Reviewer's report

Title: Mycophenolate and FK506 Have Different Effects on Kidney Allograft Fibrosis in Rats that Underwent Chronic Allograft Nephropathy

Version: 2 Date: 24 February 2012

Reviewer: Neil Gerard Docherty

Reviewer's report:

Dear Professor Luo & Colleagues,

I have reviewed your manuscript according to the criteria listed below.

General Impression As Per Journal Criteria

1. Is the question posed by the authors well defined?
   The experimental model would seem to be a good one for providing a direct comparison of the differential evolution of CAN between FK506 and MMF treatments. It is an interesting comparison.

2. Are the methods appropriate and well described?
   The CAN model methods need to be described rather than referred to.

3. Are the data sound?
   The data seem sound. However, there are some parts of the data that appear with p values but there is no evidence anywhere in the manuscript of how the end-point was quantified e.g. Trichrome staining and E-cadherin staining. Also care must be taken with areas selected for representative images.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   The discussion is rather poor as I highlight i below.

6. Are limitations of the work clearly stated?
   No

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
   There are some factual errors in the discussion that belie the authors assertions with regard to EMT etc (see later)

8. Do the title and abstract accurately convey what has been found?
   The title does not indicate directionality of effect and should.

9. Is the writing acceptable?
Some grammatical errors but nothing major

Major Compulsory Revisions (Detailed)
1. Provide a better summary paragraph on the natural history of CAN in the introduction
2. In the methods you need to describe the model not just refer to the publication
3. In methods please state clearly the criteria upon which you decided whether the allograft surgery was valid
4. For E-cadherin and Trichrome results you report results as (p<0.05) How you quantified these end-points is missing from the methods and results
5. E-cadherin in the rat marks out the distal tubule. Therefore all images for E-Cadherin should be taken from the cortex and should include a glomerulus in the field to authenticate this and should also clearly show that the staining is occurring in cells with the typical bulging nuclear morphology of the distal tubule.
6. In the discussion
- Provide more of a comment on how the MMF group retains such a comparatively favourable functional picture at 12 weks despite massive histopathological damage
- Do not refer to EMT as if it were a 100% proven phenomenon. This is a theory for which the evidence base is increasingly being critiqued. There is no need to base your results around MMF inhibiting EMT.
- You need to be very careful about how you link cause and effect. For example you say on page 8 " MMF effectively prevented fibrosis in kidney allografts by reducing the expression of CTGF..."

and on page 9 you say "FK506 dramatically upregualted the expression of fibrosis related genes...."

You have not proven this direct cause-effect relationship in either. In the former there is is an association. In the latter in fact FK506 and Vehicle are not significantly different in CTGF mRNA for example. The model therefore is the primary driver of upregulation of gene expression.

- On page 9 you state the following "Both alph-SMA and E-cadherin are fundamental extracellular matrix proteins" This is fundamentally factually inaccurate. Revise all such statements

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.