

## ANSWERING REVIEWERS

July 8<sup>th</sup> Rotterdam,

Dear Editor,

Enclosed please find the edited manuscript in Word format.

**Title:** T-cell ageing in end-stage renal disease patients; assessment and clinical relevance

**Authors:** Ruud W.J. Meijers, Michiel G.H. Betjes, Carla C. Baan and Nicolle H.R. Litjens

**Name of Journal:** *World Journal of Nephrology*

**ESPS Manuscript NO:** 11892

The manuscript has been improved according to the suggestions of reviewers and changes are highlighted throughout the manuscript. Please find below our answers to the comments and suggestions of the reviewers.



Reviewer 1:

1. Nice short review suitable for publication. Just a minor comment: In P. 15 the authors can add that although bardoxolone may attenuate T-cell ageing in ESRD patients, its clinical use is restricted due to cardiovascular side effects (Beacon Trial)
  - *This information has been added in the manuscript (highlighted on P.15) and the reference to the Beacon Trail is included.*

Reviewer 2:

## GENERAL COMMENTS

1. To apply the assessment of T cell age in renal transplantation with treatment of immunosuppressant, the relation between the age of T cell and immune rejection should be evaluated. How can you determine the dose amount of immunosuppressant on the age of T cell when you transplant the kidney to patient?
  - *Our current research is focusing on T-cell ageing parameters prior to kidney transplantation (KTx) and the risk for acute rejection afterwards. These data have not been published yet. However, more research is necessary with respect to how assessment of T-cell ageing parameters prior to KTx might contribute to dose adjustment of immunosuppressive therapy post-KTx.*
  
2. ESRD patient can be susceptible for infection based on your explanation of T cell aging. However there was no mention on the immunological strategy for overcoming the infection.
  - *As written in the manuscript, the prematurely aged T-cell system in end-stage renal disease patients caused by uremia increases the risk for infections. Rejuvenation of the T-cell system using IL-7 therapy to improve numbers of naïve T cells and increase the T cell receptor diversity (enhancing the fitness of the immune system) might decrease the risk for infections. This is mentioned in the text on P.15.*  
*After transplantation, over-immunosuppression introduces an additional risk for infections. Assessing an immunological age might prevent over-immunosuppression and thereby indirectly decrease the risk for infections.*
  
3. T cell ageing can be the important role on transplantation in ESRD patient for the reducing immunosuppressant. However, there are another immune cell such as B cell, macrophage and dendritic cell. So, the explanation of effect or role of these immune cells is required in ESRD patient. Furthermore, the explanation of effect or role of these immune cells in ESRD patient with T cell aging is required.
  - *As written in the introduction, uremia-associated ageing does not only affect the T-cell compartment but also other immune cells. For instance patients have reduced numbers of dendritic cells which have a decreased antigen presentation capacity. The number of macrophages is higher but their capacity to phagocytose is decreased. ESRD patients have reduced numbers of naïve and memory B-cells with an increased susceptibility for apoptosis. In this review, we focus on T cells in ESRD patients since they play an important role in transplantation and many immunosuppressive therapies affect T cells. In the manuscript we have now added additional references referring to studies describing effects of uremia on different immune cells.*
  
4. In page 3, it would be better to describe the full words of the abbreviation, TREC. In page 8, what is EMRA? Please describe the details of EMRA and the full words of the abbreviation, EMRA. In page 15, treatment with IL- => IL-7 ?
  - *We have now written both abbreviations in full, i.e. TREC stands for T cell receptor excision circles and EMRA refers to terminally differentiated effector memory, CD45RA+. Indeed it should be IL-7, apologies for the missing number.*

5. There are several sentences to be corrected in grammar. ex)
- A. (page 5, line 22) CD28 plays an important role in the activation of T cells and a loss of CD28 can result in insufficient activation and shorter replicative lifespan and a higher toxicity. => CD28 plays an important role in the activation of T cells and a loss of CD28 can result in insufficient activation, shorter replicative lifespan and a higher toxicity.
  - B. (page 10, line 7) Progressive loss of renal function was highly correlated with a lack of IL-7 and a loss of naïve T cells and an increase in terminally differentiated CD8+ T cells. => Progressive loss of renal function was highly correlated with a lack of IL-7, a loss of naïve T cells and an increase in terminally differentiated CD8+ T cells
  - C. (page 15, line 7) (Nrf2) which is an is an => (Nrf2) which is an
- A-C: all sentences are corrected and the changes are highlighted in the manuscript.
6. Please uniform the reference style in the text. (position of comma)
- A. Ie> (12,13). or .(12,13)
- Apologies for the inconsistent reference style. The reference style is now uniform (= . (12, 13)) throughout the manuscript.

## SPECIFIC COMMENTS

- In the part of '13 page-Premature T cell ageing and kidney transplantation', you mentioned KLOTHO gene for T cell aging. However, based on your explanation KLOTHO gene is not related with T cell aging directly. And there might be another genes related with ageing. So, I recommend you more explanation on genes which are related with T cell ageing.
- *Indeed, T-cell ageing results in several transcriptional changes which leads to an altered receptor-profile and function of T cells. In 2013, a review of Chen summarized the age-associated changes during normal ageing and tried to relate this to functional changes. We now included this reference in this manuscript. However, in this review our focus is more on uremia-associated changes and KLOTHO was affected by uremia.*
- You mentioned 'Normal ageing is associated with, epigenetic changes in HSC'. In your manuscript, you focused on T cell aging in ESRD patients. T cell is originated from HSC. By this reason, I suggest the requirement of analyzing the epigenetic status of HSC in ESRD patient.
- *I agree with this suggestion and we therefore added in the conclusion section (highlighted, P. 16) that more research is necessary to fully understand the uremia-associated premature T-cell ageing phenomenon, including also earlier developmental stages of T-cells.*
- In page 11, Please add references about the reason that increased susceptibility for apoptosis is associated with a loss of antigen-specific T cells.
- *Two references are now added in the manuscript in which it was shown that there is an increased expression of pro-apoptotic markers that are associated with a decrease in number of (antigen)-specific T-cells.*

I hope that to fully addressed the issues raised by the reviewers and that the manuscript is suitable for publication in the *World Journal of Nephrology*.

With Kind Regards,

Ruud W.J. Meijers