will be contacted to provide data. In the second step, trials without eligible data for a meta-analysis will be excluded.

Step 1: Inclusion criteria for trials

Participants: We will include studies investigating the general adult population (18 years or older) but exclude those limited to particular diseases or conditions (e.g. studies that recruited only patients with type 2 diabetes). However, we will also include studies conducted with cancer populations (18 years or older). Apart from the exclusion of non-melanoma skin cancers and benign tumors (where information is available), no restrictions will be made regarding cancer stage or tumor site, as the anti-proliferative effects of vitamin D_3 are not assumed to be specific for cancer site or stage. Therefore, we expect our results to be largely generalizable to the general population and cancer patients, respectively.

Interventions: We will focus on trials that used vitamin D_3 in any dose and any regimen (e.g. daily/weekly/monthly intake) as the intervention. However, the minimum time of the intervention shall be six months to exclude studies with one-time bolus interventions or very short intervention periods. The first reason is that cancer mortality is highly unlikely to be influenced by very brief intervention periods. The second reason is that after initiating daily, weekly, or monthly supplementation schedules, it takes three to six months for 25(OH)D levels to reach homeostasis.

Besides, we will also include studies using vitamin D_3 bioequivalent substances such as alfacalcidol, calcifediol, and calcitriol, as all of them are metabolized equally to the active vitamin D hormone $1,25(OH)_2D$.

We will exclude studies with vitamin D_2 supplementation since the Cochrane review of Bjelakovic et al. showed clearly no efficacy on mortality.(11) Co-supplementation with calcium or other dietary supplements in the intervention arm will not be an exclusion criterion. A sensitivity analysis will elucidate whether the inclusion of these studies had an impact on the overall effect estimate of the meta-analysis.

Comparators: We will include only studies, which used placebo as the comparator.

Outcomes: To be eligible for inclusion in a meta-analysis trials need to have assessed the outcome cancer mortality, cancer survival, or cancer-specific survival. In an intermediate step of the

systematic review, we will also record studies with the outcomes cancer incidence or all-cause mortality and will contact the authors if they have data for the outcomes needed for the planned meta-analyses. The definitions of all outcomes are shown in Table 2.

Table 2: Definition of outcomes

Outcome	Definition
All-cause mortality	Rate of deaths during a specific time period in population at risk
Cancer mortality	Rate of cancer deaths during a specific time period in population at risk
Cancer incidence	Rate of newly diagnosed cancer cases during a specific time period in population at risk
Overall cancer survival	Proportion of patients from a cancer population at risk alive at some point subsequent to the diagnosis of their cancer
Cancer-specific survival	Proportion of patients from a cancer population at risk who did not die of cancer at some point subsequent to the diagnosis of their cancer

Study design: We will include RCTs in which, analogous to the intervention period, the follow-up period is at least six months. The follow-up time should not be longer than the time under treatment. We will focus on parallel-group designs and exclude single-arm studies. We will further exclude all types of cohort studies and case-control studies as well as the following types of records: reviews, dissertations, theses, editorials, study protocol, clinical guidelines, commentaries, and letters.

Setting: There will be no restrictions by type of setting.

Minimum sample size: The studies need to have at least one cancer death in the verum and placebo group.

Geographical location: No restrictions are defined regarding the geographical location.

Step 2: Exclusion criteria for pooling in meta-analysis:

Studies will be excluded for pooling in the meta-analysis, if

- A 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 not reported in publication and could not be obtained from authors or individual participant data

- The trial is already included in the meta-analysis (in case of double publication from the same trial only the publication with the largest amount of information, e.g. the longest follow-up is included)

Information sources and search strategy

The search strategy will be elaborated by SK, BS, and A Heppert. Mrs. Heppert is a specialist for systematic bibliographic searches at the Central Library of the German Cancer Research Center and is not otherwise associated with the project. Finally, it will be peer-reviewed by HB and carried out by SK.

The bibliographic databases MEDLINE (Pubmed interface), ISI Web of Science (WoS; Clarivate Analytics interface), and the Cochrane Central Register of Controlled Trials (CENTRAL; OVID interface) will be searched systematically. In addition, we will also carry out a systematic search for previous systematic reviews in the Cochrane Database of Systematic Reviews (CDRS, OVID interface) and KRS Evidence (https://ksevidence.com), which are both specialized search engines for systematic reviews. RCTs included in meta-analyses on the topics vitamin D supplementation and cancer mortality, cancer incidence, all-cause mortality, or cancer survival will be extracted and merged with the hits found in the bibliographic database search. The electronic database search will be complemented by searching the WHO's International Clinical Trials Research Portal (ICTRP) to capture results from ongoing or recently completed RCTs that have not been published in scientific journals, yet. We will also scan the reference lists of eligible studies to yield additional trial articles via cross-referencing. A draft of the search strategy is presented in Figure 1.

We will search in MEDLINE, ISI WoS, and CENTRAL for medical subject headings (MeSH), free text words, synonyms, and related search terms for the concepts "vitamin D", "mortality", "cancer", "randomized controlled trial" and "placebo". Besides, standard search terms for RCTs will be used additionally wherever available. No restrictions are planned in the search strategy to prevent

overlooking important studies that have not been correctly classified in the respective bibliographic databases. All databases will be searched from inception dates. Moreover, we will not limit the search to studies in English as relevant studies might also be published in other languages. The search string for MEDLINE is shown in Table 3.

Table 3: Search string for MEDLINE

Step	Search string
1	"vitamin d"[tw] OR "vitamin D"[MeSH] OR cholecalciferol[MeSH] OR cholecalciferol*[tw] OR calciol[tw] OR hydroxycholecalciferols[MeSH] OR hydroxycholecalciferol*[tw] OR dihydroxycholecalciferol*[tw] OR “vitamin d3"[tw] OR “vitamin d 3”[tw] OR calcitriol[MeSH] OR calcitriol[tw] OR "1-hydroxycholecalciferol"[tw] OR calcifediol[MeSH] OR calcifediol[tw] OR calcidiol[tw] OR alfacalcidol[Supplementary Concept] OR alphacalcidol[tw] OR alfacalcidol[tw]
2	mortality[tw] OR mortality[MeSH] OR death[MeSH] OR death[tw] OR survival[tw] OR survival[MeSH]
3	neoplasms[MeSH] OR neoplas*[tw] OR malignanc*[tw] OR cancer*[tw] OR tumor*[tw] OR tumour*[tw] OR carcinoma*[tw]
4	(((((((((“randomized controlled trial”[pt]) OR “controlled clinical trial”[pt]) OR randomized[tiab]) OR placebo[tiab]) OR “drug therapy”[sh]) OR randomly[tiab]) OR trial[tiab]) OR groups[tiab])) NOT ((animals[mh] NOT humans[mh]))
5	placebos[MeSH] OR placebo[tw]
6	2 OR 3
7	1 AND 4 AND 5 AND 6

To search for systematic reviews in CDSR and KRS Evidence, a shortened version of the MEDLINE search string will be used. Only the first three search steps are needed because the study design is “systematic review” and not “placebo-controlled RCT”. The search string for CDSR is shown in Table 4. The literature search will be updated during the peer-review process of the publication in order to include the most up to date literature.

Table 4: Search string for the Cochrane Database of Systematic Reviews

Step	Search string
1	#1 MeSH descriptor: [Vitamin D] explode all trees #2 MeSH descriptor: [Cholecalciferol] explode all trees #3 MeSH descriptor: [Calcifediol] explode all trees #4 MeSH descriptor: [Calcitriol] explode all trees #5 MeSH descriptor: [Hydroxycholecalciferols] explode all trees #6 (("alfacalcidol") OR ("alphacalcidol") OR ("hydroxycholecalciferol*") OR ("1- hydroxycholecalciferol") OR ("hydroxyvitamin* D") OR ("calcifediol") OR (“calcidiol”) OR ("calcitriol") OR ("dihydroxycholecalciferol*") OR (“dihydroxyvitamin d*”) OR ("vitamin D") OR (cholecalciferol*) OR ("vitamin D3") OR ("vitamin D 3") OR (“calciol”)) (Word variations have been searched) #7 ("vitamin d*"):ti,ab,kw (Word variations have been searched) #8 {OR #1-#7}
2	#9 MeSH descriptor: [Mortality] explode all trees #10 MeSH descriptor: [Death] explode all trees #11 MeSH descriptor: [Survival] explode all trees #12 ("mortality" OR "death" OR "survival") (Word variations have been searched) #13 {OR #9-#12}
3	#14 MeSH descriptor: [Neoplasms] explode all trees #15 (carcinoma* OR tumour* OR tumor* OR cancer* OR malignanc* OR neoplas*) (Word variations have been searched) #16 #14 OR #15
4	#17 #13 OR #16
5	#18 #8 AND #17 in Cochrane Reviews (Word variations have been searched)

Data collection and management

Study selection and data extraction will be performed in duplicate by two reviewers. Both are blinded to each other’s decision but not to journal titles, study authors, or institutions. The screening will be conducted by entering data into blank Microsoft Word or Excel spreadsheets. The software EndNote will be used to store, organize, and manage all the references and allow a transparent and reproducible systematic search. To assure validity and high quality of the data, the data extraction will be performed by using standard and predefined data extraction forms. Both reviewers will scan independently the titles and abstracts of studies obtained by the aforementioned search strategy against the eligibility criteria. For those studies that meet the inclusion criteria or that cannot yet be fully excluded, full-text reports will be acquired and screened again towards the inclusion criteria. In

the next step, the results of both reviewers will be compared and in cases of disagreement, critical points will be discussed until a consensus is reached. If necessary, we will contact study authors to resolve questions about eligibility. We will document the reasons for excluding trials.

After completing the abstract and full-text selection with eligible studies, the two reviewers will extract independently the pre-defined data. Extracted items will include first author, publication year, country, number of participants, general population or medical condition (including cancer site and stage(s)), sex, mean/median age, race/ethnicity/skin color, mean/median BMI, mean/median 25(OH)D levels at baseline, vitamin D_3 dosing regimen, duration of vitamin D_3 supplementation, compliance, mean/median and maximum follow-up time, number of cancer deaths and effect estimates (including confidence intervals) reported for cancer mortality/cancer survival. Individual patient data for the aforementioned variables will be obtained from all trials with at least 20 cancer deaths. If summary data are not published, they shall be calculated from the obtained data. All authors will be contacted by e-mail with a maximum of three attempts sent with two weeks apart.

For the meta-analyses on cancer survival and cancer-specific survival, we will ask all authors who conducted trials in the general population to provide IPD for cancer diagnoses in the five years prior to baseline and during the trial (including cancer site with ICD-code, stage and diagnosis date). The following IPD will be additionally collected: age, sex, BMI, race/ethnicity/skin color, baseline 25(OH)D levels, compliance, randomization group allocation, baseline date, deaths during follow-up with date, cancer deaths with date, censoring dates for survival outcomes, and censoring date for patients not dying of cancer. If IPD cannot be shared, the authors of the studies will be asked to conduct the analyses in-house and to provide the summary estimates for the meta-analysis. If trial authors do not collaborate, their study cannot be included in subgroup analyses for which no effect estimates were published but the result from the total trial population will remain included in the main meta-analysis.

Quality assessment

The protocol of the systematic review with all planned statistical analyses has been registered in PROSPERO before data collection to preclude data-driven analyses and selective reporting of only statistically significant findings. The study protocol has been developed in line with the "Preferred reporting items for systematic review and meta-analysis protocols" (PRISMA-P) as well as the Institute of Medicine guideline.(20-22) We will ensure to fulfill all requirements recommended by the current PRISMA guideline when writing the publication of the systematic review.(23, 24)

The Cochrane Collaboration's tool for assessing risk of bias (table 8.5.a in the Cochrane Handbook for Systematic Review of Interventions) will be used to assess selection, performance, detection, attrition, reporting and other bias at study level.(25, 26) The following domains will be covered during the evaluation: sequence generation, allocation concealment, blinding, incomplete outcome data (e.g. withdrawals and dropouts), and selective outcome reporting. A summary assessment will be made based on the extracted items, judging whether the risk of bias in the respective study is low or high. If only insufficient data is reported, the risk of bias is "unclear" and the original study authors will be contacted for further information. The assessment will be conducted by two independent reviewers based on the criteria for judging the risk of bias (table 8.5.d in the Cochrane Handbook for Systematic Review of Interventions V.6).(26) In cases of disagreement, critical points will be discussed until a consensus is reached. The risk of bias evaluation will be incorporated into the data synthesis by performing a sensitivity analysis by excluding studies of low or unclear quality.

Descriptive analysis and meta-analysis

Measures of treatment effect

The mortality/survival outcomes shall be addressed by estimating hazard ratios (HR) and 95% confidence intervals (95%CI). Results of the intention-to-treat (ITT) approach will be used, including all patients randomized, if both ITT and per-protocol results are given.

Data synthesis

As far as study quality and differences between studies allow, effect estimates of all eligible studies with data for the following three main meta-analyses will be pooled deriving random effects results with the DerSimonian and Laird method (primary analysis) and fixed effects summary estimates using the Mantel-Haenzel method (secondary analysis).

1) Association of vitamin D_3 supplementation and cancer mortality in the general population
2) Association of vitamin D_3 supplementation and cancer-specific survival of cancer patients
3) Association of vitamin D_3 supplementation and overall survival of cancer patients

For all studies that provide IPD, unadjusted Cox proportional hazard regression models will be used to estimate HRs and 95%CIs for the main meta-analyses in which we will pool effect size data from studies who do and who do not provide IPD in a two-step approach. For studies that cannot send IPD to the coordinating center (German Cancer Research Center, Heidelberg), authors are being asked to estimate the HRs and 95%CIs themselves and send the summary data for the meta-analyses. To assess cancer survival as time-to-event data from general population cohorts, the study will be restricted to patients with a history of cancer in the five years preceding baseline or a cancer diagnosis during the trial. For the former, the survival time will be calculated from baseline to death/end of the trial, and for the latter, survival time will be counted from the date of cancer diagnosis till death/end of the trial.

With all studies that agree to send IPD data to the coordinating center or to do additional analyses in-house, we will also conduct an additional multivariate Cox proportional hazards regression model. The model for the outcome cancer mortality among general population studies will contain the variables vitamin D_3 intervention (vs. placebo), age (continuous; < 70 vs. ≥ 70 years), sex (male, female, unknown), BMI (< 25 vs. 25 – 29.9 vs. ≥ 30 kg/m² vs. unknown), skin color (white vs. black/brown vs. other), 25(OH)D baseline level (< 30 vs. 30 – 49.9 nmol/L vs. ≥ 50 nmol/L vs. unknown), diagnosis of cancer (except non-melanoma skin cancer) in five years before baseline (yes vs. no vs. unknown), and compliance (< 80% vs. ≥ 80% vs. unknown). The models for the outcomes overall and cancer-specific survival of cancer patients will be adjusted for the same variables but the cancer variable will be replaced by more specific variables for cancer stage (only

advanced stages III and/or IV vs. unknown), cancer site (prostate vs. colorectal vs. breast vs. lung vs. other vs. unknown) and time since cancer diagnosis (<1 year vs. 1-5 years). We will test for interactions of the treatment variable (vitamin D_3 vs. placebo) with these covariates to identify potential effect modifiers. Again, a two-step approach will be used for the meta-analyses, whereby the analyses are carried out on a study-specific basis, and then the effect estimates are pooled. To further explore the variation of the treatment effect by methodological or patient characteristics differences of the studies, the following subgroup analyses will be performed with IPD data and studies that published eligible data:

Subgroup analyses according to trial design

- Daily dose vs. weekly/monthly bolus dose vs. bolus dose at the beginning of the trial followed by a daily dose
- Low vs. moderate vs. high vitamin D_3 dosing (< 1000 IU vs. 1,000 – 2,000 IU vs. > 2,000 IU per day or equivalent weekly or monthly taken dose)
- Vitamin D_3 supplementation duration (< 5 vs. ≥ 5 years)
- Region (North America vs. Europe vs. Other)

Subgroup analyses according to patient characteristics

- Age (< 70 vs. ≥ 70 years)
- Sex (Male vs. female)
- Skin color (white vs. black/brown vs. other)
- BMI (< 25 vs. 25 – 29.9 vs. ≥ 30 kg/m²)
- Baseline 25(OH)D levels (< 30 vs. 30 – 49.9 nmol/L vs. ≥ 50 nmol/L)
- Compliance rate (< 80% vs. ≥ 80%)

For meta-analyses conducted in cancer patients in addition:

- Cancer stage (mix of all stages vs. only advanced stages III and/or IV)
- Cancer site (Prostate vs. colorectal vs. breast vs. lung vs. other)
- Time since cancer diagnosis (<1 year vs. 1-5 years)

Analyses in the coordinating center will be done with the statistical software SAS 9.4. The meta-analyses will be performed with Comprehensive Meta-Analysis 2.0 (Biostat, Englewood, NJ).

Assessment of heterogeneity

Heterogeneity will be presented visually by forest plots and assessed statistically by Cochran's Q test (significance level = 0.05) as well as the I^2 index (< 25% low, 25-50% moderate, > 50% high heterogeneity). Meta-Analyses will be conducted even if high heterogeneity is being detected and the results will be discussed taking the heterogeneity into consideration. Sources of heterogeneity will be explored by the subgroup analyses outlined in the previous section and the following sensitivity analyses:

- Excluding studies with a high risk of bias according to assessment with the Cochrane Collaboration's tool
- Excluding studies not reporting ITT results
- Excluding trials with co-supplementation of calcium

Assessment of publication bias

Publication bias will be accessed visually in funnel plots and tested for with Egger's test.

Dealing with missing data

In case of missing data, we will seek contact with the original investigators. If possible, we will calculate missing numerical data from the given reported data.

Strength of the body of evidence

The quality of the evidence for each outcome will be evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. The four levels of evidence comprise very low, low, moderate, and high. Evidence from RCTs starts as high quality but can be decreased for reasons such as the risk of bias, imprecision, inconsistency, indirectness, and publication bias.

Amendments

In the case of protocol amendments, we will document the date, the description of the change and the rationale in a pre-defined log sheet in Microsoft Word or Excel.

Patient and public involvement

Patients and the public were not involved in the development of the study design. Since this is a protocol for a systematic review and no participant recruitment will take place, the involvement of patients in the recruitment, the conduct of the study and the dissemination of findings to study participants are not applicable.

Dissemination

The systematic review will be published in an international journal for clinical oncology or general medicine with open access option and presented in national and international meetings. If the meta-analyses of the systematic review obtain statistically significant findings, we expect the result to be reflected in national and international guidelines and to change the current practice of tertiary prevention among cancer patients. Vitamin D_3 is already on the market in various doses and at low costs because it is not patented.

Patients will be informed via a press release from the German Cancer Research Center. Moreover, we will send a summary of the results in a language suitable for laypersons to all patient advocacy groups recommended by the Cancer Information Service of the German Cancer Research Center (up to data n = 30) for further dissemination among their members.(27) With respect to oncologists, we will disseminate the results to all German rehabilitation centers with a ward for oncologic rehabilitation, as listed in the register of the *Bundesarbeitsgemeinschaft für Rehabilitation e.V.*(28) As the topic of the review is in the field of tertiary prevention, oncologists in the rehabilitation setting are the target audience for information dissemination.

DISCUSSION

One of the strengths of this systematic review comprises the first meta-analysis on vitamin D supplementation and cancer survival and additionally the first IPD meta-analysis on this research topic. The IPD meta-analysis will allow the investigation of potential effect modifiers. Especially 25(OH)D levels at baseline, BMI, and compliance are candidates that could have had a great impact on the overall trial results.

The creation of this research protocol prompted us to plan carefully all the details of the systematic review and to anticipate and address potential problems before their actual occurrence. Arbitrary decision making concerning any procedure of this systematic review is prevented, resulting again in a decreased risk of publication bias and selective reporting bias. The protocol allows reproducible and transparent research for future reviewers.

Possible limitations of our review include a potentially insufficient number of cancer deaths in the studies and high heterogeneity, which could both negatively influence the statistical power of the meta-analyses. However, it is too early now to judge if these limitations will really occur.

The quality of selected studies will be assessed and the quality of the evidence will be judged. The ultimate goal is to ensure the reporting of highly meaningful findings for clinicians and patients. Oncologists are well aware that vitamin D deficiency and insufficiency are very common in cancer patients but there is uncertainty about whether and how they should routinely perform preventive screening and treatments. In some clinics, all cancer patients receive low-dose vitamin D with a “one dose fits all” approach, which does not take individual 25(OH)D levels of the patients into account. Since vitamin D products are readily available in pharmacies or drug stores, many patients use low-dose vitamin D supplementation as self-medication. Yet, it can be doubted whether this untargeted intervention has any effect on cancer prognosis. Consequently, evidence-based recommendations for high-dose vitamin D supplementation are highly relevant for both, clinicians and patients.

If the planned systematic review determines the efficacy of vitamin D supplementation on cancer prognosis in the expected magnitude of 10-15%, the review will be used to provide clear suggestions

on how vitamin D can be appropriately dosed to overcome vitamin D deficiency or insufficiency in cancer patients.(12) Furthermore, our systematic review would provide the evidence for statutory health insurances to cover the costs for screening for vitamin D deficiency or insufficiency in cancer patients and a subsequent vitamin D supplementation. With expected relatively large effects and very low screening and treatment costs (a vitamin D blood test costs approx. € 20, and one year of vitamin D therapy costs less than € 100), vitamin D supplementation will be highly cost-effective. The costs would be close to negligible compared to other current cancer treatment costs.

Status

At the time of submission, the study selection for the systematic review has not started.

Acknowledgements

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Authors' contribution

BS and SK are the guarantors of the systematic review. BS designed the search strategy, the selection criteria, the risk of bias assessment strategy, the data extraction criteria, and the statistical methods. SK drafted the first version of the protocol publication, which BS and HB revised.

Contributors

The following researchers have agreed to contribute to this systematic review by sharing IPD and giving intellectual input for the discussion of the results: Taisuke Akutsu (Jikei University School of Medicine. Tokyo, Japan), professor Julie E. Buring (ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor Carlos A. Camargo Jr (MD, DrPH. Massachusetts General Hospital, Harvard Medical School. Boston), Professor Nancy R. Cook (ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), professor I-Min Lee (MB, BS, ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor JoAnn E. Manson

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Data availability statement

As no new data are obtained in this systematic review, no data will be made publicly available to third parties. Decisions on data use by third parties can only be made by the principal investigators of the original studies, taking into account the votes of the responsible ethics committees.

Disclaimer

The views of the authors do not necessarily reflect those of the German Cancer Research Center.

Competing interests

None declared.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer-reviewed

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Appendix 1:

Supplementary material consisting of data extraction form, risk of bias assessment and list of individual patient data to be obtained from original researchers

Figure

Figure 1: Draft of the search strategy and study selection process

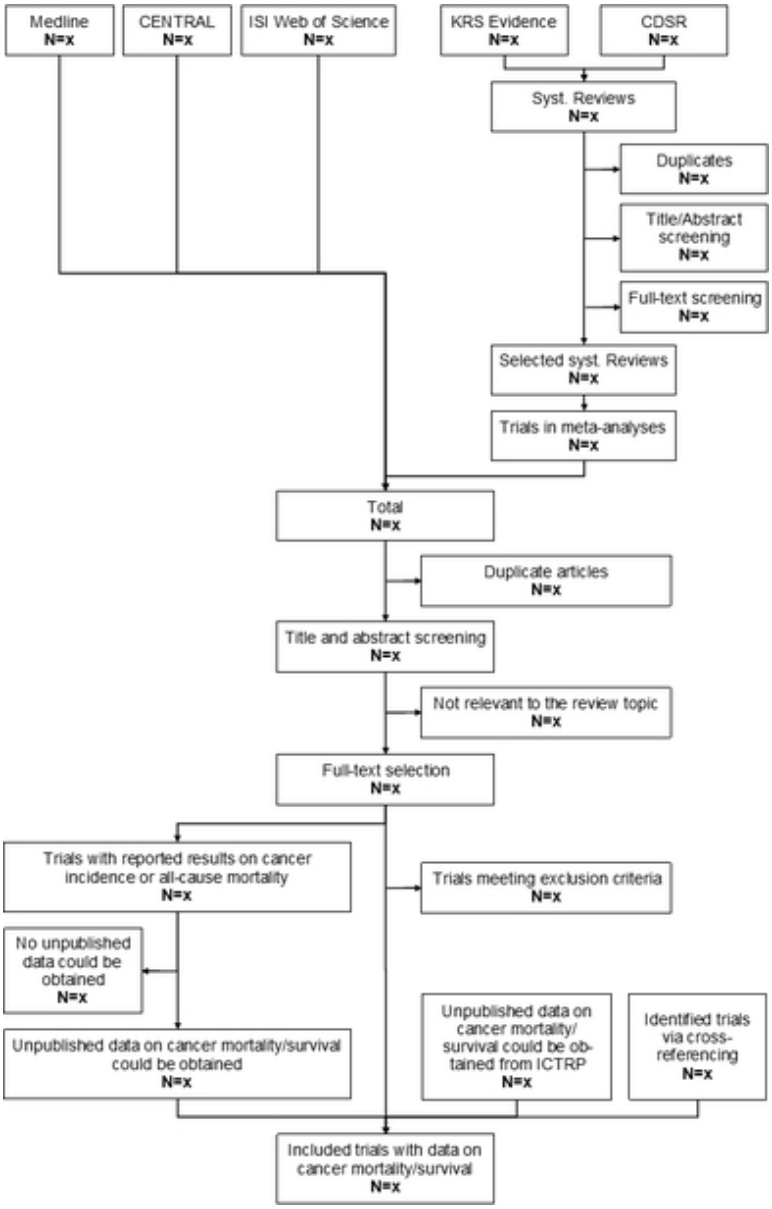

Figure 1: Draft of the search strategy and study selection process

16x25mm (600 x 600 DPI)

Appendix 1 -Data Extraction Form

Table A1 - Extraction of data from publications

Study			Population								Intervention		
First author (or study name), Year (REF)	Country	Sample size	General population (y/n)	Cancer population (y/n)	Cancer site + stage	Age (mean or median)	Sex (female %)	Race/ ethnicity/ skin color (white %)	BMI [kg/m²] (n, mean or median)	25(OH)D (n, mean or median)	Vitamin D_3 dosing regimen	Duration of vitamin D3 supplemen-tation	Compliance in intervention group (%)

Comparator		Outcomes							Comments
Placebo (y/other)	Compliance in comparator group (%)	Maximum follow-up time	Mean or median follow-up time	No of cancer deaths	Cancer mortality in general population HR (95% CI)	Cancer-specific survival of cancer patients HR (95% CI)	Overall survival of cancer patients HR (95%CI)	Covariates adjusted for	

Table A2 - Risk of Bias Assessment

First author (or study name), Year (REF)	Sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective reporting	Other bias

Table A3 - Variables to be obtained for all trial participants in order to conduct the IPD meta-analysis

No.	Variables
1	Age
2	Sex
3	BMI
4	Race/ethnicity/skin color
5	Baseline 25(OH)D levels
6	Compliance
7	Randomisation group allocation
8	Baseline date
9	Death during follow-up (y/n)
10	Date of death
11	Censoring date for survivors
12	Cancer death during follow-up (y/n)
13	Date of cancer death
14	Censoring date for non-cancer deaths
15	Cancer* diagnosis during follow-up (y/n)
16	Cancer* site for each cancer during follow-up
17	Cancer* stage for each cancer during follow-up
18	Date of cancer* diagnosis for each cancer during follow-up
19	Cancer* diagnosis in first 5 years before baseline (y/n)
20	Cancer* site for each cancer in first 5 years before baseline
21	Cancer* stage for each cancer in first 5 years before baseline
22	Date of cancer* diagnosis for each cancer in first 5 years before baseline

* Excluding non-melanoma skin cancers

PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☒	☐	1-4
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☒	n.a. (first submission)
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	63-66
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	6-34
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	438-441
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☒	☐	371-373
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	457-461
Sponsor	5b	Provide name for the review funder and/or sponsor	☒	☐	457-461
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☒	☐	459-461
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	77-123
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☒	☐	125-141
METHODS					

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☒	☐	148-188
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	198-231, 256-266
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	198-231, 223-224, 230-231, 566-569
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	233-266
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	234-245
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	246-255, 362-364, 563-565
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	289-292, 362-364
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☒	☐	126-139, 172-178, table 2
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☒	☐	268-286, 365-370
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☒	☐	294-300
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☒	☐	301-328, 347-348, 349-359
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☒	☐	329-346, 353-359
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☐	☒	n.a. (quantitative

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
					synthesis will be performed)
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☒	☐	360-361, 402-406
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	365-370

BMJ Open

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

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Primary Subject Heading:	Patient-centred medicine
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Efficacy of Vitamin D_3 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

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ABSTRACT

Introduction

Vitamin D insufficiency is much more common among cancer patients than the general population. Previous meta-analyses of controlled trials showed an approximately 15% reduction of cancer mortality by vitamin D supplementation compared to placebo or no treatment in the general population.

On top of updating the latest systematic review on vitamin D supplementation and cancer mortality in the general population, we aim to conduct the first meta-analyses of trials on vitamin D_3 supplementation and cancer-specific and overall survival of cancer patients. Besides, we will conduct for the first time subgroup analyses based on individual patient data collected from randomized controlled trials.

Methods and analysis

A systematic review and individual patient data meta-analysis will be performed on randomized placebo-controlled trials with a vitamin D_3 intervention. The addressed outcomes are cancer mortality in the general population as well as cancer-specific and overall survival of cancer patients. The quality appraisal of the studies will be evaluated by the Cochrane risk-of-bias tool for randomized trials. Trial results will be re-analyzed using adjusted and unadjusted Cox proportional hazard regression models and meta-analyses are planned. Cochran's Q-Test and the I^2 index will be used to statistically assess the level of heterogeneity, while sensitivity and subgroup analyses serve to identify potential causes of heterogeneity. Subgroup analyses will be conducted for vitamin D_3 dosing, follow-up time, age, sex, obesity, vitamin D deficiency/insufficiency, history of cancer, and compliance. Publication bias will be assessed by funnel plots and Egger's test.

Ethics and dissemination

Ethical approval is not required since no human beings are involved in this systematic review. Results will be published in a peer-reviewed journal with open access. They will be presented at conferences and sent to patient advocacy groups and German oncologic rehabilitation centers.

PROSPERO registration number

CRD42020185566

Keywords

Vitamin D, cancer, mortality, systematic review, meta-analysis

Strengths and limitations of this study

- First meta-analysis on vitamin D_3 supplementation and cancer survival as well as first individual patient data meta-analysis on this research topic.
- Results of subgroup analyses based on individual patient data allow fundamental insights for personalized medicine and may be used as guidance for future clinical trials targeting cancer patients that presumably profit most from vitamin D supplementation
- Conduction of the systematic review according to this protocol and a thorough assessment of study quality, sources of heterogeneity, and bias in meta-analyses minimize the risk of bias and will gather reproducible results.
- Number of studies with eligible data for subgroup analyses may be limited.

INTRODUCTION

Background

The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018.(1) There is accumulating evidence from epidemiological studies that a low vitamin D status goes along with increased risks of several types of cancer. Meta-analyses of observational studies reported increased risks of lung cancer, colorectal cancer, breast cancer, bladder carcinoma, and lymphoma in subjects with low 25(OH)D serum concentrations.(2-5) Furthermore, epidemiological studies have shown that low serum levels of 25-hydroxyvitamin D (25(OH)D), the acknowledged best biomarker to measure vitamin D status, were strongly associated with substantially increased cancer mortality.(6) For example, in a German population-based cohort study of older adults, the risk to die of cancer was increased by 42% in study participants with vitamin D deficiency (defined as 25(OH)D < 30 nmol/L) compared to individuals with sufficient 25(OH)D levels > 50 nmol/L (hazard ratio [HR] and 95% confidence interval (95%CI): 1.42 [1.08; 1.87]).(7)

The molecular links between vitamin D and carcinogenesis and progression have been previously described.(8) In brief, genomic mechanisms of the active hormone $1,25(OH)_2D$ impact signaling pathways that regulate cell proliferation, differentiation, and cell survival. $1,25(OH)_2D$ may primarily act as an anti-proliferative agent in many tissues and may slow down malignant cellular growth. Thus, there is biological plausibility that a sufficient vitamin D supply is especially essential for a good cancer prognosis. A causal relationship of low 25(OH)D levels and cancer mortality was furthermore supported by a Mendelian randomization study conducted within three large cohorts from Denmark.(9)

Several randomized trials with vitamin D supplementation have been conducted with mostly the aim to improve skeletal outcomes at older ages. Cancer mortality was a secondary outcome in all trials and therefore the trials were not specifically designed for this outcome.(10) Despite strong heterogeneity in study populations, intervention schemes, and other important design aspects, three out of four meta-analyses demonstrated a statistically significant reduction in cancer mortality.(11-15)

However, most trials have not been restricted to patients that were vitamin D deficient.(10) The latter is important because the association of 25(OH)D levels and adverse health outcomes is not linear.(6) 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.(16) Therefore, there is a need for a systematic review that re-analyses individual patient data (IPD) from previous trials restricted to subjects with vitamin D insufficiency (25(OH)D < 50 nmol/L) or deficiency (25(OH)D < 30 nmol/L).

Another important reason to re-analyze the previous trial data is that most studies were not restricted to cancer patients. Vitamin D deficiency or insufficiency are much more common among cancer patients than among the general population. In a study with 2,912 colorectal cancer patients, vitamin D deficiency (25(OH)D levels < 30 nmol/L) was found among 59% of colorectal cancer patients during or shortly after first-line treatment, and, in agreement with previous evidence, low 25(OH)D levels were strongly associated with poorer survival.(17, 18) Systematic reviews of observational studies on 25(OH)D levels and cancer prognosis concluded that sufficient 25(OH)D levels are associated with a better prognosis of breast and colorectal cancer, whereas there are too few studies for other cancer sites up to date to draw conclusions.(17, 19)

Further important potential effect modifiers that deserve close investigation are obesity and compliance. People with low compliance and/or obesity, who may need higher vitamin D doses because vitamin D is stored in adipocytes, might have attenuated the overall treatment effect in the trials.(20)

Objective

The objective of our systematic review is to assess the efficacy of vitamin D_3 supplementation on cancer mortality in the general population and the prognosis of cancer patients with special attention to potential effect modifiers, including baseline 25(OH)D levels, cancer at baseline, BMI and compliance.

The main outcomes include “cancer mortality in the general population”, “cancer-specific survival of cancer patients” and “overall survival of cancer patients”. These outcomes are universally used in cancer studies and do not need further refinement during the review.

In a first step, we intend to update the previous systematic reviews on vitamin D supplementation and cancer mortality in the general population by including newly published trials and unpublished data from trials with outcome data on cancer incidence or all-cause mortality by asking the authors for data on cancer mortality. Second, we will obtain data for an IPD meta-analysis. Third, we will conduct IPD meta-analyses on vitamin D_3 supplementation and overall and cancer-specific survival among cancer patients. Forth, we will conduct subgroup analyses to explore sources of heterogeneity and to identify effect modifiers. The timetable for the review is shown in Table 1.

Table 1: Proposed timetable for conducting the review

Step	**Timeframe for completion**
Literature search, abstract and full-text selection	2.5 months
Data extraction & individual patient data acquisition	2.5 months
Quality appraisal	2 months
Data analysis & meta-analysis	3 months
Writing of manuscript	2 months
Total	**12 months**

METHODS AND ANALYSIS

Study selection criteria/ Eligibility criteria

We will follow a two-step approach for the study selection: First, all trials will be selected that could potentially have published or unpublished data on the research topic. All authors of trials with potentially unpublished data on cancer mortality/survival will be contacted to provide data. In the second step, only trials with eligible data for a meta-analysis will be included.

Step 1: Inclusion criteria for trials

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).

Interventions: We will focus on trials that used vitamin D_3 in any dose and any regimen (e.g. daily/weekly/monthly intake) as the intervention. However, the minimum time of the intervention shall be six months to exclude studies with one-time bolus interventions or very short intervention periods. The first reason is that cancer mortality is highly unlikely to be influenced by very brief intervention periods. The second reason is that after initiating daily, weekly, or monthly supplementation schedules, it takes three to six months for 25(OH)D levels to reach homeostasis.

Besides, we will also include studies using vitamin D_3 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 $1,25(OH)_2D$.

We will exclude studies with vitamin D_2 supplementation since the Cochrane review of Bjelakovic et al. and other recent data showed clearly no efficacy on mortality.(10, 11, 15, 21) Co-supplementation with calcium or other dietary supplements in the intervention arm will not be an exclusion criterion. A sensitivity analysis will elucidate whether the inclusion of these studies had an impact on the overall effect estimate of the meta-analysis.

Comparators: We will include only studies, which used placebo as the comparator.

Outcomes: To be eligible for inclusion in a meta-analysis trials need to have assessed the outcome of cancer mortality, cancer survival, or cancer-specific survival. In an intermediate step of the systematic review, we will also record studies with the outcomes of cancer incidence or all-cause mortality and contact the authors if they have data for the outcomes needed for the planned meta-analyses. The definitions of all outcomes are shown in Table 2.

Table 2: Definition of outcomes

Outcome	Definition
All-cause mortality	Rate of deaths during a specific time period in population at risk
Cancer mortality	Rate of cancer deaths during a specific time period in population at risk
Cancer incidence	Rate of newly diagnosed cancer cases during a specific time period in population at risk
Overall cancer survival	Proportion of patients from a cancer population at risk alive at some point subsequent to the diagnosis of their cancer
Cancer-specific survival	Proportion of patients from a cancer population at risk who did not die of cancer at some point subsequent to the diagnosis of their cancer

Study design: We will include RCTs in which, analogous to the intervention period, the follow-up period is at least six months. The follow-up time should not be longer than the time under treatment. We will focus on parallel-group designs and exclude single-arm studies. We will further exclude all types of cohort studies and case-control studies as well as the following types of records: reviews, dissertations, theses, editorials, study protocol, clinical guidelines, commentaries, and letters.

Setting: There will be no restrictions by type of setting.

Minimum sample size: The studies need to have at least one cancer death in the verum and placebo group.

Geographical location: No restrictions are defined regarding the geographical location.

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.

Information sources and search strategy

The search strategy will be elaborated by SK, BS, and A Heppert. Mrs. Heppert is a specialist for systematic bibliographic searches at the Central Library of the German Cancer Research Center and is not otherwise associated with the project. Finally, it will be peer-reviewed by HB and carried out by SK.

The bibliographic databases MEDLINE (Pubmed interface), ISI Web of Science (WoS; Clarivate Analytics interface), and the Cochrane Central Register of Controlled Trials (CENTRAL; OVID interface) will be searched systematically. We will also carry out a systematic search for previous systematic reviews in the Cochrane Database of Systematic Reviews (CDRS, OVID interface) and KSR Evidence (https://ksevidence.com), which are both specialized search engines for systematic reviews. RCTs included in meta-analyses on the topics vitamin D supplementation and cancer mortality, cancer incidence, all-cause mortality, or cancer survival will be extracted and merged with the hits found in the bibliographic database search. The electronic database search will be complemented by searching the WHO's International Clinical Trials Research Portal (ICTRP) and clinicaltrials.gov to capture results from ongoing or recently completed RCTs that have not been published in scientific journals, yet. We will also scan the reference lists of eligible studies to yield additional trial articles via cross-referencing. A draft of the search strategy is presented in Figure 1.

We will search in MEDLINE, ISI WoS, and CENTRAL for medical subject headings (MeSH), free text words, synonyms, and related search terms for the concepts "vitamin D", "mortality", "cancer", "randomized controlled trial" and "placebo". Besides, standard search terms for RCTs will be used additionally wherever available. No restrictions are planned in the search strategy to prevent overlooking important studies that have not been correctly classified in the respective bibliographic databases. All databases will be searched from inception dates. Moreover, we will not limit the search to studies in English as relevant studies might also be published in other languages. The search string for MEDLINE is shown in Table 3.

Table 3: Search string for MEDLINE

Step	Search string
1	"vitamin d"[tw] OR "vitamin D"[MeSH] OR cholecalciferol[MeSH] OR cholecalciferol*[tw] OR calciol[tw] OR hydroxycholecalciferols[MeSH] OR hydroxycholecalciferol*[tw] OR dihydroxycholecalciferol*[tw] OR “vitamin d3"[tw] OR “vitamin d 3”[tw] OR calcitriol[MeSH] OR calcitriol[tw] OR "1-hydroxycholecalciferol"[tw] OR calcifediol[MeSH] OR calcifediol[tw] OR calcidiol[tw] OR alfacalcidol[Supplementary Concept] OR alphacalcidol[tw] OR alfacalcidol[tw]
2	mortality[tw] OR mortality[MeSH] OR death[MeSH] OR death[tw] OR died[tw] OR dead[tw] OR survival[tw] OR surviv*[tw] OR survival[MeSH]
3	neoplasms[MeSH] OR neoplas*[tw] OR malignanc*[tw] OR cancer*[tw] OR tumor*[tw] OR tumour*[tw] OR carcinoma*[tw]
4	(((((((((“randomized controlled trial”[pt]) OR “controlled clinical trial”[pt]) OR randomized[tiab]) OR placebo[tiab]) OR “drug therapy”[sh]) OR randomly[tiab]) OR trial[tiab]) OR groups[tiab])) NOT ((animals[mh] NOT humans[mh]))
5	placebos[MeSH] OR placebo[tw]
6	2 OR 3
7	1 AND 4 AND 5 AND 6

A shortened version of the MEDLINE search string will be used to search for systematic reviews in CDSR and KSR Evidence. Only the first three search steps are needed because the study design is “systematic review” and not “placebo-controlled RCT”. The search string for CDSR is shown in Table 4. The literature search will be updated during the peer-review process of the publication in order to include the most up to date literature.

Table 4: Search string for the Cochrane Database of Systematic Reviews

Step	Search string	
1	#1	MeSH descriptor: [Vitamin D] explode all trees
	#2	MeSH descriptor: [Cholecalciferol] explode all trees
	#3	MeSH descriptor: [Calcifediol] explode all trees
	#4	MeSH descriptor: [Calcitriol] explode all trees
	#5	MeSH descriptor: [Hydroxycholecalciferols] explode all trees
	#6	(("alfacalcidol") OR ("alphacalcidol") OR ("hydroxycholecalciferol*") OR ("1- hydroxycholecalciferol") OR ("hydroxyvitamin* D") OR ("calcifediol") OR (“calcidiol”) OR ("calcitriol") OR ("dihydroxycholecalciferol*") OR (“dihydroxyvitamin d*”) OR ("vitamin D") OR (cholecalciferol*) OR ("vitamin D3") OR ("vitamin D 3") OR (“calciol”)) (Word variations have been searched)
	#7	("vitamin d*"):ti,ab,kw (Word variations have been searched)
	#8	{OR #1-#7}
2	#9	MeSH descriptor: [Mortality] explode all trees
	#10	MeSH descriptor: [Death] explode all trees
	#11	MeSH descriptor: [Survival] explode all trees

	#12	("mortality" OR "dea*" OR “died” OR "survival" OR “surviv*”) (Word variations have been searched)
	#13	{OR #9-#12}
3	#14	MeSH descriptor: [Neoplasms] explode all trees
	#15	(carcinoma* OR tumour* OR tumor* OR cancer* OR malignanc* OR neoplas*) (Word variations have been searched)
	#16	#14 OR #15
4	#17	#13 OR #16
5	#18	#8 AND #17 in Cochrane Reviews (Word variations have been searched)

Data collection and management

Study selection and data extraction will be performed in duplicate by two reviewers. Both are blinded to each other’s decision but not to journal titles, study authors, or institutions. The screening will be conducted by entering data into blank Microsoft Word or Excel spreadsheets. The software EndNote will be used to store, organize, and manage all the references and allow a transparent and reproducible systematic search. To assure validity and high quality of the data, the data extraction will be performed by using standard and predefined data extraction forms (see Appendix 1). Both reviewers will scan independently the titles and abstracts of studies obtained by the aforementioned search strategy against the eligibility criteria. For those studies that meet the inclusion criteria or that cannot yet be fully excluded, full-text reports will be acquired and screened again towards the inclusion criteria. In the next step, the results of both reviewers will be compared and in cases of disagreement, critical points will be discussed until a consensus is reached. If necessary, we will contact study authors to resolve questions about eligibility. We will document the reasons for excluding trials.

After completing the abstract and full-text selection with eligible studies, the two reviewers will extract independently the pre-defined data (see Appendix 1). Extracted items will include first author, publication year, country, number of participants, general population or medical condition (including cancer site and stage(s)), sex, mean/median age, race/ethnicity/skin color, mean/median BMI, mean/median 25(OH)D levels at baseline, vitamin D_3 dosing regimen, duration of vitamin D_3

supplementation, compliance, mean/median and maximum follow-up time, number of cancer deaths and effect estimates (including confidence intervals) reported for cancer mortality/cancer survival. Individual patient data for the aforementioned variables will be obtained from all trials with at least 20 cancer deaths (see Appendix 1). If summary data are not published, they shall be calculated from the obtained data. All authors will be contacted by e-mail with a maximum of three attempts sent with two weeks apart.

For the meta-analyses on cancer survival and cancer-specific survival, we will ask all authors who conducted trials in the general population to provide IPD for cancer diagnoses in the five years prior to baseline and during the trial (including cancer site with ICD-code, stage, and diagnosis date). The following IPD will be additionally collected: age, sex, BMI, race/ethnicity/skin color, baseline 25(OH)D levels, compliance, randomization group allocation, baseline date, deaths during follow-up with date, cancer deaths with date, censoring dates for survival outcomes, and censoring date for patients not dying of cancer (see Appendix 1). If IPD cannot be shared, the authors of the studies will be asked to conduct the analyses in-house and to provide the summary estimates for the meta-analysis. If trial authors do not collaborate, their study cannot be included in subgroup analyses for which no effect estimates were published but the result from the total trial population will remain included in the main meta-analysis.

Quality assessment

The protocol of the systematic review with all planned statistical analyses has been registered in PROSPERO before data collection to preclude data-driven analyses and selective reporting of only statistically significant findings. The study protocol has been developed in line with the “Preferred reporting items for systematic review and meta-analysis protocols” (PRISMA-P, see Appendix 2), the Cochrane Handbook for Systematic Reviews of Interventions as well as the Institute of Medicine guideline.(22-25) We will ensure to fulfill all requirements recommended by the current PRISMA guideline when writing the publication of the systematic review.(26, 27)

The Cochrane risk-of-bias tool for randomized trials (RoB 2) will be used to assess various domains of bias including aspects of trial design, conduct, and reporting (see Appendix 1).(28, 29) The following domains will be covered during the evaluation: sequence generation, allocation concealment, blinding, incomplete outcome data (e.g. withdrawals and dropouts), and selective outcome reporting. A summary assessment will be made based on the extracted items, judging whether the risk of bias in the respective study is low, high, or has some concerns. If only insufficient data is reported, the risk of bias is "unclear" and the original study authors will be contacted for further information. The assessment will be conducted by two independent reviewers using the RoB 2 tool.(28, 29) In cases of disagreement, critical points will be discussed until a consensus is reached. The risk of bias evaluation will be incorporated into the data synthesis by performing a sensitivity analysis that excludes studies with high or unknown risk of bias.

Descriptive analysis and meta-analysis

Measures of treatment effect

The mortality/survival outcomes shall be addressed by estimating hazard ratios (HR) and 95% confidence intervals (95%CI). Results of the intention-to-treat (ITT) approach will be used, including all patients randomized when both ITT and per-protocol results are given.

Data synthesis

As far as study quality and differences between studies allow, effect estimates of all eligible studies with data for the following three main meta-analyses will be pooled deriving random effects results with the DerSimonian and Laird method (primary analysis) and fixed effects summary estimates using the Mantel-Haenzel method (secondary analysis).

1) Association of vitamin D_3 supplementation and cancer mortality in the general population
2) Association of vitamin D_3 supplementation and cancer-specific survival of cancer patients
3) Association of vitamin D_3 supplementation and overall survival of cancer patients

For all studies that provide IPD, unadjusted Cox proportional hazard regression models will be used to estimate HRs and 95%CIs for the main meta-analyses in which we will pool effect size data from studies who do and who do not provide IPD in a two-step approach. For studies that cannot send

IPD to the coordinating center (German Cancer Research Center, Heidelberg), authors are being asked to estimate the HRs and 95%CIs themselves and send the summary data for the meta-analyses. To assess cancer survival as time-to-event data from general population cohorts, the study will be restricted to patients with a history of cancer in the five years preceding baseline or a cancer diagnosis during the trial. For the former, the survival time will be calculated from baseline to death/end of the trial, and for the latter, survival time will be counted from the date of cancer diagnosis till death/end of the trial.

With all studies that agree to send IPD data to the coordinating center or to do additional analyses in-house, we will also conduct an additional multivariate Cox proportional hazards regression model. The model for the outcome cancer mortality among general population studies will contain the variables vitamin D_3 intervention (vs. placebo), age (continuous; < 70 vs. ≥ 70 years), sex (male, female, unknown), BMI (< 25 vs. 25 – 29.9 vs. ≥ 30 kg/m² vs. unknown), skin color (white vs. black/brown vs. other), 25(OH)D baseline level (< 30 vs. 30 – 49.9 nmol/L vs. ≥ 50 nmol/L vs. unknown), diagnosis of cancer (except non-melanoma skin cancer and benign tumors) in five years before baseline (yes vs. no vs. unknown), health status (general healthy population vs. diseased population), and compliance (< 80% vs. ≥ 80% vs. unknown). The models for the outcomes overall and cancer-specific survival of cancer patients will be adjusted for the same variables but the variable "diagnosis of cancer in five years before baseline" will be replaced by more specific variables for cancer stage (only advanced stages III and/or IV vs. unknown), cancer site (prostate vs. colorectal vs. breast vs. lung vs. other vs. unknown) and time since cancer diagnosis (<1 year vs. 1-5 years). We will test for interactions of the treatment variable (vitamin D_3 vs. placebo) with these covariates to identify potential effect modifiers. Again, a two-step approach will be used for the meta-analyses, whereby the analyses are carried out on a study-specific basis, and then the effect estimates are pooled. To further explore the variation of the treatment effect by methodological or patient characteristics differences of the studies, the following subgroup analyses will be performed with IPD data and studies that published eligible data:

<u>Subgroup analyses according to trial design</u>

- Daily dose vs. weekly/monthly bolus dose vs. bolus dose at the beginning of the trial followed by a daily dose
- Low vs. moderate vs. high vitamin D_3 dosing (< 1,000 IU vs. 1,000 – 2,000 IU vs. > 2,000 IU per day or equivalent weekly or monthly taken dose)
- Vitamin D_3 supplementation duration (< 5 vs. ≥ 5 years)
- Health status (general population vs. diseased population)
- Region (North America vs. Europe vs. Other)

Subgroup analyses according to patient characteristics

- Age (< 70 vs. ≥ 70 years)
- Sex (male vs. female)
- Skin color (white vs. black/brown vs. other)
- BMI (< 25 vs. 25 – 29.9 vs. ≥ 30 kg/m²)
- Baseline 25(OH)D levels (< 30 vs. 30 – 49.9 nmol/L vs. ≥ 50 nmol/L)
- Compliance rate (< 80% vs. ≥ 80%)

For meta-analyses conducted in cancer patients in addition:

- Cancer stage (only advanced stages III and/or IV vs. unknown)
- Cancer site (prostate vs. colorectal vs. breast vs. lung vs. other)
- Time since cancer diagnosis (<1 year vs. 1-5 years)

Analyses in the coordinating center will be done with the statistical software SAS 9.4. The meta-analyses will be performed with Comprehensive Meta-Analysis 2.0 (Biostat, Englewood, NJ).

Assessment of heterogeneity

Heterogeneity will be presented visually by forest plots and assessed statistically by Cochran's Q test (significance level = 0.05) as well as the I^2 index (< 25% low, 25-50% moderate, > 50% high heterogeneity). Meta-Analyses will be conducted even if high heterogeneity is being detected and the results will be discussed taking the heterogeneity into consideration. Sources of heterogeneity

will be explored by the subgroup analyses outlined in the previous section and the following sensitivity analyses:

- Excluding studies with a high or unknown risk of bias according to assessment with the Cochrane risk-of-bias tool for randomized trials
- Excluding studies not reporting ITT results
- Excluding trials with co-supplementation of calcium
- Excluding events in the first year of follow-up

Assessment of publication bias

Publication bias will be accessed visually in funnel plots and tested for with Egger's test.

Dealing with missing data

In case of missing data, we will seek contact with the original investigators. If possible, we will calculate missing numerical data from the given reported data.

Strength of the body of evidence

The quality of the evidence for each outcome will be evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. The four levels of evidence comprise very low, low, moderate, and high. Evidence from RCTs starts as high quality but can be decreased for reasons such as the risk of bias, imprecision, inconsistency, indirectness, and publication bias.

Amendments

In the case of protocol amendments, we will document the date, the description of the change, and the rationale in a pre-defined log sheet in Microsoft Word or Excel.

Patient and public involvement

Patients and the public were not involved in the development of the study design. Since this is a protocol for a systematic review and no participant recruitment will take place, the involvement of patients in the recruitment, the conduct of the study and the dissemination of findings to study participants are not applicable.

Ethics and dissemination

An ethics approval is not required for this systematic review because it is only a summary of already published trial data. All studies to be included in the systematic review have their own ethics approvals, which are named in the original publications. For the IPD meta-analysis, we will take care that the additional analyses are in adherence with the ethics approvals of the trials.

The systematic review will be published in an international peer-reviewed journal for clinical oncology or general medicine with open access option and presented in national and international meetings. If the meta-analyses of the systematic review obtain statistically significant findings, we expect the result to be reflected in national and international guidelines and to change the current practice of tertiary prevention among cancer patients. Vitamin D_3 is already on the market in various doses and at low costs because it is not patented.

Patients will be informed via a press release from the German Cancer Research Center. Moreover, we will send a summary of the results in a language suitable for laypersons to all patient advocacy groups recommended by the Cancer Information Service of the German Cancer Research Center (up to data n = 30) for further dissemination among their members.(30) With respect to oncologists, we will disseminate the results to all German rehabilitation centers having a ward for oncologic rehabilitation, as listed in the register of the *Bundesarbeitsgemeinschaft für Rehabilitation e.V.*(31) As the topic of the review is in the field of tertiary prevention, oncologists in the rehabilitation setting are the target audience for information dissemination.

DISCUSSION

One of the strengths of this systematic review comprises the first meta-analysis on vitamin D supplementation and cancer survival and additionally the first IPD meta-analysis on this research topic. The IPD meta-analysis will allow the investigation of potential effect modifiers. Especially 25(OH)D levels at baseline, BMI, and compliance are candidates that could have had a great impact on the overall trial results.

The creation of this research protocol prompted us to plan carefully all the details of the systematic review and to anticipate and address potential problems before their actual occurrence. Arbitrary decision making concerning any procedure of this systematic review is prevented, resulting again in a decreased risk of publication bias and selective reporting bias. The protocol allows reproducible and transparent research for future reviewers.

Possible limitations of our review include a potentially insufficient number of cancer deaths in the studies and high heterogeneity, which could both negatively influence the statistical power of the meta-analyses. However, it is still too early to judge whether these limitations occur.

The quality of selected studies will be assessed and the quality of the evidence will be judged. The ultimate goal is to ensure the reporting of highly meaningful findings for clinicians and patients. Oncologists are well aware that vitamin D deficiency and insufficiency are very common in cancer patients but there is uncertainty about whether and how they should routinely perform preventive screening and treatments. 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. Since vitamin D products are readily available in pharmacies or drug stores, many patients use low-dose vitamin D supplementation as self-medication. Yet, it can be doubted whether this untargeted intervention has any effect on cancer prognosis. Consequently, evidence-based recommendations for high-dose vitamin D supplementation are highly relevant for both, clinicians and patients.

If the planned systematic review determines the efficacy of vitamin D supplementation on cancer prognosis in the expected magnitude of 10-15%, the review will be used to provide clear suggestions on how vitamin D can be appropriately dosed to overcome vitamin D deficiency or insufficiency in cancer patients.(12) Furthermore, our systematic review would provide the evidence for statutory health insurances to cover the costs for screening for vitamin D deficiency or insufficiency in cancer patients and a subsequent vitamin D supplementation. With expected relatively large effects and very low screening and treatment costs (A vitamin D blood test costs approx. € 20, and one year of vitamin D therapy costs less than € 100.), vitamin D supplementation will be highly cost-effective. The costs would be close to negligible compared to other current cancer treatment costs.

Status

At the time of submission, the study selection for the systematic review has not started.

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Authors' contribution

All authors meet the ICMJE criteria for authorship as follows: BS and SK are the guarantors of the systematic review, therefore, are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors made substantial contributions to the conception of the work. BS and SK designed the search strategy and the risk of bias assessment strategy. BS developed the selection criteria, the data extraction criteria, and the statistical methods. SK drafted the protocol publication, which BS and HB revised critically for important intellectual content. All authors approved the final version to be published.

Contributors

The following researchers have agreed to contribute to this systematic review by sharing IPD and giving intellectual input for the discussion of the results: Taisuke Akutsu (Jikei University School of Medicine. Tokyo, Japan), Professor Julie E. Buring (ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor Carlos A. Camargo Jr (MD, DrPH. Massachusetts General Hospital, Harvard Medical School. Boston), Professor Nancy R. Cook (ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor I-Min Lee (MB, BS, ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor JoAnn E. Manson (MD, Dr. PH. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor Robert Scragg (MBBS, PhD. School of Population Health, University of Auckland. Auckland, New Zealand), John Sluyter (PhD. School of Population Health, University of Auckland. Auckland, New Zealand), Professor Mitsuyoshi Urashima (MD. Jikei University School of Medicine. Tokyo, Japan), Professor Jean Wactawski-Wende (PhD. University at Buffalo. Buffalo, New York).

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Data availability statement

As no new data are obtained in this systematic review, no data will be made publicly available to third parties. Decisions on data use by third parties can only be made by the principal investigators of the original studies, taking into account the votes of the responsible ethics committees.

Disclaimer

The views of the authors do not necessarily reflect those of the German Cancer Research Center.

Competing interests

None declared.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer-reviewed

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Appendix 1:

Supplementary material consisting of data extraction form, risk of bias assessment and list of individual patient data to be obtained from original researchers

Appendix 2:

Supplementary material consisting of PRISMA-P checklist

Figure

Figure 1: Draft of the study selection process

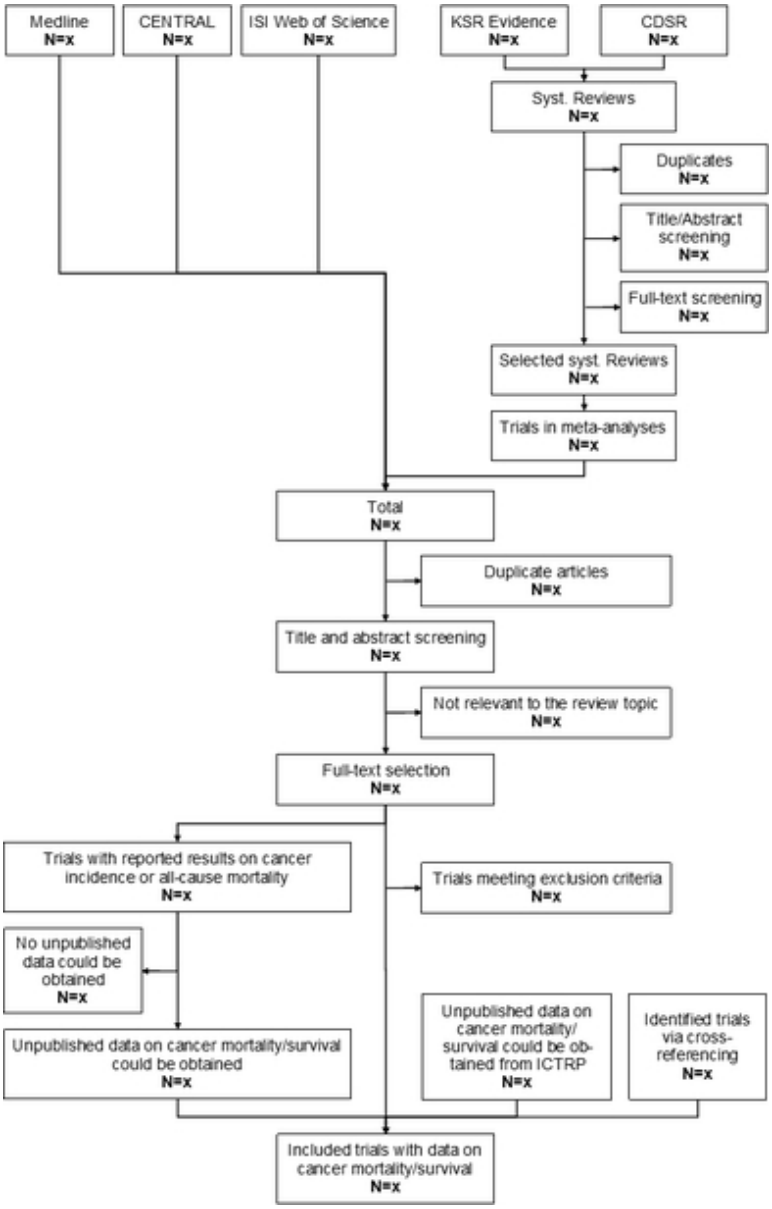

Figure 1: Draft of the study selection process

16x25mm (600 x 600 DPI)

Study			Population				
First author (or study name), Year (REF)	Country	Sample size	General population (y/n)	Cancer population (y/n)	Cancer site + stage	Age (mean or median)	Sex (female %)

			Intervention			Comp
Race/ ethnicity/ skin color (white %)	BMI [kg/m²] (n, mean or median)	25(OH)D (n, mean or median)	Vitamin D_3 dosing regimen	Duration of vitamin D3 supplementation	Compliance in intervention group (%)	Placebo (y/other)

parator	Outcomes				
Compliance in comparator group (%)	Maximum follow-up time	Mean or median follow-up time	No of cancer deaths	Cancer mortality in general population HR (95% CI)	Cancer-specific survival of cancer patients HR (95% CI)

		Comments
Overall survival of cancer patients HR (95%CI)	**Covariates adjusted for**	

First author (or study name), Year (REF)	R	D	Mi	Me	S	O

Risk of bias legend

R Bias arising from the randomiation

D Bias due to deviations from

Mi Bias due to missing outcome data

Me Bias in measurement of the

S Bias in selection of the reported

O Overall risk of bias

The following variables will be obtained for all trial participants in order to conduct the IPD meta-a

No.	Variables
1	Age
2	Sex
3	BMI
4	Race/ethnicity/skin color
5	Baseline 25(OH)D levels
6	Compliance
7	Randomisation group allocation
8	Baseline date
9	Death during follow-up (y/n)
10	Date of death
11	Censoring date for survivors
12	Cancer death during follow-up (y/n)
13	Date of cancer death
14	Censoring date for non-cancer deaths
15	Cancer* diagnosis during follow-up (y/n)
16	Cancer* site for each cancer during follow-up
17	Cancer* stage for each cancer during follow-up
18	Date of cancer* diagnosis for each cancer during follow-up
19	Cancer* diagnosis in first 5 years before baseline (y/n)
20	Cancer* site for each cancer in first 5 years before baseline
21	Cancer* stage for each cancer in first 5 years before baseline
22	Date of cancer* diagnosis for each cancer in first 5 years before baseline

* Excluding non-melanoma skin cancers and benign tumors

analysis:

PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☒	☐	1-4
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☒	n.a. (first submission)
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	63-64
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	6-34
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	443-451
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☒	☐	370-372
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	467-471
Sponsor	5b	Provide name for the review funder and/or sponsor	☒	☐	467-471
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☒	☐	467-471
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	79-124
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☒	☐	126-140
METHODS					

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☒	☐	150-190
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	192-225
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	217-218, 224-225, figure 1
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	227-262
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	227-262
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	227-262, appendix 1
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	361-363, appendix 1
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☒	☐	131-133, 168-172, table 2
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☒	☐	264-282, 347-360
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☒	☐	290-297
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☒	☐	297-325, 345-358
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☒	☐	326-344, 351-358
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☐	☒	n.a. (quantitative

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
					synthesis will be performed)
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☒	☐	359-360
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	364-369

BMJ Open

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

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Primary Subject Heading:	Patient-centred medicine
Secondary Subject Heading:	Public health
Keywords:	Nutritional support < ONCOLOGY, ONCOLOGY, NUTRITION & DIETETICS, PUBLIC HEALTH, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Epidemiology < ONCOLOGY

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Efficacy of Vitamin D_3 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

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ABSTRACT

Introduction

Vitamin D insufficiency is much more common among cancer patients than the general population. Previous meta-analyses of controlled trials showed an approximately 15% reduction of cancer mortality by vitamin D supplementation compared to placebo or no treatment in the general population.

On top of updating the latest systematic review on vitamin D supplementation and cancer mortality in the general population, we aim to conduct the first meta-analyses of trials on vitamin D_3 supplementation and cancer-specific and overall survival of cancer patients. Besides, we will conduct for the first time subgroup analyses based on individual patient data collected from randomized controlled trials.

Methods and analysis

A systematic review and individual patient data meta-analysis will be performed on randomized placebo-controlled trials with a vitamin D_3 intervention. All databases are searched from inception without time restriction. The addressed outcomes are cancer mortality in the general population as well as cancer-specific and overall survival of cancer patients. The quality appraisal of the studies will be evaluated by the Cochrane risk-of-bias tool for randomized trials. Trial results will be re-analyzed using adjusted and unadjusted Cox proportional hazard regression models and meta-analyses are planned. Cochran's Q-Test and the I^2 index will be used to statistically assess the level of heterogeneity, while sensitivity and subgroup analyses serve to identify potential causes of heterogeneity. Subgroup analyses will be conducted for vitamin D_3 dosing, follow-up time, age, sex, obesity, vitamin D deficiency/insufficiency, history of cancer, and compliance. Publication bias will be assessed by funnel plots and Egger's test.

Ethics and dissemination

Ethical approval is not required since no human beings are involved in this systematic review. Results will be published in a peer-reviewed journal with open access. They will be presented at conferences and sent to patient advocacy groups and German oncologic rehabilitation centers.

PROSPERO registration number

CRD42020185566

Keywords

Vitamin D, cancer, mortality, systematic review, meta-analysis

Strengths and limitations of this study

- First meta-analysis on vitamin D_3 supplementation and cancer survival as well as first individual patient data meta-analysis on this research topic.
- Results of subgroup analyses based on individual patient data allow fundamental insights for personalized medicine and may be used as guidance for future clinical trials targeting cancer patients that presumably profit most from vitamin D supplementation
- Conduction of the systematic review according to this protocol and a thorough assessment of study quality, sources of heterogeneity, and bias in meta-analyses minimize the risk of bias and will gather reproducible results.
- Number of studies with eligible data for subgroup analyses may be limited.

INTRODUCTION

Background

The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018.(1) There is accumulating evidence from epidemiological studies that a low vitamin D status goes along with increased risks of several types of cancer. Meta-analyses of observational studies reported increased risks of lung cancer, colorectal cancer, breast cancer, bladder carcinoma, and lymphoma in subjects with low 25(OH)D serum concentrations.(2-5) Furthermore, epidemiological studies have shown that low serum levels of 25-hydroxyvitamin D (25(OH)D), the acknowledged best biomarker to measure vitamin D status, were strongly associated with substantially increased cancer mortality.(6) For example, in a German population-based cohort study of older adults, the risk to die of cancer was increased by 42% in study participants with vitamin D deficiency (defined as 25(OH)D < 30 nmol/L) compared to individuals with sufficient 25(OH)D levels > 50 nmol/L (hazard ratio [HR] and 95% confidence interval (95%CI): 1.42 [1.08; 1.87]).(7)

The molecular links between vitamin D and carcinogenesis and progression have been previously described.(8) In brief, genomic mechanisms of the active hormone $1,25(OH)_2D$ impact signaling pathways that regulate cell proliferation, differentiation, and cell survival. $1,25(OH)_2D$ may primarily act as an anti-proliferative agent in many tissues and may slow down malignant cellular growth. Thus, there is biological plausibility that a sufficient vitamin D supply is especially essential for a good cancer prognosis. A causal relationship of low 25(OH)D levels and cancer mortality was furthermore supported by a Mendelian randomization study conducted within three large cohorts from Denmark.(9)

Several randomized trials with vitamin D supplementation have been conducted with mostly the aim to improve skeletal outcomes at older ages. Cancer mortality was a secondary outcome in all trials and therefore the trials were not specifically designed for this outcome.(10) Despite strong heterogeneity in study populations, intervention schemes, and other important design aspects, three out of four meta-analyses demonstrated a statistically significant reduction in cancer mortality.(11-15)

However, most trials have not been restricted to patients that were vitamin D deficient.(10) The latter is important because the association of 25(OH)D levels and adverse health outcomes is not linear.(6) 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.(16) Therefore, there is a need for a systematic review that re-analyses individual patient data (IPD) from previous trials restricted to subjects with vitamin D insufficiency (25(OH)D < 50 nmol/L) or deficiency (25(OH)D < 30 nmol/L).

Another important reason to re-analyze the previous trial data is that most studies were not restricted to cancer patients. Vitamin D deficiency or insufficiency are much more common among cancer patients than among the general population. In a study with 2,912 colorectal cancer patients, vitamin D deficiency (25(OH)D levels < 30 nmol/L) was found among 59% of colorectal cancer patients during or shortly after first-line treatment, and, in agreement with previous evidence, low 25(OH)D levels were strongly associated with poorer survival.(17, 18) Systematic reviews of observational studies on 25(OH)D levels and cancer prognosis concluded that sufficient 25(OH)D levels are associated with a better prognosis of breast and colorectal cancer, whereas there are too few studies for other cancer sites up to date to draw conclusions.(17, 19)

Further important potential effect modifiers that deserve close investigation are obesity and compliance. People with low compliance and/or obesity, who may need higher vitamin D doses because vitamin D is stored in adipocytes, might have attenuated the overall treatment effect in the trials.(20)

Objective

The objective of our systematic review is to assess the efficacy of vitamin D_3 supplementation on cancer mortality in the general population and the prognosis of cancer patients with special attention to potential effect modifiers, including baseline 25(OH)D levels, cancer at baseline, BMI and compliance.

The main outcomes include “cancer mortality in the general population”, “cancer-specific survival of cancer patients” and “overall survival of cancer patients”. These outcomes are universally used in cancer studies and do not need further refinement during the review.

In a first step, we intend to update the previous systematic reviews on vitamin D supplementation and cancer mortality in the general population by including newly published trials and unpublished data from trials with outcome data on cancer incidence or all-cause mortality by asking the authors for data on cancer mortality. Second, we will obtain data for an IPD meta-analysis. Third, we will conduct IPD meta-analyses on vitamin D_3 supplementation and overall and cancer-specific survival among cancer patients. Forth, we will conduct subgroup analyses to explore sources of heterogeneity and to identify effect modifiers. The timetable for the review is shown in Table 1.

Table 1: Proposed timetable for conducting the review

Step	**Timeframe for completion**
Literature search, abstract and full-text selection	2.5 months
Data extraction & individual patient data acquisition	2.5 months
Quality appraisal	2 months
Data analysis & meta-analysis	3 months
Writing of manuscript	2 months
Total	**12 months**

METHODS AND ANALYSIS

Study selection criteria/ Eligibility criteria

We will follow a two-step approach for the study selection: First, all trials will be selected that could potentially have published or unpublished data on the research topic. All authors of trials with potentially unpublished data on cancer mortality/survival will be contacted to provide data. In the second step, only trials with eligible data for a meta-analysis will be included.

Step 1: Inclusion criteria for trials

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).

Interventions: We will focus on trials that used vitamin D_3 in any dose and any regimen (e.g. daily/weekly/monthly intake) as the intervention. However, the minimum time of the intervention shall be six months to exclude studies with one-time bolus interventions or very short intervention periods. The first reason is that cancer mortality is highly unlikely to be influenced by very brief intervention periods. The second reason is that after initiating daily, weekly, or monthly supplementation schedules, it takes three to six months for 25(OH)D levels to reach homeostasis.

Besides, we will also include studies using vitamin D_3 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 $1,25(OH)_2D$.

We will exclude studies with vitamin D_2 supplementation since the Cochrane review of Bjelakovic et al. and other recent data showed clearly no efficacy on mortality.(10, 11, 15, 21) Co-supplementation with calcium or other dietary supplements in the intervention arm will not be an exclusion criterion. A sensitivity analysis will elucidate whether the inclusion of these studies had an impact on the overall effect estimate of the meta-analysis.

Comparators: We will include only studies, which used placebo as the comparator.

Outcomes: To be eligible for inclusion in a meta-analysis trials need to have assessed the outcome of cancer mortality, cancer survival, or cancer-specific survival. In an intermediate step of the systematic review, we will also record studies with the outcomes of cancer incidence or all-cause mortality and contact the authors if they have data for the outcomes needed for the planned meta-analyses. The definitions of all outcomes are shown in Table 2.

Table 2: Definition of outcomes

Outcome	Definition
All-cause mortality	Rate of deaths during a specific time period in a population at risk
Cancer mortality	Rate of cancer deaths during a specific time period in a population at risk
Cancer incidence	Rate of newly diagnosed cancer cases during a specific time period in a population at risk
Overall cancer survival	Proportion of patients from a cancer population at risk alive at some point subsequent to the diagnosis of their cancer
Cancer-specific survival	Proportion of patients from a cancer population at risk who did not die of cancer at some point subsequent to the diagnosis of their cancer

Study design: We will include RCTs in which, analogous to the intervention period, the follow-up period is at least six months. The follow-up time should not be longer than the time under treatment. We will focus on parallel-group designs and exclude single-arm studies. We will further exclude all types of cohort studies and case-control studies as well as the following types of records: reviews, dissertations, theses, editorials, study protocol, clinical guidelines, commentaries, and letters.

Setting: There will be no restrictions by type of setting.

Minimum sample size: The studies need to have at least one cancer death in the verum and placebo group.

Geographical location: No restrictions are defined regarding the geographical location.

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.

Information sources and search strategy

The search strategy will be elaborated by SK, BS, and A. Heppert. Mrs. Heppert is a specialist for systematic bibliographic searches at the Central Library of the German Cancer Research Center and is not otherwise associated with the project. Finally, it will be peer-reviewed by HB and carried out by SK.

The bibliographic databases MEDLINE (Pubmed interface), ISI Web of Science (WoS; Clarivate Analytics interface), and the Cochrane Central Register of Controlled Trials (CENTRAL; OVID interface) will be searched systematically. We will also carry out a systematic search for previous systematic reviews in the Cochrane Database of Systematic Reviews (CDRS, OVID interface) and KSR Evidence (https://ksevidence.com), which are both specialized search engines for systematic reviews. RCTs included in meta-analyses on the topics vitamin D supplementation and cancer mortality, cancer incidence, all-cause mortality, or cancer survival will be extracted and merged with the hits found in the bibliographic database search. The electronic database search will be complemented by searching the WHO's International Clinical Trials Research Portal (ICTRP) and clinicaltrials.gov to capture results from ongoing or recently completed RCTs that have not been published in scientific journals, yet. We will also scan the reference lists of eligible studies to yield additional trial articles via cross-referencing. A draft of the search strategy is presented in Figure 1.

We will search in MEDLINE, ISI WoS, and CENTRAL for medical subject headings (MeSH), free text words, synonyms, and related search terms for the concepts "vitamin D", "mortality", "cancer", "randomized controlled trial" and "placebo". Besides, standard search terms for RCTs will be used additionally wherever available. No restrictions are planned in the search strategy to prevent overlooking important studies that have not been correctly classified in the respective bibliographic databases. All databases will be searched from the inception of the databases without time restriction. Moreover, we will not limit the search to studies in English as relevant studies might also be published in other languages. The search string for MEDLINE is shown in Table 3.

Table 3: Search string for MEDLINE

Step	Search string
1	"vitamin d"[tw] OR "vitamin D"[MeSH] OR cholecalciferol[MeSH] OR cholecalciferol*[tw] OR calciol[tw] OR hydroxycholecalciferols[MeSH] OR hydroxycholecalciferol*[tw] OR dihydroxycholecalciferol*[tw] OR “vitamin d3"[tw] OR “vitamin d 3”[tw] OR calcitriol[MeSH] OR calcitriol[tw] OR "1-hydroxycholecalciferol"[tw] OR calcifediol[MeSH] OR calcifediol[tw] OR calcidiol[tw] OR alfacalcidol[Supplementary Concept] OR alphacalcidol[tw] OR alfacalcidol[tw]
2	mortality[tw] OR mortality[MeSH] OR death[MeSH] OR death[tw] OR died[tw] OR dead[tw] OR survival[tw] OR surviv*[tw] OR survival[MeSH]
3	neoplasms[MeSH] OR neoplas*[tw] OR malignanc*[tw] OR cancer*[tw] OR tumor*[tw] OR tumour*[tw] OR carcinoma*[tw]
4	(((((((((“randomized controlled trial”[pt]) OR “controlled clinical trial”[pt]) OR randomized[tiab]) OR placebo[tiab]) OR “drug therapy”[sh]) OR randomly[tiab]) OR trial[tiab]) OR groups[tiab])) NOT ((animals[mh] NOT humans[mh]))
5	placebos[MeSH] OR placebo[tw]
6	2 OR 3
7	1 AND 4 AND 5 AND 6

A shortened version of the MEDLINE search string will be used to search for systematic reviews in CDSR and KSR Evidence. Only the first three search steps are needed because the study design is “systematic review” and not “placebo-controlled RCT”. The search string for CDSR is shown in Table 4. The literature search will be updated during the peer-review process of the publication in order to include the most up to date literature.

Table 4: Search string for the Cochrane Database of Systematic Reviews

Step	Search string	
1	#1	MeSH descriptor: [Vitamin D] explode all trees
	#2	MeSH descriptor: [Cholecalciferol] explode all trees
	#3	MeSH descriptor: [Calcifediol] explode all trees
	#4	MeSH descriptor: [Calcitriol] explode all trees
	#5	MeSH descriptor: [Hydroxycholecalciferols] explode all trees
	#6	(("alfacalcidol") OR ("alphacalcidol") OR ("hydroxycholecalciferol*") OR ("1- hydroxycholecalciferol") OR ("hydroxyvitamin* D") OR ("calcifediol") OR (“calcidiol”) OR ("calcitriol") OR ("dihydroxycholecalciferol*") OR (“dihydroxyvitamin d*”) OR ("vitamin D") OR (cholecalciferol*) OR ("vitamin D3") OR ("vitamin D 3") OR (“calciol”)) (Word variations have been searched)
	#7	("vitamin d*"):ti,ab,kw (Word variations have been searched)
	#8	{OR #1-#7}
2	#9	MeSH descriptor: [Mortality] explode all trees
	#10	MeSH descriptor: [Death] explode all trees
	#11	MeSH descriptor: [Survival] explode all trees

	#12	("mortality" OR "dea*" OR “died” OR "survival" OR “surviv*”) (Word variations have been searched)
	#13	{OR #9-#12}
3	#14	MeSH descriptor: [Neoplasms] explode all trees
	#15	(carcinoma* OR tumour* OR tumor* OR cancer* OR malignanc* OR neoplas*) (Word variations have been searched)
	#16	#14 OR #15
4	#17	#13 OR #16
5	#18	#8 AND #17 in Cochrane Reviews (Word variations have been searched)

Data collection and management

Study selection and data extraction will be performed in duplicate by two reviewers. Both are blinded to each other’s decision but not to journal titles, study authors, or institutions. The screening will be conducted by entering data into blank Microsoft Word or Excel spreadsheets. The software EndNote will be used to store, organize, and manage all the references and allow a transparent and reproducible systematic search. To assure validity and high quality of the data, the data extraction will be performed by using standard and predefined data extraction forms (see Appendix 1). Both reviewers will scan independently the titles and abstracts of studies obtained by the aforementioned search strategy against the eligibility criteria. For those studies that meet the inclusion criteria or that cannot yet be fully excluded, full-text reports will be acquired and screened again towards the inclusion criteria. In the next step, the results of both reviewers will be compared and in cases of disagreement, critical points will be discussed until a consensus is reached. If necessary, we will contact study authors to resolve questions about eligibility. We will document the reasons for excluding trials.

After completing the abstract and full-text selection with eligible studies, the two reviewers will extract independently the pre-defined data (see Appendix 1). Extracted items will include first author, publication year, country, number of participants, general population or medical condition (including cancer site and stage(s)), sex, mean/median age, race/ethnicity, mean/median BMI, mean/median 25(OH)D levels at baseline, vitamin D_3 dosing regimen, duration of vitamin D_3 supplementation,

compliance, mean/median and maximum follow-up time, number of cancer deaths and effect estimates (including confidence intervals) reported for cancer mortality/cancer survival. Individual patient data for the aforementioned variables will be obtained from all trials with at least 20 cancer deaths (see Appendix 1). If summary data are not published, they shall be calculated from the obtained data. All authors will be contacted by e-mail with a maximum of three attempts sent at two-week intervals.

For the meta-analyses on cancer survival and cancer-specific survival, we will ask all authors who conducted trials in the general population to provide IPD for cancer diagnoses in the five years prior to baseline and during the trial (including cancer site with ICD-code, stage, and diagnosis date). The following IPD will be additionally collected: age, sex, BMI, race/ethnicity, baseline 25(OH)D levels, compliance, randomization group allocation, baseline date, deaths during follow-up with date, cancer deaths with date, censoring dates for survival outcomes, and censoring date for patients not dying of cancer (see Appendix 1). If IPD cannot be shared, the authors of the studies will be asked to conduct the analyses in-house and to provide the summary estimates for the meta-analysis. If trial authors do not collaborate, their study cannot be included in subgroup analyses for which no effect estimates were published but the result from the total trial population will remain included in the main meta-analysis.

Quality assessment

The protocol of the systematic review with all planned statistical analyses has been registered in PROSPERO before data collection to preclude data-driven analyses and selective reporting of only statistically significant findings. The study protocol has been developed in line with the “Preferred reporting items for systematic review and meta-analysis protocols” (PRISMA-P, see Appendix 2), the Cochrane Handbook for Systematic Reviews of Interventions as well as the Institute of Medicine guideline.(22-25) We will ensure to fulfill all requirements recommended by the current PRISMA guideline when writing the publication of the systematic review.(26, 27)

The Cochrane risk-of-bias tool for randomized trials (RoB 2) will be used to assess various domains of bias including aspects of trial design, conduct, and reporting (see Appendix 1).(28, 29) The following domains will be covered during the evaluation: sequence generation, allocation concealment, blinding, incomplete outcome data (e.g. withdrawals and dropouts), and selective outcome reporting. A summary assessment will be made based on the extracted items, judging whether the risk of bias in the respective study is low, high, or has some concerns. If only insufficient data is reported, the risk of bias is "unclear" and the original study authors will be contacted for further information. The assessment will be conducted by two independent reviewers using the RoB 2 tool.(28, 29) In cases of disagreement, critical points will be discussed until a consensus is reached. The risk of bias evaluation will be incorporated into the data synthesis by performing a sensitivity analysis that excludes studies with high or unknown risk of bias.

Descriptive analysis and meta-analysis

Measures of treatment effect

The mortality/survival outcomes shall be addressed by estimating hazard ratios (HR) and 95% confidence intervals (95%CI). Results of the intention-to-treat (ITT) approach will be used, including all patients randomized when both ITT and per-protocol results are given.

Data synthesis

As far as study quality and differences between studies allow, effect estimates of all eligible studies with data for the following three main meta-analyses will be pooled deriving random effects results with the DerSimonian and Laird method (primary analysis) and fixed effects summary estimates using the Mantel-Haenzel method (secondary analysis).

1) Association of vitamin D_3 supplementation and cancer mortality in the general population
2) Association of vitamin D_3 supplementation and cancer-specific survival of cancer patients
3) Association of vitamin D_3 supplementation and overall survival of cancer patients

For all studies that provide IPD, unadjusted Cox proportional hazard regression models will be used to estimate HRs and 95%CIs for the main meta-analyses in which we will pool effect size data from studies who do and who do not provide IPD in a two-step approach. For studies that cannot send

IPD to the coordinating center (German Cancer Research Center, Heidelberg), authors are being asked to estimate the HRs and 95%CIs themselves and send the summary data for the meta-analyses. To assess cancer survival as time-to-event data from general population cohorts, the study will be restricted to patients with a history of cancer in the five years preceding baseline or a cancer diagnosis during the trial. For the former, the survival time will be calculated from baseline to death/end of the trial, and for the latter, survival time will be counted from the date of cancer diagnosis till death/end of the trial.

With all studies that agree to send IPD data to the coordinating center or to do additional analyses in-house, we will also conduct an additional multivariate Cox proportional hazards regression model. The model for the outcome cancer mortality among general population studies will contain the variables vitamin D_3 intervention (vs. placebo), age (continuous; < 70 vs. ≥ 70 years), sex (male, female, unknown), BMI (< 25 vs. 25 – 29.9 vs. ≥ 30 kg/m² vs. unknown), ethnicity (white vs. black/brown vs. other), 25(OH)D baseline level (< 30 vs. 30 – 49.9 nmol/L vs. ≥ 50 nmol/L vs. unknown), diagnosis of cancer (except non-melanoma skin cancer and benign tumors) in five years before baseline (yes vs. no vs. unknown), health status (general healthy population vs. diseased population), and compliance (< 80% vs. ≥ 80% vs. unknown). The models for the outcomes overall and cancer-specific survival of cancer patients will be adjusted for the same variables but the variable "diagnosis of cancer in five years before baseline" will be replaced by more specific variables for cancer stage (only advanced stages III and/or IV vs. unknown), cancer site (prostate vs. colorectal vs. breast vs. lung vs. other vs. unknown) and time since cancer diagnosis (<1 year vs. 1-5 years). We will test for interactions of the treatment variable (vitamin D_3 vs. placebo) with these covariates to identify potential effect modifiers. Again, a two-step approach will be used for the meta-analyses, whereby the analyses are carried out on a study-specific basis, and then the effect estimates are pooled. To further explore the variation of the treatment effect by methodological or patient characteristics differences of the studies, the following subgroup analyses will be performed with IPD data and studies that published eligible data:

<u>Subgroup analyses according to trial design</u>

- Daily dose vs. weekly/monthly bolus dose vs. bolus dose at the beginning of the trial followed by a daily dose
- Low vs. moderate vs. high vitamin D_3 dosing (< 1,000 IU vs. 1,000 – 2,000 IU vs. > 2,000 IU per day or equivalent weekly or monthly taken dose)
- Vitamin D_3 supplementation duration (< 5 vs. ≥ 5 years)
- Health status (general population vs. diseased population)
- Region (North America vs. Europe vs. Other)

Subgroup analyses according to patient characteristics

- Age (< 70 vs. ≥ 70 years)
- Sex (male vs. female)
- Ethnicity (white vs. black/brown vs. other)
- BMI (< 25 vs. 25 – 29.9 vs. ≥ 30 kg/m²)
- Baseline 25(OH)D levels (< 30 vs. 30 – 49.9 nmol/L vs. ≥ 50 nmol/L)
- Compliance rate (< 80% vs. ≥ 80%)

For meta-analyses conducted in cancer patients in addition:

- Cancer stage (only advanced stages III and/or IV vs. unknown)
- Cancer site (prostate vs. colorectal vs. breast vs. lung vs. other)
- Time since cancer diagnosis (<1 year vs. 1-5 years)

Analyses in the coordinating center will be done with the statistical software SAS 9.4. The meta-analyses will be performed with Comprehensive Meta-Analysis 2.0 (Biostat, Englewood, NJ).

Assessment of heterogeneity

Heterogeneity will be presented visually by forest plots and assessed statistically by Cochran's Q test (significance level = 0.05) as well as the I^2 index (< 25% low, 25-50% moderate, > 50% high heterogeneity). Meta-Analyses will be conducted even if high heterogeneity is being detected and the results will be discussed taking the heterogeneity into consideration. Sources of heterogeneity

will be explored by the subgroup analyses outlined in the previous section and the following sensitivity analyses:

- Excluding studies with a high or unknown risk of bias according to assessment with the Cochrane risk-of-bias tool for randomized trials
- Excluding studies not reporting ITT results
- Excluding trials with co-supplementation of calcium
- Excluding events in the first year of follow-up

Assessment of publication bias

Publication bias will be accessed visually in funnel plots and tested for with Egger's test.

Dealing with missing data

In case of missing data, we will seek contact with the original investigators. If possible, we will calculate missing numerical data from the given reported data.

Strength of the body of evidence

The quality of the evidence for each outcome will be evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. The four levels of evidence comprise very low, low, moderate, and high. Evidence from RCTs starts as high quality but can be decreased for reasons such as the risk of bias, imprecision, inconsistency, indirectness, and publication bias.

Amendments

In the case of protocol amendments, we will document the date, the description of the change, and the rationale in a pre-defined log sheet in Microsoft Word or Excel.

Patient and public involvement

Patients and the public were not involved in the development of the study design. Since this is a protocol for a systematic review and no participant recruitment will take place, the involvement of patients in the recruitment, the conduct of the study and the dissemination of findings to study participants are not applicable.

Ethics and dissemination

An ethics approval is not required for this systematic review because it is only a summary of already published trial data. All studies to be included in the systematic review have their own ethics approvals, which are named in the original publications. For the IPD meta-analysis, we will take care that the additional analyses are in adherence with the ethics approvals of the trials.

The systematic review will be published in an international peer-reviewed journal for clinical oncology or general medicine with open access option and presented in national and international meetings. If the meta-analyses of the systematic review obtain statistically significant findings, we expect the result to be reflected in national and international guidelines and to change the current practice of tertiary prevention among cancer patients. Vitamin D_3 is already on the market in various doses and at low costs because it is not patented.

Patients will be informed via a press release from the German Cancer Research Center. Moreover, we will send a summary of the results in a language suitable for laypersons to all patient advocacy groups recommended by the Cancer Information Service of the German Cancer Research Center (up to data n = 30) for further dissemination among their members.(30) With respect to oncologists, we will disseminate the results to all German rehabilitation centers having a ward for oncologic rehabilitation, as listed in the register of the *Bundesarbeitsgemeinschaft für Rehabilitation e.V.*(31) As the topic of the review is in the field of tertiary prevention, oncologists in the rehabilitation setting are the target audience for information dissemination.

DISCUSSION

One of the strengths of this systematic review comprises the first meta-analysis on vitamin D supplementation and cancer survival and additionally the first IPD meta-analysis on this research topic. The IPD meta-analysis will allow the investigation of potential effect modifiers. Especially 25(OH)D levels at baseline, BMI, and compliance are candidates that could have had a great impact on the overall trial results.

The creation of this research protocol prompted us to plan carefully all the details of the systematic review and to anticipate and address potential problems before their actual occurrence. Arbitrary decision making concerning any procedure of this systematic review is prevented, resulting again in a decreased risk of publication bias and selective reporting bias. The protocol allows reproducible and transparent research for future reviewers.

Possible limitations of our review include a potentially insufficient number of cancer deaths in the studies and high heterogeneity, which could both negatively influence the statistical power of the meta-analyses. However, it is still too early to judge whether these limitations occur.

The quality of selected studies will be assessed and the quality of the evidence will be judged. The ultimate goal is to ensure the reporting of highly meaningful findings for clinicians and patients. Oncologists are well aware that vitamin D deficiency and insufficiency are very common in cancer patients but there is uncertainty about whether and how they should routinely perform preventive screening and treatments. 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. Since vitamin D products are readily available in pharmacies or drug stores, many patients use low-dose vitamin D supplementation as self-medication. Yet, it can be doubted whether this untargeted intervention has any effect on cancer prognosis. Consequently, evidence-based recommendations for high-dose vitamin D supplementation are highly relevant for both, clinicians and patients.

If the planned systematic review determines the efficacy of vitamin D supplementation on cancer prognosis in the expected magnitude of 10-15%, the review will be used to provide clear suggestions on how vitamin D can be appropriately dosed to overcome vitamin D deficiency or insufficiency in cancer patients.(12) Furthermore, our systematic review would provide the evidence for statutory health insurances to cover the costs for screening for vitamin D deficiency or insufficiency in cancer patients and a subsequent vitamin D supplementation. With expected relatively large effects and very low screening and treatment costs (A vitamin D blood test costs approx. € 20, and one year of vitamin D therapy costs less than € 100.), vitamin D supplementation will be highly cost-effective. The costs would be close to negligible compared to other current cancer treatment costs.

Status

At the time of submission, the study selection for the systematic review has not started.

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Authors' contribution

All authors meet the ICMJE criteria for authorship as follows: BS and SK are the guarantors of the systematic review, therefore, are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors made substantial contributions to the conception of the work. BS and SK designed the search strategy and the risk of bias assessment strategy. BS developed the selection criteria, the data extraction criteria, and the statistical methods. SK drafted the protocol publication, which BS and HB revised critically for important intellectual content. All authors approved the final version to be published.

Contributors

The following researchers have agreed to contribute to this systematic review by sharing IPD and giving intellectual input for the discussion of the results: Taisuke Akutsu (Jikei University School of Medicine. Tokyo, Japan), Professor Julie E. Buring (ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor Carlos A. Camargo Jr (MD, DrPH. Massachusetts General Hospital, Harvard Medical School. Boston), Professor Nancy R. Cook (ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor I-Min Lee (MB, BS, ScD. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor JoAnn E. Manson (MD, Dr. PH. Brigham and Women's Hospital and Harvard Medical School. Boston), Professor Robert Scragg (MBBS, PhD. School of Population Health, University of Auckland. Auckland, New Zealand), John Sluyter (PhD. School of Population Health, University of Auckland. Auckland, New Zealand), Professor Mitsuyoshi Urashima (MD. Jikei University School of Medicine. Tokyo, Japan), Professor Jean Wactawski-Wende (PhD. University at Buffalo. Buffalo, New York).

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Data availability statement

As no new data are obtained in this systematic review, no data will be made publicly available to third parties. Decisions on data use by third parties can only be made by the principal investigators of the original studies, taking into account the votes of the responsible ethics committees.

Disclaimer

The views of the authors do not necessarily reflect those of the German Cancer Research Center.

Competing interests

None declared.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer-reviewed

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Appendix 1:

Supplementary material consisting of data extraction form, risk of bias assessment, and list of individual patient data to be obtained from original researchers

Appendix 2:

Supplementary material consisting of PRISMA-P checklist

Figure

Figure 1: Draft of the study selection process

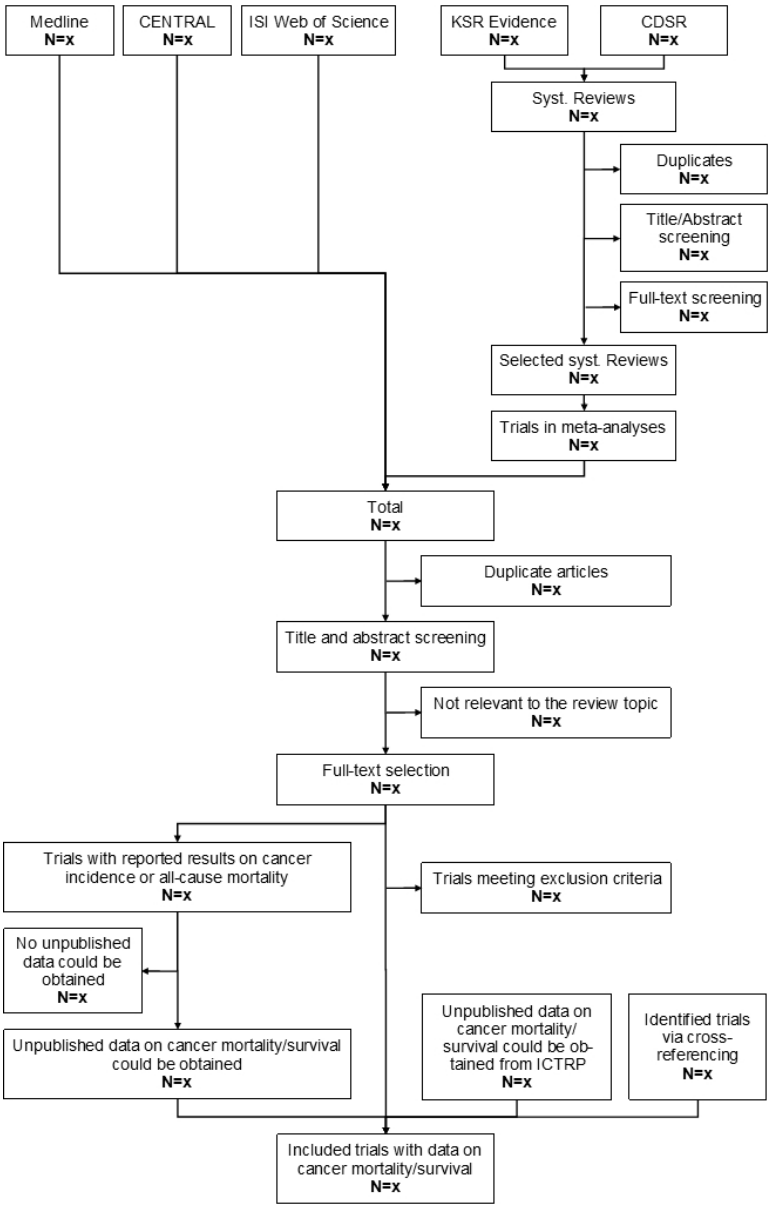

Figure 1: Draft of the study selection process

16x25mm (1000 x 1000 DPI)

Study			Population				
First author (or study name), Year (REF)	Country	Sample size	General population (y/n)	Cancer population (y/n)	Cancer site + stage	Age (mean or median)	Sex (female %)

			Intervention			Comp
Race/ ethnicity/ skin color (white %)	BMI [kg/m²] (n, mean or median)	25(OH)D (n, mean or median)	Vitamin D_3 dosing regimen	Duration of vitamin D3 supplementation	Compliance in intervention group (%)	Placebo (y/other)

parator	Outcomes				
Compliance in comparator group (%)	Maximum follow-up time	Mean or median follow-up time	No of cancer deaths	Cancer mortality in general population HR (95% CI)	Cancer-specific survival of cancer patients HR (95% CI)

		Comments
Overall survival of cancer patients HR (95%CI)	**Covariates adjusted for**	

First author (or study name), Year (REF)	R	D	Mi	Me	S	O

Risk of bias legend

R Bias arising from the randomiation

D Bias due to deviations from

Mi Bias due to missing outcome data

Me Bias in measurement of the

S Bias in selection of the reported

O Overall risk of bias

The following variables will be obtained for all trial participants in order to conduct the IPD meta-a

No.	Variables
1	Age
2	Sex
3	BMI
4	Race/ethnicity/skin color
5	Baseline 25(OH)D levels
6	Compliance
7	Randomisation group allocation
8	Baseline date
9	Death during follow-up (y/n)
10	Date of death
11	Censoring date for survivors
12	Cancer death during follow-up (y/n)
13	Date of cancer death
14	Censoring date for non-cancer deaths
15	Cancer* diagnosis during follow-up (y/n)
16	Cancer* site for each cancer during follow-up
17	Cancer* stage for each cancer during follow-up
18	Date of cancer* diagnosis for each cancer during follow-up
19	Cancer* diagnosis in first 5 years before baseline (y/n)
20	Cancer* site for each cancer in first 5 years before baseline
21	Cancer* stage for each cancer in first 5 years before baseline
22	Date of cancer* diagnosis for each cancer in first 5 years before baseline

* Excluding non-melanoma skin cancers and benign tumors

analysis:

PRISMA-P 2015 Checklist

This checklist has been adapted for use with protocol submissions to *Systematic Reviews* from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 **4**:1

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
ADMINISTRATIVE INFORMATION					
Title					
Identification	1a	Identify the report as a protocol of a systematic review	☒	☐	1-4
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	☐	☒	n.a. (first submission)
Registration	2	If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract	☒	☐	63-64
Authors					
Contact	3a	Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author	☒	☐	6-34
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	☒	☐	443-451
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	☒	☐	370-372
Support					
Sources	5a	Indicate sources of financial or other support for the review	☒	☐	467-471
Sponsor	5b	Provide name for the review funder and/or sponsor	☒	☐	467-471
Role of sponsor/funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	☒	☐	467-471
INTRODUCTION					
Rationale	6	Describe the rationale for the review in the context of what is already known	☒	☐	79-124
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	☒	☐	126-140
METHODS					

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
Eligibility criteria	8	Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review	☒	☐	150-190
Information sources	9	Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage	☒	☐	192-225
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	☒	☐	217-218, 224-225, figure 1
STUDY RECORDS					
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	☒	☐	227-262
Selection process	11b	State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis)	☒	☐	227-262
Data collection process	11c	Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	☒	☐	227-262, appendix 1
Data items	12	List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications	☒	☐	361-363, appendix 1
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	☒	☐	131-133, 168-172, table 2
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	☒	☐	264-282, 347-360
DATA					
Synthesis	15a	Describe criteria under which study data will be quantitatively synthesized	☒	☐	290-297
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., I^2, Kendall's tau)	☒	☐	297-325, 345-358
	15c	Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression)	☒	☐	326-344, 351-358
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	☐	☒	n.a. (quantitative

Section/topic	#	Checklist item	Information reported		Line number(s)
			Yes	No	
					synthesis will be performed)
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies)	☒	☐	359-360
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (e.g., GRADE)	☒	☐	364-369