Reviewer's report

Title: Protocol and baseline data for a Prospective open-label explorative randomized single centre comparative study to determine the effects of various intravenous iron preparations on markers of oxidative stress and kidney injury in chronic kidney disease (CKD). (IRON-CKD)

Version: 0 Date: 27 Nov 2018

Reviewer: Christos Argyropoulos

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In this protocol, Ziedan et al propose a protocol and provide the baseline data for the prospective, open label assessment of the effects of three intravenous iron preparations on the markers of oxidative stress and kidney injury in kidney disease. This protocol rests on the hypothesis that the different iron preparations will result on variable generations rates of catalytically active iron, which in turn will affect oxidative stress, endothelial function and possible levels of kidney damage. The latter will be assessed by two markers of renal damage: proteinuria and levels of NGAL. The interventions include the use of three different preparations of iron (dextran, sucrose and isomaltoside) which will be delivered as infusions of 200mg for each product except the isomaltoside preparation in which 1000mg will also be used. Forty patients will be enrolled in this study and will be randomized 1:1:1:1 to the different iron preparations/doses. The (numerous) primary outcomes include measurement of markers of renal function, damage, endothelial function, inflammation and oxidative stress.

The premise of the study in an interesting one, considering the agressive "marketing" of these pleotropic effects of iron preparations, the variable costs associated with each product and the renewed interest in iron therapy as an Erythropoeitin Stimulating Agent sparing therapy. The introduction is well written and provide an interesting summary of existing studies. The statistical methodology is well written and the authors avoid the "lethal sin" of analyzing changes from baseline for the statistically correct approach of ANCOVA adjusted for baseline measures.

I have a few comments for the author consideration prior to publication of this study protocol

1) The small sample size justifies the labeling of this study as "exploratory" and thus it is highly unlikely that the study will provide definitive data: at best it will demonstrate the feasibility of a larger randomized controlled trial in this domain. Although the authors acknowledge the exploratory nature of their study, they suggest that the primary objective of the study is to provide valuable information on the multiple outcomes (lines 154-158). This is highly speculative and in fact impossible. In my mind, the only thing that this pilot can show is that these outcomes can be collected; the small sample size precludes the generation of valuable information or inference. Please rewrite this section and also the statement in lines 371-372 of the discussion that alludes to the possibility of generating data on relative efficacy of these agents
2) I am somewhat concerned about the presentation of the study flow: whereas 521 were identified as potential participants, only 40% were contacted and eventually only forty were randomized. By design, the authors would not have been able to recruit and retain non-local participants (237 per line 332). This is a major limitation due to the complexity of the protocol that limits its "translation" to a bigger study. The authors may want to comment about this aspect in the discussion and offer ways in which the complexity of the study procedures may be reduced in a future replication.

3) The discussion should include a section on the future use of the data from this study and how it can be used to power/design a study which allow the investigators to compare these iron preparations in a statistically and clinically meaningful fashion.

4) The decision to use NGAL rather than the many other markers of renal toxicity investigated by the HESI and PSTC consortiums and qualified by the FDA and EMEA as biomarkers of renal toxicity should be discussed. In fact NGAL is not one of the qualified biomarkers of kidney toxicity (see the papers by Fuchs et al https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3231866/ and Dieterle et al https://www.nature.com/articles/nbt.1622 which describe the qualified biomarkers (e.g. clusterin, beta 2 microglobulin, clusterin, Cystatin C, TFF3 and RPA-1). NGAL is not a qualified biomarker at the present time. This is a major limitation of the study protocol concept since any regulatory implications of this study will only have to rely on one biomarker ie urinary albumin.

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