PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Safety and efficacy of vitamin D3 supplementation with imatinib in chronic phase-chronic myeloid leukemia: an exploratory, placebo-controlled randomized trial
AUTHORS	Bandyopadhyay, Arkapal; Palepu, Sarika; Dhamija, Puneet; Nath, Uttam; Chetia, Rituparna; Bakliwal, Anamika; Vaniyath, Sudeep; Chattopadhyay, Debranjani; Handu, Shailendra

VERSION 1 – REVIEW

REVIEWER	Bagchi, Basab All India Institute of Medical Sciences - Patna, Medical Oncology Haematology
REVIEW RETURNED	06-Aug-2022

GENERAL COMMENTS	1. Please elaborate on CHR assessment in the methodology. If some cases were assessed remotely, then how their spleen size was measured at 3months(for CHR)? 2. In the page 15 paragraph 2 , it is mentioned that majority of patients were vitamin D deficient initially and at 3 months, but percentage is 35.5% at the beginning and 33.9% at 3months- which seemed incorrect 3. An inherent weakness of the study is that, at the time of assessment(3 months) majority of patients in the intervention group were still vitamin D deficient.

REVIEWER	Marcinkowska, Ewa University of Wroclaw
REVIEW RETURNED	28-Aug-2022

GENERAL COMMENTS	The paper by Arkapal Bandyopadhyay et al. reports results from a small clinical trial in which supplementation of vitamin D (cholecalciferol) was tested in patients with chronic myeloid leukemia (CML). The trial was randomized, placebo controlled and double blind. The patients in chronic phase were treated against CML using standard therapy, namely Immatinib. The trial was conducted in a rather small group of patients, and observation lasted 3 months. Cholecalciferol was given in high doses (60 000 IU) once a week, and such supplementation appeared to be safe for patients. Surprisingly, not all patients in vitamin D receiving group were vitamin D-sufficient at the end of the trial. There were no statistically significant differences in early molecular response rates, complete hematological response rates, and times to complete hematological response between the study groups. The most important message from this study is that supplementation of vitamin D in doses of 60 000 IU/week is safe

	for the patients. The other results obtained, raise some concerns and questions. 1. The description of vitamin D supplementation on page 14, and in Table 3 is hard to understand. If I understand correct, there were patients in placebo group whose vitamin D levels rose during the study. Did these patients supplement vitamin D on their own? If this was the case, the results of the study are not valid. 2. The title of Table 3 says that “vitamin D levels” are presented in this table. However, in order to assess vitamin D status, 25-hydroxyvitamin D (calcifediol) is usually measured. Are this title and description correct? Moreover, the values in the table are presented without units, and this should be corrected. 3. There is discrepancy in the Abstract. In section Results there is a statement “patients with vitamin-D3 supplementation were more likely to achieve complete hematological response in comparison with placebo group”, while the Conclusion states that “supplementation of vitamin-D3 with imatinib therapy did not have significant effect on … complete hematologic response”. 4. Introduction should be expanded. The metabolism of vitamin D in human body, as well as actions of its active metabolite should be described. The role of sun exposure should be also discussed, because this is an important factor, which may affect results of any vitamin D supplementation trial.

REVIEWER	Mitchell, Cassie Emory University School of Medicine
REVIEW RETURNED	19-Dec-2022

GENERAL COMMENTS	This is an interesting randomized study examining the association of Vitamin D3 supplementation with imatinib (a first-line TKI) efficacy in treatment-naive chronic phase CML patients. The study is well-designed and statistical analysis appears sound. Some additional details and clarifications are needed to improve clarity and reproducibility. The authors should better clarify in their study objective that they are examining an association of Vitamin D3 with imatinib treatment efficacy versus looking at any causal factors. All analysis examines association only, which is fine, but this needs to be clearly stated. While the primary analysis appears solid, there are a few additional aspects of the analysis that could be improved or minimally clarified in the text. First, the authors state in their protocol that logistic regression was utilized to look at confounding variables, but I could not find the results of that analysis in the provided file documentation. A short summary of such analysis would be interesting. Second, the authors performed Kaplan Meier to examine differences in the response curves. However, the authors did not specify what statistical test was used to determine the presence/absence of significant difference in the Kaplan Meier curves (e.g. log rank test, etc.). This should be stated in the Methods in line 47. Kaplan Meier is a simple and acceptable method to assess for a possible significant differences in temporal response between the two treatment groups. However, Cox regression would enable a better assessment of relative variable association with hematological or cytogenetic response. For example, how many other variables were relatively more important than Vitamin D3 in determining therapeutic response?

	Vitamin D3 deficiency varies based on many factors, including geography. If possible, it would be helpful if the authors could provide an age and gender-matched general population or non-CML population assessment of Vitamin D3 insufficiency in their treating hospital's region or country (e.g. a location that most closely resembles the patients enrolled in the study). This would provide more context to assess if Vitamin D3 deficiency was more significant in the assessed CML population compared to age and gender-matched general or non-CML population in this region. Finally, there is one recent reference that could be helpful to include in this article. A recent study found a predicted association between vitamin deficiencies and TKIs, specifically Vitamin D deficiency. Whether TKIs exacerbate vitamin D deficiency or whether patients taking TKIs are innately more susceptible to vitamin D deficiency is an important question for future research. The suggested study citation is Mehra, et. al. 2022, in the open-access journal, Cancers. https://www.mdpi.com/2072-6694/14/19/4686 MINOR: There are some small English corrections that need to made - some run-on sentences (example: the line 16 sentence starting with Majority.... should be "The majority.."), or improper/missing wording (example: line 59 of abstract).

VERSION 1 – AUTHOR RESPONSE

Response to reviewer's comments:

Sl No	Comments	Response	Page number
1	*For all trials that started after January 2019, we require that a data sharing plan is included in the clinical trial registry. This appears to be missing from the registry page for your study. Please update the registry page to indicate your IPD sharing plan for the study – we will not be able to consider the manuscript further until this is done.	Data sharing plan will be included in trial registry	
2	*We note that the trial registry entry includes a third secondary outcome ("To correlate the levels of 25(OH)2D3 levels with treatment response") that is not mentioned in the manuscript. Is there a reason this outcome is not reported here? Even if it cannot be reported, it should be mentioned in	Modified	Page – 8 and Page – 16

	the main text Methods section, with an explanation as to why it is not reported.		
3	*The dates between which the study took place should be mentioned in both the abstract and the main text. Please revise to include this information.	Modified	Abstract – Page 5 Main text – Page 9
4	*Throughout the manuscript, please revise to avoid reporting non-significant results as if they represent a real difference. For example, in the abstract, the sentence reading “Patients with vitamin-D3 supplementation were more likely to achieve complete haematological response in comparison with placebo group” is not appropriate, as this is not true because the difference was not significant. Please revise here and in all similar instances throughout the manuscript.	Modified	Abstract – Page 5
5	*In the abstract, in the sentence starting “Significant difference in vitamin-D3 levels from baseline”, please revise to include full numerical data for this finding (a p value alone is not informative for interpretation).	Modified	Abstract – Page 5
6	*Please revise the ‘Strengths and limitations of this study’ section of your manuscript (after the abstract). This section should contain up to five short bullet points, no longer than one sentence each, that relate specifically to the methods. The novelty, aims, results or expected impact of the study should not be summarised here.	Modified and added	Page 5-6
7	*In the main text ‘Sample Size Calculation’ section, please provide more details about the basis of the sample size calculation (eg, what difference was the study designed to be powered to detect? What assumptions were made in the calculation?).	Modified	Page - 10

8	*Please ensure that the main text 'Limitations of the study' section includes detailed discussion of all study limitations, including the key limitation(s) highlighted in the 'Strengths and limitations of this study' section (eg, lack of data on long-term treatment outcomes).	Modified	Page - 18
9	*Please change the heading 'Financial support and sponsorship' to 'Funding' and please revise the text to clarify if the support received was for the present study.	Modified A meagre grant amount of Rs 50000 (INR) was received from ICMR as a part of DM (Clinical Pharmacology) dissertation programme. The remaining expenses to conduct the study were borne by the investigators.	Page - 19
10	*Please complete a thorough proofread of the text and correct any spelling and grammar errors that you identify. It may be useful to ask a native English-speaking colleague to assist you or to enlist the help of a professional copy-editing service, if possible, to ensure any English grammar issues or problems with respect to clarity of meaning are identified and addressed.	Modified	
11	*Please delete the 'Competing interests' and 'Financial support' statements from after the 'Strengths and limitations of this study' section, as this information is already reported at the end of the manuscript.	Modified	Page 6
12	*Please change the main text heading 'Methodology' to 'Methods'.	Modified	Page 8
13	We note that the primary outcome here is a surrogate one, and it was not clear to us what would be a clinically meaningful difference between groups. Please clarify.	Since CML CP is chronic disease measuring a relevant surrogate would be beneficial in long term management. The only plausible objective measurement of quantitative *BCR-ABL* has been well established and has a direct relation with	

		long term treatment response and disease progression.	
14	What were the exact starting and end dates of the trial? This should be reported anyway but is particularly important to specify as the authors indicate that the trial covered COVID lockdown periods.	Mentioned in main text	Page 9
15	Related to the overlap with COVID etc, perhaps the authors might want to use the CONSERVE statement to revise their paper, to ensure reporting standards for studies impacted by the pandemic are met. Please see https://jamanetwork.com/journals/jama/fullarticle/2781397 for the CONSERVE statement.	The laboratory investigations were obtained from national accredited laboratories. Hence, there was a negligible requirement to use the CONSERVE statement for the present study	
16	Please elaborate on CHR assessment in the methodology. If some cases were assessed remotely, then how their spleen size was measured at 3months (for CHR)?	Intermittent follow ups were done remotely in a few patients. But the final follow-up at 3 months was done at study site for all patients. This has been mentioned in the text.	
17	In the page 15 paragraph 2 , it is mentioned that majority of patients were vitamin D deficient initially and at 3 months, but percentage is 35.5% at the beginning and 33.9% at 3 months- which seemed incorrect	Modified in page – 15	
18	An inherent weakness of the study is that, at the time of assessment(3 months) majority of patients in the intervention group were still vitamin D deficient.	This finding is already mentioned in page – 18	
19	The description of vitamin D supplementation on page 14, and in Table 3 is hard to understand. If I understand correct, there were patients in placebo group whose vitamin D levels rose during the study. Did these patients supplement vitamin D on their own? If this was the case, the results of the study are not valid.	To the best knowledge of the authors, no vitamin D supplementation was taken by placebo group as enquired in follow up visits regarding concomitant medications intake. Fluctuating vitamin levels in this group can be due to complex pathophysiological mechanisms which needs further research. Imatinib therapy has several mechanisms of vitamin D modulation, thereby altering the levels.	

20	The title of Table 3 says that “vitamin D levels” are presented in this table. However, in order to assess vitamin D status, 25-hydroxyvitamin D (calcifediol) is usually measured. Are this title and description correct? Moreover, the values in the table are presented without units, and this should be corrected.	25 (OH) vitamin D3 has been evaluated in the study. Modification done	Page - 16
21	There is discrepancy in the Abstract. In section Results there is a statement “patients with vitamin-D3 supplementation were more likely to achieve complete hematological response in comparison with placebo group”, while the Conclusion states that “supplementation of vitamin-D3 with imatinib therapy did not have significant effect on … complete hematologic response”.	Modified	Page 5
22	Introduction should be expanded. The metabolism of vitamin D in human body, as well as actions of its active metabolite should be described. The role of sun exposure should be also discussed, because this is an important factor, which may affect results of any vitamin D supplementation trial.	Modified	Page – 8
23	The authors should better clarify in their study objective that they are examining an association of Vitamin D3 with imatinib treatment efficacy versus looking at any causal factors. All analysis examines association only, which is fine, but this needs to be clearly stated.	Imatinib treatment efficacy is determined by CHR and EMR, which is already stated in the objectives. To be more explicit, efficacy with and without Vitamin D is mentioned in the objectives.	
24	While the primary analysis appears solid, there are a few additional aspects of the analysis that could be improved or minimally clarified in the text. First, the authors state in their protocol that logistic regression was utilized to look at confounding variables, but I could not find the results of that analysis in the provided file documentation. A short summary of such	Modified – Kaplan Meier curve (Logistic regression findings were insignificant and hence were not included in methods and results section)	Page - 11

	analysis would be interesting. Second, the authors performed Kaplan Meier to examine differences in the response curves. However, the authors did not specify what statistical test was used to determine the presence/absence of significant difference in the Kaplan Meier curves (e.g. log rank test, etc.). This should be stated in the Methods in line 47. Kaplan Meier is a simple and acceptable method to assess for a possible significant differences in temporal response between the two treatment groups. However, Cox regression would enable a better assessment of relative variable association with hematological or cytogenetic response. For example, how many other variables were relatively more important than Vitamin D3 in determining therapeutic response?		
25	Vitamin D3 deficiency varies based on many factors, including geography. If possible, it would be helpful if the authors could provide an age and gender-matched general population or non-CML population assessment of Vitamin D_3 insufficiency in their treating hospital's region or country (e.g. a location that most closely resembles the patients enrolled in the study). This would provide more context to assess if Vitamin D_3 deficiency was more significant in the assessed CML population compared to age and gender-matched general or non-CML population in this region.	Reports ranging from 40-90% deficiency have been reported in India. As Imatinib itself is associated with modulation of Vitamin D. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6060930/ The authors could not find any age, gender and region matched data after extensive literature search.	
26	Finally, there is one recent reference that could be helpful to include in this article. A recent study found a predicted association between vitamin deficiencies and TKIs, specifically Vitamin D deficiency. Whether TKIs exacerbate vitamin D deficiency or whether patients taking TKIs are innately more susceptible to vitamin D deficiency is an important question for future research. The suggested study citation is Mehra, et. al. 2022, in	Reference added in the discussion section	Page - 19

	the open-access journal, Cancers. https://www.mdpi.com/2072-6694/14/19/4686		
27	There are some small English corrections that need to made - some run-on sentences (example: the line 16 sentence starting with Majority.... should be "The majority.."), or improper/missing wording (example: line 59 of abstract).	Modified in text	

VERSION 2 – REVIEW

REVIEWER	Marcinkowska, Ewa University of Wroclaw
REVIEW RETURNED	22-Feb-2023

GENERAL COMMENTS	The paper has been substantially corrected after the first round. However, there are still some minor errors which need corrections. These are: p.4 l. 49: The sentence "Vitamin-D3, a fat-soluble vitamin transforms to Vitamin-D3 after various steps." should be replaced by "A fat-soluble Vitamin-D3 is produced in few steps." p.5 l. 6: replace "(D3)" with "D3". p.6 l.40: replace "Vitamin-D3" with "calcidiol".

REVIEWER	Mitchell, Cassie Emory University School of Medicine
REVIEW RETURNED	16-Feb-2023

GENERAL COMMENTS	The authors have made improvements to the manuscript clarity.

VERSION 2 – AUTHOR RESPONSE