nature portfolio

Peer Review File

Remdesivir, mAb114, REGN-EB3, and ZMapp partially rescue nonhuman primates infected with a low passage Kikwit variant of Ebola virus

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

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript by Prasad et al, comparing Remdesivir, mAb114, REGN-EB3 and ZMapp in EBOV Kikwit infected Rhesus Macaques is an extremely important contribution to the field. As such, my overall recommendation is for this article to be published. This study models the PALM trial, wherein humans were treated with these four therapies at a late stage of disease. Previous NHP EBOV studies using these treatments have used different protocols and in some cases treatment was initiated too early to be comparable to the human situation. Here, the authors initiate treatment at 5 days post-infection which is an extremely high bar in this model. Indeed, one of the animals on the study (in the remdesivir treatment group) succumbs on the day treatment was initiated. This manuscript is well written, thorough and establishes several very important findings, and does so convincingly:

1) Treatment with Remdesivir, mAb114, REGN-EB3 or ZMapp in EBOV Kikwit infected Rhesus Macaques at 5 dpi is not fully protective, but each treatment results in similar survival rates.

2) Survival with each treatment is correlated with the viral load at the time of treatment.

3) mAb114 treatment resulted in a trend of greater escape mutants, bolstering a movement in the field towards combining therapeutics (i.e. mAb combined with a small molecule such as Remdesivir).

Recognizing that studies in ABSL-4 are difficult to conduct and NHPs are a scare resource, there are nonetheless some aspects of the study that are not ideal. However, these issues are not serious enough to discount the results and conclusions made by the authors. These potential limitations are as follows:

1) The study was conducted in separate iterations. However, the authors describe that all procedures and reagents, the challenge dose, and personnel conducting the study were the same, which minimizes the impact.

2) Very few controls were used in these iterations. Additionally, the control for the remdesivir arm of the study was “untreated” rather than treated by daily i.v. infusion with vehicle. While this is not ideal, vehicle treatment alone would not be expected to change the outcome (animals would not be expected to survive).

3) The authors do not state whether the study was blinded.

4) It is not stated whether statistical tests were described in a study protocol prior to the conduct of the study (rather than after the fact).

Other concerns:

5) There is a large age and weight range of NHP subjects. However, age or weight of NHPs is not known to significantly impact the disease course EBOV in NHPs.

6) The blood collection schedule and anesthesia events are not described (and whether these were consistent between iterations).

7) Very little information is provided about the 14 historical control animals. Of note, the average time to death is greater in this group of animals than the controls across the iterations.

8) The male/female breakdown of groups is not described in the text of the article (although this is given in Table S1).

9) It would be nice if the authors would compare the treatment doses/schedule for their NHP studies with that of the human protocols in the PALM trial (how were these similar/different in terms of dose/frequency and timing)? Perhaps this could be described in a table.

10) For the animal scoring, it would be nice to see a more detailed description of how each factor is weighted in the scoring scheme. Is it possible for an animal to reach a score of 9 without having a contribution of impaired posture and activity level?

11) For figures 2C and 2D, it would be nice to see which of the dots correspond to various treatments (perhaps through color coding). Did certain treatments show a trend of better protection at the higher 5 dpi viral loads? (Recognize that the numbers may not be sufficient to make this determination.).

Reviewer #2

(Remarks to the Author)
In this study, the authors evaluated the therapeutic potential of four drugs—remdesivir, mAb114, REGN-EB3, and ZMapp—all of which were clinically tested and compared during the 2018-2020 outbreak of Ebola virus disease (EVD) in the Democratic Republic of the Congo (DRC). Previous studies concluded that mAb114 and REGN-EB3 were superior to remdesivir and ZMapp in reducing EVD mortality while also highlighting challenges in fairly randomizing patients during outbreaks. The present study demonstrated that these therapeutics exhibited similarly suboptimal efficacy in a uniformly lethal and well-controlled nonhuman primate model of EVD. The authors extensively and carefully discussed their results in relation to existing literature, highlighting both strengths and weaknesses and suggesting potential ways forward to maximize overall therapeutic effectiveness and reduce the risk of escape mutant emergence. The manuscript is well written, and the information presented in this paper is highly important and valuable. Below are suggestions for improving the manuscript:

Specific comments
1. Title: While this reviewer acknowledges the importance of using a low-passage 7U-dominant variant as an EVD animal model, this information could be omitted from the title, as most readers of this journal are unlikely to be familiar with this niche topic.
2. L40-41: The 2018–2020 EVD outbreak occurred in DRC, specifically in the North Kivu and Ituri provinces, but not in Uganda. In Uganda, only imported cases were reported, with no active transmission. Could the authors verify the accuracy of this statement?
3. L87-127: Were the treatment doses (i.e., 50 mg/kg for antibodies and 5 mg/kg for remdesivir) appropriate and consistent with those used in the PALM trial? Additionally, why was treatment administered only on day 5 post-infection? Information on the rationale behind selecting these regimens for comparison should be provided.
4. L146: "vRNA" should be spelled out here, as it is first introduced as an abbreviation on L147.
5. L181-277: The pathology section is unnecessarily long and redundant. It should be condensed.
6. L195-196: A new sentence should begin with “The two remaining survivors (ZMP-2 and ZMC-1).”
7. L278-300: Are the described substitutions actually associated with escape from antibody-mediated neutralization? This information should be clarified. If no such association has been reported, the possibility should at least be discussed.
8. L425-436: If the authors have any information on differences in viral genome persistence between the treatments, it would be interesting to include it.

Reviewer #3

(Remarks to the Author)
The manuscript by Prasad et al. directly compares efficacy of 3 different monoclonal antibody treatments and remdesivir for post-Ebola virus-infection treatment of rhesus monkeys. The study is a direct comparison of treatments using the same virus preparation at the same facilities to conduct a head-to-head test. Although the data are potentially interesting, there are concerns of the applicability and novelty of the data.

Major concerns
1) The manuscript states that “Remdesivir, mAb114, REGN-EB3, and ZMapp equally and only partially rescue nonhuman primates.” However, this is impossible to prove with 4-5 animals per group. For example, 50% of animals survived with CHO-derived ZMapp, but only 25% survived with plant-derived ZMapp. This could be a very interesting finding, but it is not statistically significant since there are only 4 animals per group. It is likely that differences between mA114 or REGN-EB3 and remdesivir or ZMapp in humans would not have been found with a low number of treated human cases. In fact, the very fact that there were observed differences between the human studies and the animal data reported in this manuscript highlights the difficulty in concluding these treatments are equally effective, as this conflicts with data with hundreds of

human cases. While it is completely understandable that not more animals per group were challenged due to the excessive cost and difficulty in obtaining macaques, and while the authors briefly discuss the low number of animals in the discussion, the data do not support the stated conclusion of the manuscript.

2) The data combine 3 separate experiments, which lessens the direct comparison between the treatments.

3) Animals received 50 mg/kg of the ZMapp treatments, but 150 mg/kg of REGN-EB3 (50 mg/kg of each of the 3 mAbs in the cocktail). The mAb114 treated animals received 50 mg/kg of this mAb. It is not stated in the manuscript why these doses were chosen, but it can be assumed these were chosen since it is the dose used in the PALM trial. However, in effect, you have 50 mg/kg of each of 3 mAbs in REGN-EB3, 50 mg/kg of the single mAb in mAb114, and 16.67 mg/kg of each of the mAbs in the ZMapp treatment regimen. This could confound the direct comparison analysis.

4) The remdesivir dose (10 mg/kg loading dose, 5 mg/kg daily maintenance dose) appears to be much high than the PALM trial (200 mg total loading dose (roughly 2.8 mg/kg) and 100 mg total daily maintenance dose (~1.4 mg/kg).

5) Figure 2C and 2D show that animals that survived infection had lower viremias at the time of treatment onset then animals that died. Therefore, this clouds the interpretation of the data in comparing efficacy of the different treatments.

6) Figure 4, there is no information on what time points the mutations were found and if the mutations were found in the animals that succumbed to infection or those that survived.

7) There is no discussion of if the mutations in Figure 4 mapped to known binding sites of the mAb cocktails.

8) There should be statistical analysis conducted on the hematology and serum biochemistry data.

Minor concerns
1) Lines 63-66 state “A separate trial conducted toward the end of the epidemic demonstrated that ZMapp, a cocktail of three EBOV glycoprotein (GP)-specific mAbs19 64 , was 91.2% more effective in preventing Ebola virus disease (EVD) mortality than standard supportive care alone, although this fell short of the pre-specified study goal of 97.5%.” However, this is incorrect, as the referenced paper stated “The observed posterior probability that ZMapp plus the current standard of care was superior to the current standard of care alone was 91.2%, falling short of the prespecified threshold of 97.5%.” Therefore, the % listed in paper deals with probability, not effectiveness. This should be revised.

2) The lack of difference in viral RNA load in the eyes between the treatment and control groups is intriguing, as is the CNS persistence in surviving animals. This should be expanded on in the discussion as it relates to long-term disease and/or viral persistence in human survivors.

3) There should be a paragraph summarizing the extensive results on pathology on the tissues from the different treatment groups—are there any significant differences between the groups?

4) Lines 414-418, the authors compare survival rates of their mAb-or remdesivir-treated animals with that of humans treated similarly, but this is not a valid comparison, given the 100% lethality of the untreated animals compared with the much lower lethality rate in the human study.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I feel that the authors responses and edits to the reviewers' critiques are thorough and that they have rectified all concerns that were possible to address. This is an extremely important body of work and I recommend publication of this article.

Reviewer #2

(Remarks to the Author)
The manuscript has been revised accordingly.

Reviewer #3

(Remarks to the Author)
The authors are to be commended for their thorough response to the reviewers' concerns. While the data are worth publishing, there is not enough impact or novelty for publication in Nature Communications. The major finding of the manuscript--that remdesivir, mAb114, ZMAPP, and REGN-EB3 all partially protect nonhuman primates from EBOV infection--have already been confirmed in NHP and/or human trials.

The main novelty from the manuscript seems to be that these studies a) were performed with the same viral stock, b)

contradicted some previously published studies that showed complete (not partial) protection from EBOV from some of these treatments, and c) found some EBOV GP mutations after treatment. However, since these treatments have been tested in relatively large numbers of humans, the importance of point a) is lessened. Furthermore, point c) is less important since all of the point mutations were found in animals that died, suggesting that no important escape mutants were identified.

Overall, this manuscript is worth publishing but does not reach the impact that would be required for publication in Nature Communications.

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Reviewer 1:

1) The study was conducted in separate iterations. However, the authors describe that all procedures and reagents, the challenge dose, and personnel conducting the study were the same, which minimizes the impact. We acknowledge this potential limitation highlighted by the Reviewer and agree that efforts to minimize other sources of variation between the experiments mitigate the potential impact on the interpretation of the data.

2) Very few controls were used in these iterations. Additionally, the control for the remdesivir arm of the study was "untreated" rather than treated by daily i.v. infusion with vehicle. While this is not ideal, vehicle treatment alone would not be expected to change the outcome (animals would not be expected to survive). In accordance with the "3R's" principle of ethical use of animals in research ("replacement, reduction, refinement"), and in accordance with UTMB IACUC, we use the minimum number of animals, including controls, to achieve the research aims. Typically, one or two control animals per challenge period are used to confirm lethality of challenge material used in the treatment studies where-in clinical parameters are compared with historical control animals derived from other experiments using the identical challenge material (i.e., exact same virus seed stock, route, and dose). This allows for an ethical reduction in animal number use for data with highly predictable, lethal outcomes. As the in-study control for the remdesivir and REGN-EB3 studies was shared between the studies, it was left untreated as opposed to "treated" with vehicle. However, as the Reviewer notes, this would not be expected to, and indeed, did not, affect the outcome of the challenge.

3) The authors do not state whether the study was blinded. The study was not blinded. A specific statement reading "Due to the academic nature of this study, as well as the inherent challenges in designing and staffing blinded studies in ABSL-4 settings, blinding was not performed for this study, as it was not necessary to answer the questions set forth by the study." is included in the accompanying Reporting Summary as required by the Journal.

4) It is not stated whether statistical tests were described in a study protocol prior to the conduct of the study (rather than after the fact). The minimum size for each cohort was determined prior to each study (in the IACUC approved study protocol) to detect with > 80% power a difference between treatment group and the control group (0% survival) by Fisher's exact test, assuming alpha 0.05. The Mantel-Cox log-rank test is a standard test for testing significance in survival curves between treatment and control groups. We have included information as required in the Reporting Summary regarding the study design.

Other concerns:

5) There is a large age and weight range of NHP subjects. However, age or weight of NHPs is not known to significantly impact the disease course EBOV in NHPs. Unfortunately, due to the increased cost and decreased availability of rhesus and cynomolgus macaques for research due to

the COVID-19 pandemic, it has become increasingly difficult to closely match animals across studies. The Reviewer is however correct in stating that age and weight are not known to significantly affect the disease course or outcome of EBOV infection in NHPs; particularly by uniformly lethal challenge doses and routes such as i.m, as used in this study.

6) The blood collection schedule and anesthesia events are not described (and whether these were consistent between iterations). We have included a statement in the Methods (under "Nonhuman primate challenge and treatment") detailing the sampling schedule for each study, which were identical except that blood was sampled 3 DPI for the ZMapp study only, and at 35 DPI for the remdesivir, REGN-EB3, and mAb114 studies only (the ZMapp study endpoint was 28 DPI).

7) Very little information is provided about the 14 historical control animals. Of note, the average time to death is greater in this group of animals than the controls across the iterations. We have added citations for the historical control animals (line 132 in the Results) which come from a combination of published and unpublished studies. Importantly, all control animals were challenged with the same virus stock at the same dosage via the same route, and the studies were conducted by the same research personnel as the current study.

8) The male/female breakdown of groups is not described in the text of the article (although this is given in Table S1). We have included a statement in the Methods (under "Nonhuman primate challenge and treatment") stating that efforts were made to balance the sex distribution and pointing the reader to Supplementary Tables 1-3 for information regarding the sex of individual animals.

9) It would be nice if the authors would compare the treatment doses/schedule for their NHP studies with that of the human protocols in the PALM trial (how were these similar/different in terms of dose/frequency and timing)? Perhaps this could be described in a table. We have added text in the Methods section (under "Nonhuman primate challenge and treatment") stating that treatment dosages and dosing schedules were designed in congruence with the PALM trial and/or previous NHP studies assessing each therapeutic individually. As three of the four therapeutics (ZMapp, REGN-EB3, and mAb114) were administered at the same dosage and frequency as both the NHP studies and the PALM trial, and remdesivir was given at a dosage utilized in a previous NHP study (cited and discussed within the manuscript), we do not feel that an additional table would be especially informative and have elected not to include one.

10) For the animal scoring, it would be nice to see a more detailed description of how each factor is weighted in the scoring scheme. Is it possible for an animal to reach a score of 9 without having a contribution of impaired posture and activity level? Unfortunately, UTMB policy prevents us from divulging the specific details of our IACUC-approved disease scoring protocols. However, in our experience, changes in posture (e.g. hunching at early stages, potentially progressing to recumbency) and activity level are typically the first overt indications of disease progression in NHPs infected with EBOV. While we cannot provide the exact scoring criteria in the manuscript, Supplemental Tables 1-3 provide descriptions of disease progression made for each animal throughout the study duration.

11) For figures 2C and 2D, it would be nice to see which of the dots correspond to various treatments (perhaps through color coding). Did certain treatments show a trend of better protection at the higher 5 dpi viral loads? (Recognize that the numbers may not be sufficient to make this determination.). We have revised Figure 2C and 2D to include color coding denoting the treatment cohort each data point belongs to. Unfortunately, the Reviewer is correct in that, due to the small number of survivors from each treatment cohort, statistical or trend analysis was not possible.

Reviewer 2:

1. Title: While this reviewer acknowledges the importance of using a low-passage 7U-dominant variant as an EVD animal model, this information could be omitted from the title, as most readers of this journal are unlikely to be familiar with this niche topic. We have removed "7U-dominant" from the title as suggested.

2. L40-41: The 2018–2020 EVD outbreak occurred in DRC, specifically in the North Kivu and Ituri provinces, but not in Uganda. In Uganda, only imported cases were reported, with no active transmission. Could the authors verify the accuracy of this statement? The Reviewer is correct in that the cases in Uganda involved cross-border movement of infected persons. We have removed the mention of Uganda from this statement.

3. L87-127: Were the treatment doses (i.e., 50 mg/kg for antibodies and 5 mg/kg for remdesivir) appropriate and consistent with those used in the PALM trial? Additionally, why was treatment administered only on day 5 post-infection? Information on the rationale behind selecting these regimens for comparison should be provided. The treatment doses for ZMapp, REGN-EB3, and m114 were consistent with what was administered to human patients in the PALM trial as well as efficacy studies in NHPs as cited in the manuscript. The dosage of remdesivir we administered in the current study differed from that in the PALM trial, which was not based on individual patient body mass in adults but rather as an initial loading dose of 200 mg followed by daily maintenance doses of 100 mg, which as stated by Reviewer 3 may be assumed to translate to ~2.8 mg/kg loading dose and ~1.4mg/kg maintenance doses for an average (~70 kg) adult. However, as mentioned in the discussion, the doses of remdesivir we utilized in the present study are identical or within range of those used in NHP efficacy studies with this antiviral. As this study was conducted in NHPs and not humans, and to be consistent with the published literature, we chose to administer remdesivir at the stated dosages. Likewise, the frequency of each treatment was designed to mimic that of both the PALM trial as well as the published studies in NHPs. Specifically, in the PALM trial ZMapp was administered to patients every third day beginning on day 1 of enrollment (NHPs in our study received ZMapp 5 DPI, 8 DPI, and 11 DPI); while REGN-EB3 and mAb114 were singly administered on day 1 of enrollment. In the PALM trial, remdesivir was administered daily beginning at day 1 of enrollment and continuing for a consecutive 9-13 days; likewise, NHPs in our study received remdesivir for a total duration of 12 days.

For all of our studies, treatment was administered at 5 DPI as it is a point of advanced disease in this model. We have included text in the Methods section of the revised manuscript under "Nonhuman primate challenge and treatment" stating that the dosage and frequency regimen of each treatment was designed in congruence with the PALM and/or preclinical animal studies.

4. L146: "vRNA" should be spelled out here, as it is first introduced as an abbreviation on L147. We thank the Reviewer for catching this omission and have corrected it as suggested.

5. L181-277: The pathology section is unnecessarily long and redundant. It should be condensed. We have done our best to present the data as thoroughly as possible, which necessitates some repetition of similar findings in different animals and across treatment groups. We have presented pathology results similarly across dozens of publications with no prior negative feedback, so we take that to indicate this is a subjective opinion of the Reviewer and as such we have elected to keep the pathology results section as is.

6. L195-196: A new sentence should begin with "The two remaining survivors (ZMP-2 and ZMC-1)." We thank the Reviewer for catching this typo and have corrected it as suggested.

7. L278-300: Are the described substitutions actually associated with escape from antibody-mediated neutralization? This information should be clarified. If no such association has been reported, the possibility should at least be discussed. We were unable to find references to the specific high-frequency mutations found in our sequencing data in the existing literature; however, the three non-synonymous mutations we identified from the m114-treated group occur within the receptor binding domain (RBD) of GP1, which is the region targeted by mAb114. We have added text to both the "Variant analysis" section of the Results as well as the Discussion specifying this.

8. L425-436: If the authors have any information on differences in viral genome persistence between the treatments, it would be interesting to include it. We have discussed the presence of EBOV vRNA in tissues from survivors in the Results section (under the "Quantification of viral burden in blood and tissues by RT-qPCR and plaque titration" subheading); as noted there, vRNA abundance was relatively consistent in survivors across treatment groups, though the small number of survivors from each group prevented statistical analysis.

Reviewer 3:

Major concerns:

1) The manuscript states that "Remdesivir, mAb114, REGN-EB3, and ZMapp equally and only partially rescue nonhuman primates." However, this is impossible to prove with 4-5 animals per group. For example, 50% of animals survived with CHO-derived ZMapp, but only 25% survived with plant-derived ZMapp. This could be a very interesting finding, but it is not statistically

significant since there are only 4 animals per group. It is likely that differences between mA114 or REGN-EB3 and remdesivir or ZMapp in humans would not have been found with a low number of treated human cases. In fact, the very fact that there were observed differences between the human studies and the animal data reported in this manuscript highlights the difficulty in concluding these treatments are equally effective, as this conflicts with data with hundreds of human cases. While it is completely understandable that not more animals per group were challenged due to the excessive cost and difficulty in obtaining macaques, and while the authors briefly discuss the low number of animals in the discussion, the data do not support the stated conclusion of the manuscript. We understand and respect the Reviewer's concerns. We have softened the wording of the title to **"Remdesivir, mAb114, REGN-EB3, and ZMapp partially rescue nonhuman primates infected with a low passage Kikwit variant of Ebola virus"**. We also strongly emphasize that this manuscript was not intended to invalidate the results of the PALM trial, nor the previous studies assessing each therapeutic individually in NHPs. While we discuss certain potential limitations or confounding factors in the PALM trial, such as the increased viral burden and delayed time to treatment initiation in the ZMapp and remdesivir treated groups (which are mentioned in that paper by the authors themselves), we make no claims that the present study in NHPs somehow improves upon a human clinical trial conducted during a natural outbreak. Rather, we demonstrate that all four treatments demonstrate statistically significant, although incomplete ("suboptimal") protection in NHPs compared to untreated positive control animals. In the PALM trial, there was no untreated control arm, and all comparisons were made to ZMapp. Our results do contrast with those of previous NHP studies assessing these therapeutics individually, all of which showed complete or near complete protection against EBOV. As we mention in the manuscript, this may be due to a number of factors, not limited to differences in the virus isolate, passage history, NHP species/origin, 7U/8U content, and scoring/euthanasia criteria. The overall purpose of this study was to assess each of these four treatments with these variables reduced or eliminated. Therefore, we stand by the overall conclusions of the study.

2) The data combine 3 separate experiments, which lessens the direct comparison between the treatments. We respectfully disagree with the Reviewer on this point. As the Reviewer is likely aware, NHP experiments in ABSL-4 conditions are constrained by a multitude of factors, including cost, space, and staffing limitations, which can impose difficulties in conducting a single study of this size. As we mention in the manuscript, one of the goals of this study was to assess these therapeutics with as few variables as possible, which we describe and believe we have accomplished. The actual inoculum dose poses the biggest challenge to replicate independently across experiments; however, as we state in the Methods section of the manuscript, while the target dosage of virus was 1000 PFU i.m. for each study, the actual dosages were back-titered as 1075 PFU (for the ZMapp study), 888 PFU (for the remdesivir and REGN-EB3 studies), and 1163 PFU (for the mAb114 study), differences that ours and other labs have consistently demonstrated do not impact disease progression, outcome, or clinical pathology. Thus, while we can agree with the Reviewer that conducting these studies separately may be less than ideal, we do not feel it lessens the comparisons made or the conclusions of the study.

3) Animals received 50 mg/kg of the ZMapp treatments, but 150 mg/kg of REGN-EB3 (50 mg/kg of each of the 3 mAbs in the cocktail). The mAb114 treated animals received 50 mg/kg of this mAb. It is not stated in the manuscript why these doses were chosen, but it can be assumed these were chosen since it is the dose used in the PALM trial. However, in effect, you have 50 mg/kg of

each of 3 mAbs in REGN-EB3, 50 mg/kg of the single mAb in mAb114, and 16.67 mg/kg of each of the mAbs in the ZMapp treatment regimen. This could confound the direct comparison analysis. The dosages used in the current study were chosen based on dosages used in previous NHP studies assessing the efficacy of these therapeutics individually (all of which are cited in the manuscript), which for the three mAb/mAb-cocktails were also congruent with the dosages used in the PALM trial. We have added text in the Methods section under the "Nonhuman primate challenge and treatment" subheader explaining this. However, we must re-emphasize that this study was not intended as an exact duplication of the PALM trial in NHPs (which would not be possible), but rather, an assessment of the therapeutic efficacy of each treatment in NHPs under identical or near-identical test conditions. While the reviewer is correct in that the total dosage of mAbs differs between the mAb cocktails (ZMapp and REGN-EB3) and the individual mAb (mAb114), these are the dosages which have been previously assessed as both safely tolerated and efficacious in preclinical and clinical trials. Moreover, the Reviewer's critique regarding the comparison of therapeutic efficacy between mAbs with different dosage formulations as applied here could also be applied to the PALM trial itself. In short, the dosages chosen here were done so with the intent to be consistent with the existing literature involving these therapies.

4) The remdesivir dose (10 mg/kg loading dose, 5 mg/kg daily maintenance dose) appears to be much high than the PALM trial (200 mg total loading dose (roughly 2.8 mg/kg) and 100 mg total daily maintenance dose (~1.4 mg/kg). As stated above, the dosages for each therapeutic in the current study were primarily informed by the dosages used in previous preclinical studies in NHPs, which for the three mAb/mAb-cocktails, were also those used in the PALM trial. Again, we must re-iterate that an exact duplication of the PALM trial in NHPs was not the intent of these studies.

5) Figure 2C and 2D show that animals that survived infection had lower viremias at the time of treatment onset then animals that died. Therefore, this clouds the interpretation of the data in comparing efficacy of the different treatments. The Reviewer is correct in that we found an association (and discuss this within the manuscript) between individual animals within cohorts that had lower viremia and survival following treatment. However, the geometric mean titer (assessed by both RT-qPCR and plaque titration) for each cohort did not differ significantly, which is the criteria used when comparing cohorts to one another. The association between lower viral load and serum biochemical markers of disease (e.g., creatinine, ALT, AST) and survival was also observed during the PALM trial, irrespective of treatment.

6) Figure 4, there is no information on what time points the mutations were found and if the mutations were found in the animals that succumbed to infection or those that survived. We have added information in the "Variant analysis" section of the Results that mentions which animals these mutations were found; as they were all from animals that succumbed to disease and were sequenced from tissues, they are from the terminal timepoint for each animal.

7) There is no discussion of if the mutations in Figure 4 mapped to known binding sites of the mAb cocktails. We were unable to find references to the specific high-frequency mutations found in our sequencing data in the existing literature; however, the three non-synonymous mutations we identified from the m114-treated group occur within the receptor binding domain (RBD) of GP1, which is the region targeted by mAb114. We have added text to both the "Variant analysis" section of the Results as well as the Discussion specifying this.

8) There should be statistical analysis conducted on the hematology and serum biochemistry data. We have included data as a Supplementary Figure comparing the 5 DPI (day of treatment initiation) fold change (compared to baseline) values of serum ALT, AST, and creatinine (CRE), which are known markers of filovirus disease in humans and NHPs, with additional text detailing this analysis in the Results and Discussion sections of the revised manuscript.

Minor concerns

1) Lines 63-66 state "A separate trial conducted toward the end of the epidemic demonstrated that ZMapp, a cocktail of three EBOV glycoprotein (GP)-specific mAbs19 64 , was 91.2% more effective in preventing Ebola virus disease (EVD) mortality than standard supportive care alone, although this fell short of the pre-specified study goal of 97.5%." However, this is incorrect, as the referenced paper stated "The observed posterior probability that ZMapp plus the current standard of care was superior to the current standard of care alone was 91.2%, falling short of the prespecified threshold of 97.5%." Therefore, the % listed in paper deals with probability, not effectiveness. This should be revised. We thank the Reviewer for catching this error. The statement has been revised to read "…, was 91.2% more likely to have prevented Ebola virus disease (EVD)-associated mortality than standard supportive care alone…".

2) The lack of difference in viral RNA load in the eyes between the treatment and control groups is intriguing, as is the CNS persistence in surviving animals. This should be expanded on in the discussion as it relates to long-term disease and/or viral persistence in human survivors. We have added text to the Discussion regarding this important point as suggested by the Reviewer.

3) There should be a paragraph summarizing the extensive results on pathology on the tissues from the different treatment groups—are there any significant differences between the groups? We have added text to the end of the "Gross lesions and histopathology" section briefly summarizing the findings as suggested. As described, animals that survived infection lacked gross lesions at the study endpoint (with the exception of mild lymphadenomegaly in a few subjects), while animals which succumbed displayed gross lesions consistent with EVD. However, given the low number of animals from each treatment group and clinical outcome, it is not possible to make statistical comparisons.

4) Lines 414-418, the authors compare survival rates of their mAb-or remdesivir-treated animals with that of humans treated similarly, but this is not a valid comparison, given the 100% lethality of the untreated animals compared with the much lower lethality rate in the human study. We believe the Reviewer is mistaken here. While our study design compared the efficacy of each therapeutic to untreated control animals, whereas the PALM trial made comparisons of remdesivir, REGN-EB3, and mAb114 to ZMapp, the statement the Reviewer is referring to compares the similar therapeutic efficacy for between the treatment cohorts in our study to human subjects in the PALM trial; e.g., the proportion of surviving animals in each treatment cohort in our study, which was designed to model treatment at an advanced stage of disease, was congruent with the proportion of patients with advanced disease (as defined in the PALM study) surviving EBOV infection following treatment with mAb114 or REGN-EB3; e.g., ~ 40%. Moreover, this is a generalized statement with no claims of statistical significance (as no such comparison was made)

that descriptively compares the similarity of results of our study in NHPs to that of a clinical trial in humans.

Additional changes to the manuscript:

1) In the "Variant analysis" section of the Results, we reported a high frequency mutation (C7669T : Thr544Ile, referred to erroneously in the original manuscript as Thr203Ile). This mutation was reported in error as it was present in the challenge inoculum at a similar frequency as in tissue it was recovered from. We have removed this mutation from the text of the "Variant analysis" section of the Results in the revised manuscript as well as from Figure 4. The removal of this data from the manuscript does not impact the conclusions of the studies.