Author's response to reviews

Title: Effects of BIS076 in a model of osteoarthritis induced by anterior cruciate ligament transection in ovariectomised rats

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We have performed all the indicated corrections, and they are marked in red in the manuscript.

"Major Compulsory Revision:
1) Although the quality of the images shown in figure 1 are improved, the histology is still poor and raises questions. This reviewer is not convinced by the quality of the histology shown in Figure 1 is still poor. The Safranin-O stained images in Figure 2 show better quality histology, but it looks like this has been overstained (an with no counterstain) making it difficult to determine the extent of proteoglycan loss (which is one component of the OARSI histologic scoring system). NB the methods also still state that histologic scoring was performed on HE sections only."

We have changed the images for others of a better resolution. In any case, the saved images are worse than real images of microscopic observation of histology slides used for scoring. The reviewer is right: in methods although it is indicated "sections were stained with haematoxylin and eosin (general morphology) or safranin O (proteoglycan)" afterwards safranin O was not mentioned for scoring. We have cleared up this point in the text.

"2) Clarification of animal groups. The authors have clarified the experimental groupings. This has highlighted that their sham control animal is both sham for
ovariectomy and ACLT surgery. Since they are looking at the effects of BIS076 on preventing OA-like joint damage, the most appropriate “sham” control would have been ovariectomy + Sham ACLT. The absence of this control prevents the determination of what bone is lost due to the ovariectomy and what alterations are due to ACLT."

This control was in fact performed in this work. As the purpose of the study was to assess the effect of BIS076 on this model of ACLT which affects different tissues and can give more information than partial methods, and not the description of the method itself which is already known, we did not include it in the previous manuscript for the sake of clarity. We have added the information concerning this group (Ovx). These results suggest that changes in metaphysis are dependent on ovariectomy and there is also a contribution of ovariectomy to cartilage changes.

"3) MicroCT analysis of bone structural changes. The review agrees that T2 treatment does improve metaphyseal bone structure, however the evidence for similar changes in the epiphysis is not strong (not even a trend in change). Therefore the suggestion that treatment does improve epiphyseal bone structure should be removed."

We have not suggested that treatment improves epiphyseal bone structure:

-In page 12 it was indicated:
"Effect of BIS076 on bone micro-architecture
µCT analysis indicated loss of bone mass and structural alterations in control rats compared with sham-operated animals (Table 2 and Figure 4). Main bone changes were observed in metaphysis, with significant reductions in bone volume fraction (BV/TV), bone surface density (BS/TV), trabecular number (Tb.N) and volumetric bone mineral density (vBMD) whereas trabecular factor (Tb.Pf) significantly increased. A similar tendency was observed in the parameters measured in epiphysis although these changes did not reach statistical significance."

This statement compared the groups control and Sham, there is no reference to treatment. Changes in epiphysis in control versus Sham were similar to changes in metaphysis, with reductions in bone volume fraction (BV/TV), bone surface density (BS/TV), trabecular number (Tb.N) and volumetric bone mineral density (vBMD) whereas trabecular factor (Tb.Pf) increased although these changes were not significant in epiphysis.

We have now added: "(Ovx or Control versus Sham)". Also, in page 15 we have added "in metaphysis" and in page 16: "metaphyseal" and in "Abstract": "metaphyseal" to clear up this point in the text.
"Minor Essential Revision:
1) MicroCT methodology: The authors have provided most of the details for their microCT analysis, but have neglected to include details of the thresholds and algorithms used to binarise their images in CTANalyser for determination of structural properties. These should be included."

We have added more information on this methodology (pages 9-10).