Reviewer’s report

Title: Epoetin beta pegol for treatment of anemia ameliorates deterioration of erythrocyte quality associated with chronic kidney disease

Version: 0 Date: 04 Jul 2017

Reviewer: Peter Van Buren

Reviewer’s report:

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1. I am not certain of the conclusions that can be made about the "life-span" of the erythrocytes based on the biotinization. It appears as if this is estimating an absolute number of marked erythrocytes at various points in time, but we are never provided with the overall number of baseline erythrocytes in any group. While data on the hemoglobin is provided, one must take into consideration that Hgb will be relative to extracellular volume, which is presumably expanding in the CKD animals. Therefore, part of the decrease in Hgb in the CKD-vehicle animals may be in part due to decreased erythropoiesis, increased destruction, as well as hemodilution. We see similar Hgb in the CKD-CERA and sham rats, but the overall erythrocyte count is not provided. Therefore, it is possible if the CKD-CERA animals are becoming more volume overloaded that the actually erythrocyte count would be higher than sham animals, but distributed in a larger extracellular volume. As the biotinized erythrocytes are calculated from marked erythrocytes to total erythrocytes, the relative preservation of marked erythrocytes compared to the CKD-vehicle may have simply been a reflection of higher baseline erythrocyte mass. This is important to consider in the context of a failure to demonstrate differences in hemolysis.

Specific questions related to this are:

A) How did the animals weights change over time?

B) Was there any assessment of body composition over time?

C) Was there any other assessment of hemolysis ascertained (LDH)?

D) Can the authors provide any further validation of the biotin methods for assessing RBC lifespan
2. I agree with the authors comments that all of the findings in the Epogen group may be related to simply the relative young erythrocyte age from enhanced erythropoiesis.

3. I am curious how the results would have differed in rats with CKD from a different model. The process of removing a single kidney likely effects the overall erythropoietin producing capability more drastically so than other disease processes that cause glomerular disease (ie DM, GN, etc). This study fails to address the response to the intervention in the context of the presence of two diseased kidneys (much more commonly encountered in the clinical setting), as opposed to one diseased kidney, in a uninephrectomized animal.

4. Can the authors comment on the clinical implications of these findings? Should RBC function be further assessed in CKD patients receiving ESA as opposed to following Hgb? What would account for differences between Hgb and RBC function. In other words is the difference in elongation in CKD-CERA and Sham (despite similar Hgb) related to the effects of CKD on old erythrocytes only or would be expect CKD to also impact newly formed erythrocytes?

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable

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I am currently a local principal investigator of a multicenter randomized Phase III clinical trial comparing the benefits in non-dialysis CKD patients of the anemia treatments Aranesp vs vadadustat. However, I have no financial association with the sponsor.

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