Author's response to reviews

Title: Phase 1 safety and tolerability study of BMP-7 in symptomatic knee osteoarthritis.

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Author's response to reviews: see over
Dear Editor:

I wish to thank you and your reviewers for the careful review and the constructive comments on the revised manuscript which has resulted in further revision. We think this has improved the clarity of the work and its quality. Below is attached a list of the reviewer’s comments (*italics*) and our corrections and detailed answers to the reviewers’ comments, point-by-point, in order of their appearance. Enclosed please find the revised version of the manuscript, with and without track changes.

I sincerely hope that the revised manuscript has met the referees’ suggestions and is now acceptable for publication.

Sincerely yours,

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The authors report a phase 1 placebo controlled dose ranging study on intraarticular injection of BMP 7 in patients with symptomatic knee osteoarthritis (OA). As tell in the introduction, we do need to explore new avenues in the treatment of knee OA and the intra articular route to administer molecules such as BMP-7 is in theory logical; therefore this kind of “pioneer” study should be encouraged. However there a lot of limitations especially in the methods of this study which at least should be clarified. The main criticism concerns the small number of patients per group, even if as tell by the authors the main goal was a safety and tolerability study. The efficacy results are secondary end points which should be interpreted with caution.

We appreciate the reviewer’s support of the need for such trials and readily agree that this needs further trial data to support efficacy in larger cohorts. Consistent with the concerns raised we have tempered the discussion and conclusions based around the secondary endpoints.

The second main criticism concerns the safety end points which are not made clear in the paper. What is the main end point in terms of safety? At which time? It is very important after a local intra articular injection to visit the patients at least in the 48 or 72hours following this injection. Local AE after hyaluronic acid injections occurred mainly in the first 72 h following the injections. One concern is the possible local synovitis induced by this kind of molecules which could be clinically defined by a synovial fluid effusion plus an increase in the level of pain. However the authors describe several side effects such as joint swelling, injection site pain, injection site swelling without clear definition of those AE.

Thus, joint swelling should be recorded at day 2 by a senior physician during a physical examination and not only by just a telephone call. There is at least 3 patients: 2 in the 0.03 mg and 1 in the 1 mg group, who presented joint swelling but we don’t when it occurred (at which visit) and we have no idea of the intensity of this AE. Otherwise the authors do not present any results from the laboratory and immunogenicity data: why? Which kind of tests have been performed?

There are a lot of caveats in the methods and in the presentation of results.

The thoughtful critique of the methods presentation is helpful and we have edited this section to make this clearer. After an observation period of at least 1 hour, participants were released and contacted via telephone on Day 2 to query for adverse events (AEs) and concomitant medications. Additional follow-up visits were done on Days 7, 14, 28, 56, 84 and 168 at which time safety and efficacy parameters were performed. We did not see participants on Day 2 however all participants were seen by a physician who performed the physical examination at each of the listed visits. The onset of joint swelling was different for all three participants, however, all three episodes were classified as mild and all resolved prior to their final study visit. Two subjects were randomized to the 0.03 dose group, the other to 1.0 mg. Specifics details noted below: 01-001, (0.03 mg dose group), onset of joint swelling same day of IA knee injection, classified as mild, resolved within 7 days.
02-001, (0.03 mg dose group), onset of joint swelling one day post-IA knee injection, classified as mild, resolved in 23 days.

02-018, (1.0 mg dose group), onset of joint swelling 19 days post-IA knee injection, classified as mild, resolved within 2 days.

These details have been added to the manuscript.

At each of the study visits, the following clinical laboratory evaluations were performed:

- Hematology: WBC count, RBC count, hemoglobin, hematocrit, platelet count, WBC differential count.
- Coagulation: PT, aPTT, INR
- Clinical chemistry: Albumin, alkaline phosphatase, ALT (SGPT), AST (SGOT), bicarbonate, bilirubin (total), calcium, phosphorus, chloride, creatinine, glucose, potassium, sodium, total protein, urea (BUN), uric acid.
- Urinalysis: Color, appearance, specific gravity, pH, protein, glucose, ketones, blood, microscopic examination (RBC, WBC, casts, crystals).

Laboratory measures were compared with their corresponding normal ranges, and the incidence of abnormally high and of abnormally low laboratory values was calculated for each relevant protocol-specified laboratory test.

Random, minor changes in laboratory values occurred without any apparent trends or clinically significant abnormalities.

At each visit, patient samples were screened for anti-OP-1 IgM and IgG antibodies. Anti-OP-1 neutralizing activity was determined only in samples positive for anti-OP-1 binding antibodies. No patients developed anti-OP-1 binding antibodies during the study.

Blood samples for serum were obtained just prior to injection, immediately post-injection, 1 hour post-injection and at the 7 day visit to assess op-1 drug levels. Results in this study demonstrated that values seen at 1 hr post-dose were very low (near the level of detection of our PK assay) and sporadic, and had no relationship to dose group. Most were non-detectable but a few were near the limit of detection thus not an observable measurement of systemic exposure.

These details have been added to the methods and results section of the manuscript.

**Page 5: inclusion criteria**

**Which dose of acetaminophen was authorized?**

Participants were asked to discontinue NSAIDs and all other analgesics except for acetaminophen, up to 1.0 gram every 6 hours as needed for 5 days prior to KOOS/WOMAC assessments (subsequent protocol amendment revised the NSAID washout to 2 days for the majority of the study)

This has been added to the methods section of the manuscript.

**Page 6:**

**How was the first dose of BMP-7 chosen?**

Preclinical safety evaluation of single intraarticular OP-1 injections in dog stifle joints was assessed at doses of 0.3, 1.0, 3.0, or 10.0 mg. Doses of 1.0, 3.0 and 10.0 mg led to local tissue reactions noted microscopically that included chronic inflammation of tissue
surrounding the administered stifle joint, along with fibro-osseous and fibro-chondroid metaplasia). Clinical signs associated with OP-1 dosing included swelling at the administration site and transient disuse and limping in the 3.0- and 10.0-mg animals. The no-observed-adverse-effect level (NOAEL) under conditions of this study was considered to be 0.3 mg OP-1 intraarticular administration. If maximal systemic exposure occurred with all intraarticularly administered OP-1 entering the bloodstream, and using allometric scaling from dog to human, the starting dose of 0.03 mg is 25.5-fold less than the equivalent systemic NOAEL dose in humans (assuming subject weight = 70 kg). Considering the opposite theoretical scenario, where all OP-1 is deposited in and stays in the joint space, and that there is a 5 to 10-fold greater volume in the human knee as compared to the dog stifle joint, then the starting dose of 0.03 mg is 50-100-fold less than the equivalent local NOAEL for humans.

Page 7: test product and mode of administration could be easily reduced (there are repeated sentences)

Consistent with the reviewers concern this section has been reduced.

Page 8: safety assessment: see previous remarks
Concomitant treatments: why acetaminophen was permitted in the 48 h before each visit? This could unmask the response.

We recognize that there are merits and disadvantages to a flare design when assessing symptomatic response [1]. Given that the primary purpose of the current study was to assess safety and tolerability some masking of therapeutic response was considered acceptable.

How were the concomitant treatments collected?
There is no data reporting analgesics and NSAIDs consumption? Thus how could we be sure that there was no difference between groups?
All concomitant treatment medications were collected at each visit. Patient diaries however were not utilized in the study. There was varied and sporadic usage of rescue medications throughout the study without any clear trends or safety concerns identified. There appeared to be no clinically relevant differences between treatment groups. The most common medications administered were ibuprofen and paracetamol. Early versions of the protocol required a 5-day washout of NSAIDs prior to efficacy assessments. This was later decreased to a 48-hr washout.

Were the X rays performed in the same center and how the X rays were read?

X-rays were performed in the 3 recruiting centres and sent for central reading to a musculoskeletal radiologist with osteoarthritis expertise.

The authors speak about MRI performed in some patients without presenting any results?
The MRI results were exploratory and given concerns over sample size, adequate powering and also the fact that we did not find anything (either favourable or adverse) these were not further presented due to space limitations.

**Page 9: results**

*Lack the following points which should be precised:*

**The exact description of the mean level of womac pain with SD in each sub group.**

The mean WOMAC pain score is 8.6 for active vs 8.63 placebo.

Mean WOMAC pain score at baseline for the 0.03 dose group = 7.57

0.1 = 10.5

0.3 = 8.17

1.0 = 8.33

Placebo = 8.63

**The Kellgren Lawrence classification or one other methods of radiological grading to be sure that there is no difference between groups.**

The mean K-L Grade at Baseline was 2.8 in the active vs 3.4 for placebo.

The mean baseline K-L Grade for the 0.03 dose group = 3

0.1 = 3

0.3 = 2.5

1.0 = 2.5

Placebo = 3.4

**The % of patients with bilateral knee OA in each group** This data was not specifically captured in the study as the index knee was the primary focus of K-L Grade scoring.

**The distribution of concomitant treatments in each group. The duration of knee OA**

The mean duration of illness for the placebo group (9.1 years) and active (3.9 years).

These details have been added to the results section.

*All these points are important to be sure that there is no difference at baseline between groups.*

**Some AE like lymphadenopathy in 2 patients receiving BMP-7 are intriguing. Could the authors comment?**

In preclinical animal models, there is no data that are suggestive of lymphadenopathy in any species at doses many fold the dose used in our clinical trials. Additionally, we see no evidence of meaningful changes in circulating white blood cell counts.

**The results section on efficacy are not interpretable and we suggest to be more cautious and to reduce this section; first the trial is not designed to look for efficacy; the number of patients is too small; The authors say in the discussion that there is no difference in terms of efficacy, but no statistical test are presented!**

Consistent with the reviewers concern the number of tables has been reduced from 5 to 4 with omission of the function results and the text describing the findings has similarly been reduced consistent with a safety/ tolerability study as opposed to an efficacy study.
Moreover the results are really surprising: How the authors explain the low rate of placebo response at week 4 (12%) and then an increase effect at week 8 up to week 24 (which not in line with other reports on placebo response using the i.a route of administration).

Given the small number of participants in this study and the desire not to over interpret the data it is surprising although difficult to ascertain what meaningful conclusion can be drawn from this.

The results in BMP groups are divergent and the worst result observed with the highest dose is unexpected. Could the authors comment this result?

Preclinical data are roughly equivalent in pattern compared to the reported clinical results; i.e. as expected, there are low doses that are ineffective, higher doses that scale to a similar dose in humans that are effective and very high doses that are either less effective or ineffective. A non-linear dose response is not atypical to see with biologic therapy.

Similarly how the authors may explain a prolonged effect in BMP groups at week 24 owing the half life of this growth factor? Is it not purely a placebo effect?

It is possible that this is a placebo effect although well-designed and appropriately powered studies are needed to test this. These are now underway.

Alternatively this study may include patients with a low level of pain at baseline.
The baseline pain levels were not low and are now reported in the results.

It is very speculative as this study stands (follow up on osteophyte formation based on X Rays at 6 months) to tell that i.a of BMP-7 is safe in terms of the risk of bony formation; the authors should at least comment this potential risk of differentiation of local stem cells to an osteogenic lineage, under BMP-7 stimulation.

We do know (via unpublished preclinical data) that there is the potential for differentiation of stem cells to the osteogenic lineage under BMP-7 stimulation. Preclinical studies performed with BMP-7 include a single IA injection study in dogs, two multiple (4 injection) IA injection studies (one in dogs and one in monkeys), and a 28-Day Intravenous study in monkeys. The only consistent observation among the various IA studies was the sporadic formation of small foci (generally no larger than 1-5 mm in diameter) of bone along the needle track between the skin and the outside of the joint capsule. Such foci are likely the end result of residual product at the needle tip. The relatively small size of the dog and non-human primates compared to man makes it difficult to employ techniques that prevent the loss of dosing solution along the needle track (e.g., needle with stopcock to prevent backflow and adequate flushing to remove traces of protein from the needle; this technique was successfully used in the earlier clinical study 06-OA-001 with BMP-7). Needle track bone alone was not considered to be an adverse effect in preclinical studies because no clinical signs, such as lameness or swelling were observed in the treated animals.

To clarify, with regards to the 06-OA-001 human study, the 6 month x-rays were evaluated for both osteophytes changes and evidence of ectopic bone formation. No patients had any evidence of ectopic bone formation at six months.
Referee 2

The study by Hunter DJ et al. evaluated the safety and tolerability as well as dose-limiting toxicity and maximal tolerated dose of intra-articular BMP-7 administration. Clinical laboratory tests, immunogenicity data and radiographic assessments were also included in this study.

**Major Compulsory Revisions.**

1. Please explain any abbreviation used in the text, for example AP, OP-1 and AE. Not each reader may be familiar with these abbreviations.

The list of abbreviations has been extended to include these omissions.

2. Section of Test product, dose and mode of administration, requires an extensive revision because is very redundant.

Thank-you for highlighting this-this has now been trimmed and edited.

3. How do the authors measure immunogenicity? It does not explain in the materials and methods. Why the immunogenicity results are not mentioned in the results section?

At each visit, patient samples were screened for anti-OP-1 IgM and IgG antibodies. Anti-OP-1 neutralizing activity was determined only in samples positive for anti-OP-1 binding antibodies.

4. Why the results of urinalysis, hematology, chemistry, and biomarker assessments are not mentioned in the results section?

Random, minor changes in laboratory values occurred without any apparent trends or clinically significant abnormalities captured in AE summaries.

5. Were patients provided with instructions on a set of standard physiotherapy exercises to be performed throughout the study?

The participants were not instructed on standard physiotherapy exercises during this study so as not to contaminate the intervention tested.

6. Explain the rationale to use only one administration of BMP-7.

As with many growth factors, there is no clear linkage between the pharmacodynamic effects of BMP-7 and its temporal presence in the blood or at the site of action. BMP dimers bind to specific receptors on the surface of mesenchymal stem cells and initiate a cascade of events that eventually leads to the differentiation of new tissues in the following weeks and months. We have evidence that a single injection/treatment of BMP-7 is sufficient to trigger a cascade of bone formation from months to years. Thus, these findings are not surprising.

This has been added to the discussion.
7. Why authors are not mentioned if there were modifications in joint space narrowing, osteophytes and cartilage evaluated by X-ray?

The length of the radiologic assessment period was too short to assess for any positive effects on joint space which using x-ray likely requires 2 years. The major intent of the x-rays was to assess for any adverse effects of the injection of which none were found.

8. In table 1 please add, disease duration as well as analgesic usage or concomitant medication (tablets/day) and range in all the evaluations.

Per the reviewers request this text has now been added to the results section.

Minor Essential Revisions:
The article will be greatly enhanced by including the following references in the next revision:

These references have been added to the paper.

Reference List