PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol: A Phase II randomised controlled trial of high-dose Vitamin D supplementation to prevent progression in localised prostate cancer cases with low-intermediate risk of progression on active surveillance (ProsD).
AUTHORS	Nair-Shalliker, Visalini; Smith, David; Gebski, Val; Patel, Manish; Frydenberg, Mark; Yaxley, John; Gardiner, Robert; Espinoza, David; Kimlin, Michael; Fenech, Michael; Gillatt, David; Woo, H; Armstrong, Bruce; Rasiah, Krishan; Awad, Nader; Symons, James; Gurney, Howard

VERSION 1 – REVIEW

REVIEWER	Francesco Schettini University of Naples Federico II, Italy and August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Spain.
REVIEW RETURNED	20-Sep-2020

GENERAL COMMENTS	The proposed protocol describes a randomized phase II trial that should preliminarily test the efficacy of high dose vitamin D in prolonging the time to active therapy for prostate cancer in low-intermediate risk men affected by prostate cancer and in active surveillance. Despite being interesting and potentially impactful on future clinical practice, I believe there are some major concerns that should be addressed, as well as some minor revisions to the protocol. Here are as follows: 1) The major issue lies in the statistical plan, which is very confusing. This is a randomized trial with two arms and several time-dependent primary and secondary endpoints (e.g. the proposed variants of the time to active therapy). However, a Simon-2-step design, with a proportion as endpoint (% of change from active surveillance to active therapy), has been taken into account to calculate the number of patients needed for recruitment of the experimental arm. Subsequently, the authors state that no formal comparison will be done between the two arms and thus never propose a statistical plan to actually calculate the ATFS nor the differences between patients in active surveillance and patients receiving vitamin D. Considering that patients have been already recruited I understand that it won't be possible to significantly amend the plan, but a statistical revision is highly recommended to change the primary endpoint accordingly and throughout the whole protocol, and then provide time-dependent endpoints as secondary/tertiary outcomes, with a statistical plan that includes consistent analyses for time dependent endpoint (Kaplan Meier, log rank test, Cox regression...). 2) It is stated that patients should compile surveys. A survey form

	should be presented within the protocol. 3) How will the DNA damage be evaluated? 4) The pre-planned blood biomarker analyses should be clearly detailed (which analyses will be performed? When will be performed? How will be performed?) in the protocol. On the contrary, the information provided in the Participant Information Sheet seem to be adequate for the patients, in terms of both quantity and quality of information. 5) Additionally, it is not necessary to divide in primary, secondary and tertiary endpoints. I suggest to clearly state the primary endpoint and subsequently state all secondary endpoints without further divisions. 6) There are several typos here and there that should be corrected. 7) Appendix figure 1 presents some irregularities in the format. Better create a figure file (e.g. a pdf file should be ok) and insert it in the appendix instead of the multiple text boxes and lines format that has currently been adopted.

REVIEWER	L. Venderbos Department of Urology, Erasmus University Medical Center, Rotterdam, the Netherlands.
REVIEW RETURNED	22-Sep-2020

GENERAL COMMENTS	The authors are conducting a trial with which they aim to determine if oral high dose vitamin D supplementation taken monthly for 2 years can prevent PCa progression in cases with low-intermediate risk of progression. I have a few questions which the authors may be able to clarify: - Could the authors clarify a bit more why they have chosen for a study population of localized PCa patients who are at low-intermediate risk of progression? Why not include patients with low risk of progression too? - Page 9, line 18: The referenced studies ran for 1 year and 10 weeks. The authors state that this short duration of follow-up may have resulted in the lack of significant findings in these 2 referenced studies. Can the authors clarify a bit more why they have chosen for an intervention period of 2 years in the ProsD trial? And how do the authors relate to the objective of the ProsD study and the limitation on page 6, line 18/19 saying that 'follow-up is 2 years and therefore a benefit or lack of benefit of Vitamin D may not be appreciated this time, given the natural slow progression of PCa'. - Page 9, line 24: A word is missing between 'trials may'. Please add. - Page 10, 'Objectives and outcomes': Is it worthwhile of adding some objectives on the feasibility and adherence of the participants in the study group to the intervention? The mode of delivery of the intervention? - Page 13, line 28: It says that there are no absolute requirements for conversion from AS to curative therapy, but that the authors count on the discretion of the urologist. Although guidelines are given on when active therapy may be considered, I wonder how the authors feel about the possible variation and protocol-deviation it may lead to? - Page 15, line 14: How many biopsies are patients likely to undergo

	in the 24-month study period? And what if patients 'miss' a biopsy moment or do not want to undergo one? - What happens after the study intervention period? Depending on the outcome, can participants keep taking vitamin D supplementation? Will the placebo group be offered the opportunity to start Vitamin D supplementation? Will patients be followed-up after the intervention period of 24 months? - Page 15, 'Study progress': The authors indicate that the maximum age cut-off increased from 75 to 79. May you then also be including men, aged 76, 77, 78 or 79 who are on AS, but actually are on WW? - On page 6, line 15/16 the authors indicate that the results of this phase II trial may inform a phase III trial in the future. Have they already thought about how such a trial will look like?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1.1 The major issue lies in the statistical plan, which is very confusing. This is a randomized trial with two arms and several time-dependent primary and secondary endpoints (e.g. the proposed variants of the time to active therapy). However, a Simon-2-step design, with a proportion as endpoint (% of change from active surveillance to active therapy), has been taken into account to calculate the number of patients needed for recruitment of the experimental arm. Subsequently, the authors state that no formal comparison will be done between the two arms and thus never propose a statistical plan to actually calculate the ATFS nor the differences between patients in active surveillance and patients receiving vitamin D. Considering that patients have been already recruited I understand that it won't be possible to significantly amend the plan, but a statistical revision is highly recommended to change the primary endpoint accordingly and throughout the whole protocol, and then provide time-dependent endpoints as secondary/tertiary outcomes, with a statistical plan that includes consistent analyses for time dependent endpoint (Kaplan Meier, log rank test, Cox regression...).

Response: In the definitions of outcomes and endpoints we (inadvertently) stated 'time to switching to active therapy', whereas we should have stated 'proportion of participants switching to active therapy'. This has now been corrected in the manuscript. We thank the reviewer for the comments regarding this. As this is a non-comparative phase II design, any comparisons between groups would be purely exploratory (described in Lines 405-407). We will be ascertaining the activity through estimation and confidence intervals within each group rather than comparisons between them.

We have made the following amendments in the manuscripts to reflect this:

Page 7 Lines 180-187

"Primary outcome:

The primary trial outcome is the time to switch from active surveillance to active therapy, for clinical or non-clinical reasons, within 2-years of AS.

Secondary outcomes:

(a) Time to switch from AS to active therapy, specifically for clinical reasons, within 2-years of AS.

(b) Time to switch from AS to active therapy, specifically for non-clinical reasons, within 2-years of AS.

"

Page 10 Line 292:

"Primary Endpoint

The primary endpoint will be the time to the proportion of participants opting for active therapy for PC (Active therapy-free survival, ATFS). There will be no absolute requirements for conversion to active therapy which will be at the discretion of the treating urologist. However, as a guideline, active therapy may be considered in the following situations. "

Page 11 Lines 307 and 309

"Secondary Endpoints

• time to proportion of participants opting for active therapy for PC for non-cancer progression (e.g. anxiety) or
• time to proportion of participants opting for active therapy for PC for clinical reasons“
1.2 It is stated that patients should compile surveys. A survey form should be presented within the protocol.
Response: We have attached a copy of the survey as Appendix 3, although we have removed the Brief Symptom Inventory (BSI18) questions due to licensing restrictions.
1.3 The pre-planned blood biomarker analyses should be clearly detailed (which analyses will be performed? When will be performed? How will be performed?) in the protocol. On the contrary, the information provided in the Participant Information Sheet seem to be adequate for the patients, in terms of both quantity and quality of information. How will the DNA damage be evaluated?
Response: We have included a section that specifically describes blood collection and processing in Page 12 Lines 342-354. We have included a section that describes blood biomarker analyses for markers of vitamin D metabolites (25(OH)D and 1,25(OH)2D), DNA damage, and Ki67 proliferation in Pages 12-13 Lines 364-391.
1.4 Additionally, it is not necessary to divide in primary, secondary and tertiary endpoints. I suggest to clearly state the primary endpoint and subsequently state all secondary endpoints without further divisions.
Response: We have retained the endpoints as primary, secondary and tertiary to keep it consistent with the trial protocol (version 7).

1.5 There are several typos here and there that should be corrected.
Response: These typos have been corrected

1.6 Appendix figure 1 presents some irregularities in the format. Better create a figure file (e.g. a pdf file should be ok) and insert it in the appendix instead of the multiple text boxes and lines format that has currently been adopted.
Response: This has been corrected

Reviewer: 2
2.1. I have a few questions which the authors may be able to clarify:
- Could the authors clarify a bit more why they have chosen for a study population of localized PCa patients who are at low-intermediate risk of progression? Why not include patients with low risk of progression too?
Response: Our selection of low-intermediate risk cases is mainly because they have a higher risk of disease progression than low-risk cases and are therefore more likely to experience events (disease progression) in the 2 years of follow-up, as well as to exclude the likelihood of including indolent low risk cases. Additionally, we have chosen to exclude low-risk patients based on evidence that show no or a weak association between vitamin D and low risk PC (Schenk 2012; Nair-Shalliker 2020).
Reference:
Schenk et al CEBP 2014 doi: 10.1158/1055-9965.EPI-13-1340
Nair-Shalliker et al Sci Rep 2020 doi: 10.1038/s41598-020-62182-w.

2.2. Page 9, line 18: The referenced studies ran for 1 year and 10 weeks. The authors state that this short duration of follow-up may have resulted in the lack of significant findings in these 2 referenced studies. Can the authors clarify a bit more why they have chosen for an intervention period of 2 years in the ProsD trial?
Response: Rising levels of PSA are an indication of disease progression and, if present, progression is confirmed by a mpMRI scan and biopsy. Results from the follow-up of the UCSF PC cohort showed that low-risk PC cases reported a median time interval between surveillance biopsies for low-risk cases of ~13months and, for intermediate-risk cases, 14.5months (Cooperberg et al). Our design for a 2-year follow-up was to provide sufficient time for progression to occur and to be confirmed by follow-

up biopsy.
Reference: Cooperberg et al JCO 2011 doi: 10.1200/JCO.2010.31.4252

2.3. And how do the authors relate to the objective of the ProsD study and the limitation on page 6, line 18/19 saying that 'follow-up is 2 years and therefore a benefit or lack of benefit of Vitamin D may not be appreciated this time, given the natural slow progression of PCa'.
Response: We understand longer follow-up could make a smaller gain detectable but present funding permits only two years. Ideally, we would like to run this trial for 5 years, but this is a Phase 2 and aims to capture a signal for vitamin D activity. We will consider 5 years if we observe any benefits of supplementation after 2 years.

2.4. Page 9, line 24: A word is missing between 'trials may'. Please add.
Response: This has been corrected

2.5. Page 10, 'Objectives and outcomes': Is it worthwhile of adding some objectives on the feasibility and adherence of the participants in the study group to the intervention? The mode of delivery of the intervention?
Response: It was always the intention to monitor and report adherence as this is key to delivering a good trial. We have revised the 'Objectives and Outcomes" in Page 7 Lines 177-179 as follows:
"We will assess the feasibility and safety of an initial oral loading dose of 500,000IU followed by a monthly oral dose of 50,000IU of cholecalciferol, for 2 years of AS."

2.6 Page 13, line 28: It says that there are no absolute requirements for conversion from AS to curative therapy, but that the authors count on the discretion of the urologist. Although guidelines are given on when active therapy may be considered, I wonder how the authors feel about the possible variation and protocol-deviation it may lead to?
Response: This is a pragmatic description of a urologists' clinical practice. For patients on active surveillance, the thresholds to tip a man (from AS) to curative intent will differ between patients, generally due to non-cancer specific risks of mortality or other morbidities. As the men on AS age, the threshold for initiating curative intent lifts. This is one of the great strengths of an AS program, as it minimizes the over-treatment of prostate cancer in men for whom the disease will be clinically insignificant. Additionally, issues such as PSA velocity, the presence of pattern 4 cancer, cribriform pattern malignancy, number of positive cores and amount of core involvement are all important factors when considering patient management. The standardisation of these pathological features as a trigger for curative treatment in current international AS protocols is not robust. Patients are influenced by many non-pathological or biological factors such as family history, opinions of their partners and family and personal acceptance of the potential complications of treatment. Unfortunately, whilst clinical judgement on a case-by-case basis does pose some limitations in extrapolating our findings into a formal protocol, this discretion is also a truer reflection of the realities of a clinical practice aiming for better outcomes.

2.7 Page 15, line 14: How many biopsies are patients likely to undergo in the 24-month study period? And what if patients 'miss' a biopsy moment or do not want to undergo one?
Response: When the trial initially commenced all participants were required to have a repeat biopsy at 12 months and 24 months. However, it is now standard practice to omit the 12 month standard biopsy if the 12-month mpMRI scan and 3-monthly PSA results showed no signs of disease progression. Hence most patients are likely to have a biopsy at baseline and a follow-up at 24 months. If a patient misses a biopsy at 24 months this will not impact the primary or secondary end point.
The protocol was amended to reflect this practice. Participants are now requested to have at least one repeat biopsy during the 24 months of follow-up. This has been now been revised in Figures 1 and 2, and in the manuscript in Page 13 Lines 383-384 and in Page 16 Lines 483-485.
"Additionally, the original requirement to have biopsies at 12- and 24-months was changed to having

at least one biopsy in the 24 months of follow-up.".

2.8 What happens after the study intervention period? Depending on the outcome, can participants keep taking vitamin D supplementation? Will the placebo group be offered the opportunity to start Vitamin D supplementation? Will patients be followed-up after the intervention period of 24 months?
Response: There is no cross over in this study. The outcome for this trial will only be known after the last patient follow-up is complete and therefore no participants will be given vitamin D supplements after they have completed their 24-months of follow-up. All participants will be requested to destroy all un-used supplements when their trial follow-up is complete. If we find evidence for vitamin D activity, we will progress to a phase 3 trial. Participants who have completed the trial will be passively followed-up through their clinical records. They will be at liberty to obtain vitamin D supplements from a non-study source after the completion of the trial. We note though that high dose vitamin D (50,000IU) can only be obtained with a doctor's prescription in Australia.
This has now been revised in:
Page 9 Lines 265-266
"At the completion of intervention period, all participants will be requested to destroy all remaining supplements."
Page 13 Lines 384-386
"This figure describes from the timepoint a participant is flagged as potentially eligible, through to the completion of the trial, after which all participants will continue to be followed-up passively, through their clinical records obtained from their treating urologists."

2.9 Page 15, 'Study progress': The authors indicate that the maximum age cut-off increased from 75 to 79. May you then also be including men, aged 76, 77, 78 or 79 who are on AS, but actually are on WW?
Response: The rigour of monitoring these men 3-monthly for PSA levels, annually with mpMRI and the requirement to have at least one biopsy in the 24 months of follow-up for disease progression would characterise them as being on AS rather than WW. We have finished recruitment and no patients were on WW prior to study entry.

2.10 On page 6, line 15/16 the authors indicate that the results of this phase II trial may inform a phase III trial in the future. Have they already thought about how such a trial will look like?
Response: Yes, we are currently thinking about a phase III trial but it is premature to outline trial design or endpoints. Our current experience highlights the difficulty in recruiting this cohort of participants. As such a phase III trial would seek to engage international collaborators.

VERSION 2 – REVIEW

REVIEWER	Lionne Venderbos Department of Urology, Erasmus University Medical Center Rotterdam, the Netherlands.
REVIEW RETURNED	04-Jan-2021

GENERAL COMMENTS	I thank the authors for the changes they made to the manuscript. Perhaps it is not a requirement for the publication of a study protocol, or I missed it, but I did not find an authors reply to the reviewers comments/questions. That would have been helpful.