Format for ANSWERING REVIEWERS

March 3, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14710 revision.doc).

Title: Modern Approaches to Incompatible Kidney Transplantation

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Name of Journal: *World Journal of Nephrology*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Page 5: At the end of the first paragraph it would be interesting if the authors could give a difference between Rituximab effects on bone marrow-derived plasmocytes compared to those from the spleen, and the possible clinical consequences.

Response: Rituximab depletes CD20+ B-cells in the bone marrow, spleen and lymph nodes. It does not deplete plasma cells as they are CD20-. Rituximab may have some effect on plasmablasts that emerge primarily from the spleen. Data from Montgomery et al suggest that splenectomy is effective in treating antibody mediated rejection because it removes DSA secreting plasmablasts that are the primary source of DSA production.

This has been added to the fourth paragraph of the section entitled *Intravenous Immunoglobulin (IVIG) and Rituximab (anti-B cell).*

(2) Page 5, third paragaph: Please provide the treatment given to overcome the ABMR episodes.

Response: AMR was treated with pulse steroids, IVIG and rituximab. Plasma exchange was administered for severe AMR. This information has been added to the paragraph.

(3) Page 6: Explain cPRA

Response: "….a calculated PRA (cPRA), which provides an estimate of the percentage of deceased organ donors that will be crossmatch incompatible for a candidate, greater than 50%. This has been added to the corresponding paragraph.

(4) Page 6, second paragraph: The dose of IVIG is not detailed.

This information in now detailed in the corresponding paragraph.

(5) Page 6: About bortezomib, the dose and schedule should be provided. I would recommend authors to give these details when referring to bortezomib and other drugs in the manuscript.

Response: These six patients were treated with PLEX, IVIG (100mg/kg after each PLEX and 300-400 mg/kg for 1-2 days after the last PLEX with a cumulative dose of 1g /kg), steroids, and single-dose rituximab (375 mg/m2) along with bortezomib (1.3 mg/m2). This is now added to the paragraph.

(6) Page 7: Please rewrite the sentence starting with "Reghavan et al...".

Response: This sentence has now been revised to the following: Reghavan et al reported a kidney transplant recipient with a weak binding DSA who successfully received a deceased-donor kidney transplant after using bortezomib in combination with rituximab. The patients cPRA was reduced from 57% to 31% and the DSA, became undetectable after transplant [20].

(7) Page 7 last paragraph: Please comment briefly on the relevance of complement fixing HLA-antibodies. Are not these the anti-HLA antibody titers the ones to pursue and treat, rather the anti HLA anibodies without knowing its complement interactions?. In other words, are non-complement fixing antiHLA DSAs pathogenic?

Response:
The reduction in complement fixing antibodies is significant since they are mostly responsible the acute presentation of c4d positive ABMR and are difficult to modify. However, non-complement binding antibodies acting via antibody-dependent cell-mediated cytotoxcity are equally deleterious leading to chronic ABMR and transplant glomerulopathy.

(8) Page 8: Please make a brief comment on the most serious dise effects of bortezomib.
Response: The main adverse effect of bortezomib is peripheral neuropathy that may occur in about 30% of treated patients. Severe events noted with bortezomib therapy include thrombocytopenia (28%) and neutropenia (11%) resulted from bone marrow suppression.
This is noted in the second paragraph on page 8.

(9) Page 8 about Eculizumab: "This is expected since C5 is located....". This statement is true. But eculizumab in unable to remove already deposited complement fractions as C5a, C4d, etc with pro-inflammatory actions. Include this concept in the article and use this useful reference: Zuber et al Nat Rev Nephrol 2012; 8: 643-657.

Response: Although the C5 epitope bound by eculizumab is located far from the C5a portion of C5), eculizumab can block C5 cleavage effectively. Eculizumab prevents the entry of the substrate molecule C5 into the C5 convertase, which means that C5 cleavage and the formation of C5a and C5b–9 are inhibited,

resulting in blockade of the pro-inflammatory, pro-thrombotic and lytic functions of complement. The inhibition of complement activation at the level of C5 creates a functional C5 deficiency.
This is added to the first paragraph on page 9. The reference is also included.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Nephrology*.

Sincerely yours,