Author's response to reviews

Title: Changes in fibroblast growth factor 23 levels in normophosphatemic patients with chronic kidney disease stage 3 treated with lanthanum carbonate: results of the PREFECT study, a phase 2a, double blind, randomized, placebo-controlled trial

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**Author's response to reviews:** see over
We thank the editor and reviewers for their comments. Please see responses to the individual points raised below.

Reviewer 1: Bjorn Meijers

The manuscript entitled “Changes in FGF23 levels in normophosphatemic patients with CKD stage 3 with lanthanum carbonate: results of the PREFECT study” by Urena-Torres et al describes the results of a randomized placebo-controlled trial with lanthanum carbonate in patients with CKD stage 3. The authors conclude that, while phosphaturia was adequately reduced, lanthanum carbonate did not lead to sustained reductions in iFGF23, suggesting that factors other than phosphate burden may be responsible for increases of FGF23.

The study design is adequate and the results are well presented and interpreted. Limitations of the study (mainly small number of patients) are addressed by the authors. Some comments and suggestions are given below.

Major Compulsory Revisions

-P6: how was completeness of 24h urine samples assessed?

The following has been added to the methods (p6):

‘One day prior to the visit, each patient was asked to discard their first morning urination, to note the time on the urinary bottle, and then to collect the urine of all subsequent micturitions for 24h. The time of the last urination was also noted on the bottle.’

We did not observe any significant intra-individual variation in the volume of urine collected at the baseline, week 2 and week 12.

-P8: were the observed differences (e.g., age, proportion of men/black patients, …) statistically significant? Please add p-values.

No statistical comparisons were performed to compare these groups. We feel that it would not be suitable to test for statistical significance between these characteristics retrospectively, first because the blinding and randomization of the trial was satisfactory, and secondly because the protocol did not specify this statistical comparison and the study was not powered for it. The text has been amended in the Results (p8) to indicate that these were numerical differences, rather than statistically significant differences.

‘… there were some numerical differences between the lanthanum carbonate and placebo groups’
-P12: safety evaluation: were the differences in adverse events also statistically significant? Again, please add p-values.

Again, for the reasons specified in response to the previous comment, no statistical comparisons were performed to compare these groups. This sentence in the Results (p12) describing the adverse event data has been re-phrased to reflect this:

‘More patients in total, 30.4% of patients experienced adverse events in the lanthanum carbonate group (30.4%) than compared with 16.7% in the placebo group (16.7%).’

-In the discussion, could you add a paragraph describing the effect of other calcium- and non-calcium-based phosphate binders on FGF23 levels in previous studies? Could the lack of effect with lanthanum be drug-specific? Differences in treatment period? Based on these results, are there any clinically significant differences between non-calcium based phosphate binders? It may be useful to add a Table describing all available studies, number of patients, treatment, result

Please see the table below comparing available studies on the topic. This could be added to the paper if desired. The following text has been added to the discussion (p14):

‘The effect of lanthanum carbonate treatment on circulating FGF23 levels in patients with CKD has been reported in several previous studies. Gonzalez-Parra et al. demonstrated a decrease in FGF23 in patients with CKD stage 3 after 4 weeks of treatment with lanthanum carbonate [19]. Isakova et al. saw a reduction in FGF23 values after 12 weeks of treatment with lanthanum carbonate in patients with CKD stages 3 or 4, but only if administered in combination with a phosphate-restricted diet [20]. Other studies on lanthanum carbonate in patients with CKD stages 3–4 have shown no significant change in circulating FGF23 levels using cFGF23 or iFGF23 after 2 weeks [24] or 9 months [31]. FGF23 has also been assessed following treatment with sevelamer or calcium-based phosphate binders [18, 31, 32]. Sevelamer treatment led to a reduction in circulating FGF23 levels in patients with CKD stages 3–4 after 6 weeks [18], and 9 months of treatment [31], and after 8 weeks in patients with CKD stage 4 [32]. There was a less consistent effect of calcium-based phosphate binders on FGF23 in these studies. The inconsistent results between these publications suggest that further studies are required, using a larger sample size and controlled dietary phosphate, to clearly determine whether there is any differing effect between intestinal phosphate binder types and FGF23. Notably, the exact stage of CKD varies between the studies discussed here and this may have a substantial effect on the FGF23 response.’
Minor Essential Revisions
- Additional figure 1 is not visible

This has been corrected
Reviewer 2: Carmine Zoccali

This a small trial showing that lanthanum carbonate, notwithstanding a clearcut reduction urine phosphate (a sign of reduced intestinal absorption), does not lower FGF23.

The initial FGF23 fall IS more apparent than real and it is just a post-hoc finding. I suggest the authors to better de-emphasise this observation.

Thank you for this suggestion. The following has been removed from the introductory paragraph of the discussion (p13), so that this is not presented as such a key observation:

‘However, post hoc analysis demonstrated that lanthanum carbonate treatment was associated with a statistically significant reduction in iFGF23 levels in the first week of treatment, compared with placebo.’

It is possible that the phosphate binder being used be important. Indeed in a previous study by Yilmaz et al., (AJKD 59:177, 2012, which is not quoted in this paper) a 27% reduction in FGF23 was registered in a Group of 47 stage 4 CKD patients randomized to Sevelamer.

Previous papers should be all quoted and arranged in a clear table and findings in this study should framed in the context of available knowledge (previous clinical trials).

Please see below a table that could be added to the paper if required. The following has been added to the discussion (p14) describing the results of available studies:

‘The effect of lanthanum carbonate treatment on circulating FGF23 levels in patients with CKD has been reported in several previous studies. Gonzalez-Parra et al. demonstrated a decrease in FGF23 in patients with CKD stage 3 after 4 weeks of treatment with lanthanum carbonate [19]. Isakova et al. saw a reduction in FGF23 values after 12 weeks of treatment with lanthanum carbonate in patients with CKD stages 3 or 4, but only if administered in combination with a phosphate-restricted diet [20]. Other studies on lanthanum carbonate in patients with CKD stages 3–4 have shown no significant change in circulating FGF23 levels using cFGF23 or iFGF23 after 2 weeks [24] or 9 months [31]. FGF23 has also been assessed following treatment with sevelamer or calcium-based phosphate binders [18, 31, 32]. Sevelamer treatment led to a reduction in circulating FGF23 levels in patients with CKD stages 3–4 after 6 weeks [18], and 9 months of treatment [31], and after 8 weeks in patients with CKD stage 4 [32]. There was a less consistent effect of calcium-based phosphate binders on FGF23 in these studies. The inconsistent results between these publications suggest that further studies are required, using a larger sample size and controlled dietary phosphate, to clearly determine whether...’
there is any differing effect between intestinal phosphate binder types and FGF23. Notably, the exact stage of CKD varies between the studies discussed here and this may have a substantial effect on the FGF23 response.
Reviewer 3: Jessica Kendrick

This is a very interesting study examining the effect of lanthanum carbonate on FGF23 levels in patients with CKD stage 3. The study is limited by its small sample size. Overall it was a negative study as FGF23 levels did not decrease but phosphate excretion did decrease.

Major comments:

1. If the main study endpoint was iFGF23 levels then why were C-terminal (cFGF23) levels used for study inclusion? In their tertiary endpoint cFGF23 levels were reduced compared to placebo. These may be different so I would like the authors to comment on why they chose cFGF23 for inclusion.

We chose cFGF23 values rather than iFGF23 as an inclusion criterion because more published data are available regarding normal and reference ranges of cFGF23 values for classifying CKD stage than for iFGF23. We also had more experience and personal data on cFGF23 than on iFGF23 values which allowed us to estimate a cFGF23 threshold value above which we considered that most patients would have abnormally high cFGF23.

We used iFGF23 instead of cFGF23 as the main endpoint because the assay for intact FGF23 provides a more accurate measurement of the bioactive FGF23 molecule. The measurement of cFGF23, by definition is assessing carboxy-terminal fragments of FGF23, which include FGF23 molecules that have not yet been cleaved and other fragments of FGF23 containing the C-terminus of the molecule in addition to the bioactive molecule.

2. What is their power calculation based on? i.e. where did they get the 40% reduction in FGF23 levels they were expecting to see? This is a large reduction in FGF23 and it is likely the study was underpowered to show a difference in FGF23 levels from placebo with such small numbers of patients.

This power calculation was based on previous work by Dr Prié in which fasting FGF23 levels in patients with CKD were seen to be log-normally distributed with a coefficient of variation (CV) of the logged data of 0.13. Using this CV, 33 patients would be sufficient to detect a ratio of 1.15 placebo to lanthanum carbonate in the means of the logged data at Week 12. When transformed back to the original scale of measurement, this represents an approximate reduction in mean FGF23 levels with lanthanum carbonate of 40% compared with placebo at 12 weeks. The following explanation has been added to the methods (p7):

‘Previous work by one of the authors (DP) showed fasting FGF23 levels to be log-normally distributed with a coefficient of variation of the logged data of 0.13 [author’s unpublished data]. A sample size of 33 participants randomized in a 2:1 ratio (22 receiving lanthanum carbonate and 11 receiving placebo) was estimated to be
sufficient to detect a ratio of 1.15 in the logged means, or a 40% reduction in mean iFGF23 levels in the lanthanum carbonate group compared with the placebo group at 12 weeks. This assumed 80% power and a two-sided significance level of 5%.

3. The authors did not give information on compliance in the study? Did participants tolerate and take the lanthanum carbonate? This has been an issue with previous studies.

Patients were instructed to bring their unused investigational product and empty used containers to every visit and compliance was assessed based on remaining investigational product in the containers. Compliance outside the range 60%–120% was considered a major protocol deviation and led to exclusion from the analysis. Mean compliance was similar between treatment groups. The following has been added to the Results section (p8) to demonstrate this.

‘Mean treatment compliance, measured by the proportion of tablets left in the medicine bottle at the treatment visit, was 87.5% (SD: 20.1%) in the lanthanum carbonate group and 91.0% (SD: 13.0%) in the placebo group.’

4. Not having information on dietary phosphorus intake is a significant limitation to the study. The authors do note this in the discussion. We entirely agree with this point and it will be important to record these data in future studies.

5. The results are somewhat conflicting with a previous study examining lanthanum carbonate in patients with CKD stage 3 as the previous study found a decrease in FGF23 levels. Although the present study did find a decrease in cFGF23 levels. Again, I am not sure why cFGF23 levels were not the main outcome given that they used cFGF23 levels to determine eligibility. The authors need to comment on this.

We partially agree with this comment. We accept that mean cFGF23 values decreased at all weeks in the lanthanum carbonate group, whereas these values increased or were slightly reduced in the placebo group. However, the between-group difference was only statistically significant 2 and 8 weeks after the start of treatment, but not after 1 or 12 weeks. As the pre-specified objective was the comparison between the two groups at week 12, using either iFGF23 or cFGF23 did not change the final results.

Please see response to comment 1 for an explanation of why cFGF23 was used as an inclusion criterion, and iFGF23 as the main endpoint.
Editorial Requests:

1) In the methods section, please can you include the full name of the ethical committee.

   This has been added to the Methods (p5):
   
   ‘The study protocol and informed consent documents received ethical approval from the institutional ethical review committee: Comité de Protection des Personnes, Ile de France II, Necker Hospital, Paris, France, the institutional review board or independent ethics committee.’

2) Please can you remove all images from the main manuscript document. These should be uploaded separately on the submission system. Please note that figure legends should not be included with the image, but appear after the References under the section 'Figure Legends'

   The figures have now been uploaded separately from the main text.

3) Please can you remove the Tables from the uploaded figures files. Tables should appear in the main manuscript document after the References section.

   The uploaded tables have now been removed and are included in the main manuscript document.

4) Please can you adhere to CONSORT reporting guidelines. Please can you upload the CONSORT checklist as an additional file.

   This has been completed and uploaded as an additional file. Changes to the manuscript made whilst completing the checklist are described below.

   A description of the randomisation sequence generation (8a, 8b) has been added to methods (p5):

   ‘Participants were screened within a 2-week period, then returned for a baseline visit at which their eligibility for inclusion in the study was confirmed before they were randomized, by sequential allocation of a unique 2-digit number by the investigator that matched a 2-digit number on the study medication. Treatment was assigned by a randomization schedule and patients received either lanthanum carbonate (FOSRENOL®, Shire, Nyon, Switzerland) 1000 mg three times daily, or a placebo equivalent for 12 weeks (Figure 1A), details of which were in the form of code-break envelopes held at the investigational site.’

   The dates on which the study was carried out (14a) have been added to the methods (p5):

   ‘This was a phase 2a, double-blind, placebo-controlled, proof-of-concept study carried out from November 2010 to May 2012.’
### Table comparing different studies that assess the effect of phosphate binders on FGF23

<table>
<thead>
<tr>
<th>Reference</th>
<th>Intervention</th>
<th>iFGF23/ cFGF23</th>
<th>N</th>
<th>CKD stage</th>
<th>Duration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isakova et al, CJASN, 2013¹</td>
<td>Placebo; LC; P-restricted diet + placebo; P-restricted diet + LC</td>
<td>cFGF23 only</td>
<td>39</td>
<td>3–4</td>
<td>12 weeks</td>
<td>Significant 35% reduction in FGF23 in the P-restricted + LC group at week 12 vs baseline. No significant change in FGF23 from baseline in other groups.</td>
</tr>
<tr>
<td>Yilmaz et al, AJKD, 2012²</td>
<td>Sevelamer; Calcium acetate</td>
<td>iFGF23</td>
<td>100</td>
<td>4</td>
<td>8 weeks</td>
<td>Sevelamer group: reduction (27.1%) at week 8. Calcium acetate group: increase (3.5%) at week 8. Significance of changes from baseline not specified.</td>
</tr>
<tr>
<td>Oliveira et al, CJASN, 2010³</td>
<td>Calcium acetate; Sevelamer hydrochloride</td>
<td>iFGF23</td>
<td>40</td>
<td>3–4</td>
<td>6 weeks</td>
<td>For patients with stage 3 CKD: Sevelamer group: significant reduction in FGF23 at week 6 (34.6%). Calcium group: non-significant reduction in FGF23 at week 6 (24.7%).</td>
</tr>
<tr>
<td>Isakova et al NDT, 2010⁴</td>
<td>LC; Placebo (both on P-controlled diet either 750mg or</td>
<td>cFGF23</td>
<td>16</td>
<td>3–4</td>
<td>2 weeks</td>
<td>No significant change from baseline. Slight increase from baseline in the group ingesting 1500mg P + placebo.</td>
</tr>
</tbody>
</table>

¹ Isakova et al, CJASN, 2013
² Yilmaz et al, AJKD, 2012
³ Oliveira et al, CJASN, 2010
⁴ Isakova et al NDT, 2010
<table>
<thead>
<tr>
<th>Block, JASN, 2012&lt;sup&gt;5&lt;/sup&gt;</th>
<th>Placebo; Calcium acetate; Sevelamer; LC</th>
<th>cFGF23 and iFGF23</th>
<th>148</th>
<th>3–4</th>
<th>9 months</th>
<th>No significant differences in cFGF23 Change from baseline of iFGF23: Sevelamer: small but significant reduction Calcium acetate: small but significant increase LC and Placebo: no change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonzalez-Parra NDT 2011&lt;sup&gt;6&lt;/sup&gt;</td>
<td>LC</td>
<td>cFGF23</td>
<td>18</td>
<td>3</td>
<td>4 weeks</td>
<td>Significant reduction (21.8%) from baseline of cFGF23</td>
</tr>
</tbody>
</table>

**References**