

Rapamycin treatment for Alzheimer's disease and related dementias: a pilot phase 1 clinical trial

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Communications Medicine.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Gonzales et al. report the results of the first pilot study of rapamycin in MCI/AD. The topic in general is of very high interest given the scarcity of trials of rapamycin in age-related disease (non-transplant patients), and the high promise of TOR inhibitors based on preclinical and early clinical data. This feasibility pilot builds on previous work by these authors in healthy individuals and is an important practical foundational study for future research on rapamycin in AD. Data on the primary outcome (CSF levels of rapamycin) were negative but still provide important information for the design of future trials. Similarly, the clinical results provide potentially important signals and are interpreted reasonably within the constraints inherent to the methodological limitations of small study size and lack of a placebo. I had previously reviewed an earlier version of this manuscript for a related journal, and all of my methodological and reporting suggestions are sufficiently addressed in the current version. I have no further concerns. I believe the manuscript is appropriate for publication.

Reviewer #2

(Remarks to the Author)

The statement "Our secondary endpoints were to examine...target engagement by evaluating baseline to posttreatment changes in CSF and plasma ADRD" is slightly misleading. It would seem that measuring phosphorylation of mTOR or downstream substrates would be the readout to determine if rapamycin engaged with its intended target (mTOR). As the others note later, it is unclear if rapamycin had a detrimental effect on these biomarkers or whether that is the natural course of ADRD. Is there anyway the authors are able to measure these biomarkers in patients with similar disease status that were not treated with rapamycin to distinguish if rapamycin is having detrimental or no impact on ADRD biomarkers?

The authors should remove this statement "In light of rapamycin's pleiotropic effects on key processes linked to ADRD pathogenesis, there has been a call for clinical trials¹⁷". The reference is authored by a co-author of the trial and therefore a natural bias. The authors can still get their point across of the potential need for testing rapamycin on ADRD with a different approach.

Ln 205-206 "Rapamycin is one of the best studied longevity-enhancing drugs." Should be clearly noted that the longevity effects have only been studied/established in pre-clinical/animal models.

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RESPONSE TO REVIEWERS (NMED-A133195 -- NCOMMS-24-54840-T -- COMMSMED-24-1242-A)

We thank the Reviewers for their review of manuscript. We have taken the opportunity to revise the manuscript in response to the Reviewers' feedback as described below.

Reviewer #1

Remarks to the Author: Gonzales et al. report the results of the first pilot study of rapamycin in MCI/AD. The topic in general is of very high interest given the scarcity of trials of rapamycin in age-related disease (non-transplant patients), and the high promise of TOR inhibitors based on preclinical and early clinical data. This feasibility pilot builds on previous work by these authors in healthy individuals and is an important practical foundational study for future research on rapamycin in AD. Data on the primary outcome (CSF levels of rapamycin) were negative but still provide important information for the design of future trials. Similarly, the clinical results provide potentially important signals and are interpreted reasonably within the constraints inherent to the methodological limitations of small study size and lack of a placebo. I had previously reviewed an earlier version of this manuscript for a related journal, and all of my methodological and reporting suggestions are sufficiently addressed in the current version. I have no further concerns. I believe the manuscript is appropriate for publication.

Response: We thank the reviewer for highlighting the importance of this work and our revisions to appropriately acknowledge the study limitations.

Reviewer #2

Remarks to the Author:

1. **Comment:** The statement "Our secondary endpoints were to examine...target engagement by evaluating baseline to posttreatment changes in CSF and plasma ADRD" is slightly misleading. It would seem that measuring phosphorylation of mTOR or downstream substrates would be the readout to determine if rapamycin engaged with its intended target (mTOR).

Response: We understand this concern and have removed all references to "target engagement" in the revised manuscript (please see Lines 144, 161, and 348).

2. **Comment:** As the others note later, it is unclear if rapamycin had a detrimental effect on these biomarkers or whether that is the natural course of ADRD. Is there anyway the authors are able to measure these biomarkers in patients with similar disease status that were not treated with rapamycin to distinguish if rapamycin is having detrimental or no impact on ADRD biomarkers?

Response: We agree that the lack of a control group and our inability to distinguish between natural changes in disease course and potential adverse effects of the study drug is a significant limitation. We have summarized the literature on longitudinal ADRD biomarker changes; however, differences in patient populations, assays, and/or other methodological factors may contribute to variability across studies. As stated beginning on Line 484 in the revised manuscript "Over a mean of 2.1 years, Lleo *et al.* examined longitudinal changes in p-tau 181 using the Fujirebio Europe INNOTEST assay and NfL using the Uman Diagnostics assay. When restricting the sample to individuals with AD, CSF p-tau 181 levels remained unchanged and NfL increased at an annual rate of 4%⁵⁵. Other studies have similarly reported stability in p-tau 181 in individuals with AD over a 6-month follow up period⁵⁶."

We also highlight this limitation beginning on Line 387 of the revised manuscript. “Without a placebo-controlled group, we are unable to clearly determine if the outcomes observed are due to the study drug, natural disease progression, and/or other unexplored factors, highlighting the need for further research.”

3. **Comment:** The authors should remove this statement "In light of rapamycin's pleiotropic effects on key processes linked to ADRD pathogenesis, there has been a call for clinical trials¹⁷". The reference is authored by a co-author of the trial and therefore a natural bias. The authors can still get their point across of the potential need for testing rapamycin on ADRD with a different approach.

Response: We have revised the statement and have added a citation from an independent team. As stated on Lines 116-117 of the revised submission “In light of rapamycin's pleiotropic effects on key processes linked to ADRD pathogenesis, it has been proposed as a potential, yet, untested translational therapeutic” (Hou SJ, Zhang SX, Li Y, Xu SY. Rapamycin Responds to Alzheimer's Disease: A Potential Translational Therapy. Clin Interv Aging. 2023 Oct 2;18:1629-1639. doi: 10.2147/CIA.S429440).

4. **Comment:** Ln 205-206 "Rapamycin is one of the best studied longevity-enhancing drugs." Should be clearly noted that the longevity effects have only been studied/established in pre-clinical/animal models.

Response: Thank you for suggesting this important clarification. We have revised the statement to indicate that data on longevity have been derived from animal models (please see Line 380 in the revised submission).