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CONTRACTING ORGANIZATION: University of New Mexico Health Sciences Center

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The overall research idea of this proposal is to ascertain the feasibility of providing short-term protection against Ebola virus infection while co-administering Ebola virus vaccines to provide long-term protection against disease. Ebola virus vaccine candidates (a live replicating vesicular stomatitis virus (VSV) expressing Ebola-GP and a replication-deficient adenovirus (AdV) expressing Ebola GP) have been used in humans. For therapeutic purposes, monoclonal antibodies (ZMapp, mAb114) and antiviral small molecules (Remdesivir) are also being tested in human infections. However, neither antibody therapy nor small molecule inhibitors of Ebola virus replication will protect against infection long-term, and vaccines do not provide immediate protection. To that end, this proposal will test multiple strategies to combine therapeutic drugs with vaccines to generate rapid short-term as well as long-term protection against Ebola virus. In the first and second year we found that administration of neutralizing antibodies had an effect on the short-term immunogenicity of both VSV and AdV vaccines and that VSV elicited substantially higher IgG titers than AdV vaccine. In year three, we found there was no significant impact on antibodies elicited by VSV vaccine after treatment with the antiviral small molecule remdesivir. In the no-cost extension, we attempted to conduct BSL-4 experiments with live EBOV challenge in mice but were unable to acquire these services despite submitted paperwork to two different facilities.

**15. SUBJECT TERMS**

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**REPORT OUTLINE**

**1. INTRODUCTION:** Ebola virus causes severe disease and often death in human infections. Recent large outbreaks have demonstrated that the virus is a serious emerging threat to human health. The overall research idea of this proposal is to ascertain the feasibility of providing short-term protection against Ebola virus infection while co-administering Ebola virus vaccines to provide long-term protection against disease. Two Ebola virus vaccine candidates, a live vesicular stomatitis virus expressing Ebola glycoprotein (VSV-GP) and a single-cycle adenovirus vector expressing Ebola glycoprotein (AdV-GP) have been extensively tested in humans, and the VSV-GP has been used as a ring vaccine in Ebola virus outbreaks. The use of monoclonal antibodies has been very promising in nonhuman primate studies and have been used experimentally in human infections, as have the small molecules Favipiravir (T-705) and Remdesivir (GS-5734). However, neither antibody therapy nor small molecule inhibitors of Ebola virus replication will protect against infection long-term, and vaccines do not provide immediate protection. What is needed for military personnel and health care workers responding to filovirus outbreaks is a regimen to protect in the short-term against infection, and in the long-term as well. To that end, this proposal will test multiple strategies to combine therapeutic drugs with vaccines to generate rapid short-term as well as long-term protection against Ebola virus. Specific Aim 1 was to test the effects of time of administration of Ebola virus neutralizing antibody cocktails on immunogenicity of advanced Ebola virus vaccines, and to test how these regiments affect the neutralizing antibody cocktail levels. Specific Aim 2 was to determine whether functional non-neutralizing anti-GP antibodies, and selective anti-sGP antibodies, affect vaccine immunogenicity. Specific Aim 3 was designed to compare the effects of antiviral small molecule administration on vaccination.

**2. KEYWORDS:** Ebola, virus, vaccination, vaccine, filovirus, antibody, monoclonal, therapeutics, drugs, Remdesivir, Favipiravir, ZMAPP, mAb114, glycoprotein, MIL77, GP.

## 3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

  - Major Task 1: Test the effects of time of administration of Ebola virus neutralizing antibody cocktails on immunogenicity of advanced Ebola virus vaccines

    - Subtask 1: Local IRB/IACUC Approval
    - Subtask 2: Determine the effects of timing of passive neutralizing antibody administration on vaccine immunogenicity
    - Subtask 3: Determine the effects of vaccine administration on longevity of passively-administered neutralizing antibody in vivo
    - Subtask 4: Assessment of protection against live virus challenge
    - Milestone 1: HRPO/ACURO Approval
    - Milestone 2: Establishment of optimal regiment for administering Ebola vaccines and neutralizing antibodies

  - Major Task 2: Determine whether functional non-neutralizing anti-GP1,2 antibodies, and selective anti-sGP antibodies, affect vaccine immunogenicity.

    - Subtask 1: Determine effects of protective non-neutralizing antibody administration on vaccine immunogenicity
    - Subtask 2: Determine the effects of anti-sGP antibody administration on vaccine immunogenicity
    - Subtask 3: Assessment of protection against live virus challenge
    - Milestone 1: Establishment of optimal regimen for administering Ebola vaccines and non-neutralizing antibodies

  - Major Task 3: Determine whether antivirals effective against Ebola virus affect vaccine immunogenicity.

    - Subtask 1: Determine effects of Remdesivir on vaccine immunogencity
    - Subtask 2: Determine the effects of Favipiravir on vaccine immunogenicity
    - Subtask 3: Assessment of protection against live virus challenge
    - Milestone 1: Establishment of optimal regimen for administering Ebola vaccines and antivirals

- **What was accomplished under these goals?**

### a) Major activities

*Acquisition of Ebola virus neutralizing monoclonal antibodies.* The goal of this aim was to test how Ebola virus neutralizing antibodies affected immunogenicity of two Ebola virus vaccines. The antibodies to be tested were the monoclonal antibody mAb114 (1) and the 3-antibody cocktail ZMAPP (2), both of which have been used in human trials. However, we were unable to acquire ZMAPP, as it was unavailable to researchers since it is being funded for human clinical trials. Therefore, we purchased MIL77 from MAPP Biopharmaceuticals.

MIL77 contains three antibodies with the same variable regions of those in ZMAPP and are manufactured in modified Chinese hamster ovary (CHO) cells. This cocktail is therefore very comparable to ZMAPP and was used in the below experiments. Similarly, we were not able to acquire mAb114, as it was being tested in clinical trials. To bypass this issue, we contracted the generation of mAb114 by using the publicly-available sequence of this antibody. mAb114 was successfully generated in ExpiCHO cells and purified at a usable concentration (2.77 mg/mL, 36 mg total antibody), as shown in Figure 1. This antibody was used in the below experiments.

*Acquisition of Ebola virus vaccines.* We acquired the VSV-GP vaccine through the generosity of Dr. Heinz Feldmann and used this for the mouse experiments. However, we were not able to acquire the ChAd3-GP vaccine, as it is being used in clinical trials and we were not given permission to acquire this vaccine. We therefore contracted the generation of a replication-defective AdV5-GP vaccine, since no ChAd3 backbone was commercially available. We screened several AdV serotype 5-GP viruses by western blot and picked a clone that expressed GP at a high level. We received virus at $5 \times 10^{12}$ particles/mL, sufficient for the *in vivo* experiments.

*Assessment of the effects of vaccination on Ebola virus vaccine immunogenicity and the effects of vaccines on monoclonal antibody persistence.* The results of these experiments are described in subsection c below.

*Acquisition of Ebola virus non-neutralizing monoclonal antibodies*. The goal of this aim was to test how Ebola virus functional but non-neutralizing antibodies affected immunogenicity of two Ebola virus vaccines. We used two well characterized non-neutralizing antibodies, 6D8 and 13c6, both of which were a gift from Dr. John Dye at the United States Army Medical Research Institute of Infectious Diseases. These antibodies were used in the below experiments.

*Acquisition of Ebola virus antivirals*. The goal of this aim was to test how Ebola virus vaccines are impacted by concurrent and offset timed treatments with established and potential Ebola antiviral drugs. Remdesivir and was acquired from MedChemExpress, and reconstituted according to manufacturer's instructions. Originally, favipiravir was to be tested; however, clinical trials conducted after the beginning of this project suggested that favipiravir was not protective in humans. Therefore, we did not perform experiments with this drug.

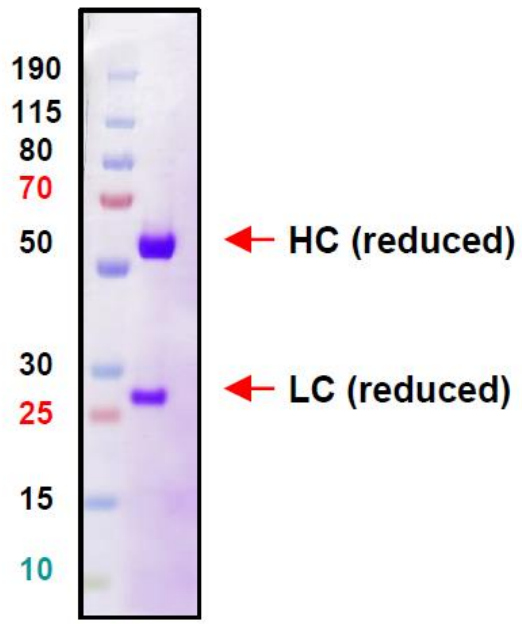
*Assessment of the effects of antiviral treatment on Ebola virus vaccine immunogenicity.* The results of these experiments are described in subsection c below.

**b) Specific objectives**

The specific objectives were:

<u>Major Task 1: Test the effects of time of administration of Ebola virus neutralizing antibody cocktails on immunogenicity of advanced Ebola virus vaccines.</u>

- Subtask 1: Local IRB/IACUC Approval



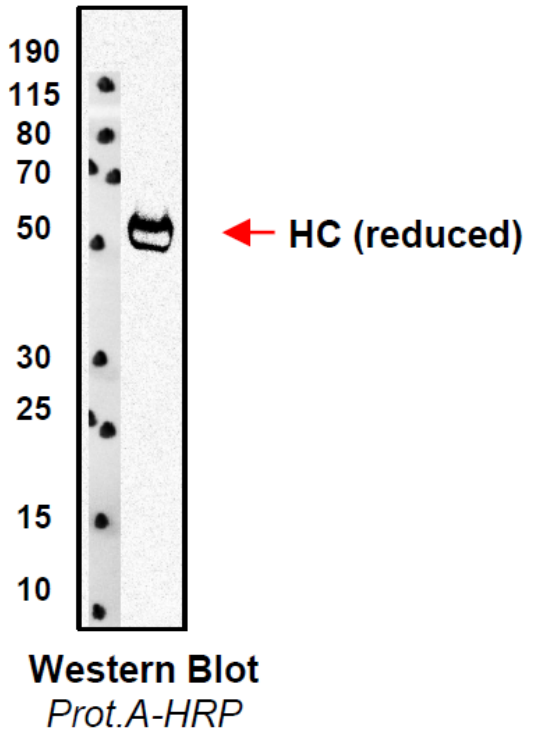




Figure 1. Production of mAb114.

**c) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)**

Major Task 1: Test the effects of time of administration of Ebola virus neutralizing

- Subtask 2: Determine the effects of timing of passive neutralizing antibody administration on vaccine immunogenicity
- Subtask 3: Determine the effects of vaccine administration on longevity of passively-administered neutralizing antibody in vivo
- Subtask 4: Assessment of protection against live virus challenge

Major Task 2: Determine whether functional non-neutralizing anti-GP1,2 antibodies, and selective anti-sGP antibodies, affect vaccine immunogenicity.

Subtask 1: Determine effects of protective non-neutralizing antibody administration on vaccine immunogenicity

Subtask 2: Determine the effects of anti-sGP antibody administration on vaccine immunogenicity

Subtask 3: Assessment of protection against live virus challenge

Major Task 3: Determine whether antivirals effective against Ebola virus affect vaccine immunogenicity.

Subtask 1: Determine effects of Remdesivir on vaccine immunogenicity

Subtask 2: Determine effects of Favipiravir on vaccine immunogenicity

Subtask 3: Assessment of protection against live virus challenge

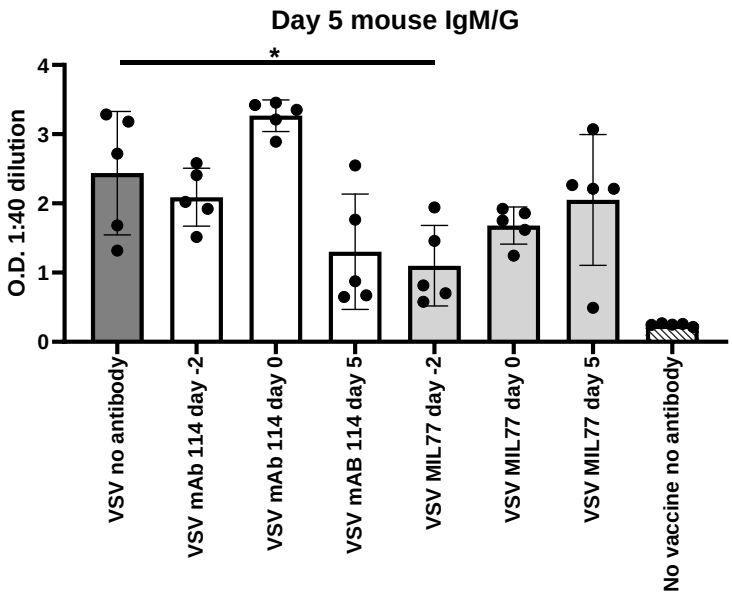



Figure 3. Day 5 mouse IgM and IgG antibody responses after VSV-GP vaccination and mAb114 or MIL77 treatment. *p<0.05

antibody cocktails on immunogenicity of advanced Ebola virus vaccines.

*Subtask 1 (Local IRB/IACUC Approval).* We obtained our local IACUC approval for this project prior to Month 1 (protocol # 19-200880-HSC) entitled “"Effects of passive immunization on immunogenicity of filovirus vaccines in mice.”

*Milestone 1: HRPO/ACURO Approval.* We received ACURO approval in Month 1 of this grant (protocol PR182064.e001 entitled, "Effects of Passive Immunization on Immunogenicity of filovirus vaccines in mice.”)

*Subtask 2: Determine the effects of timing of passive neutralizing antibody administration on vaccine immunogenicity*

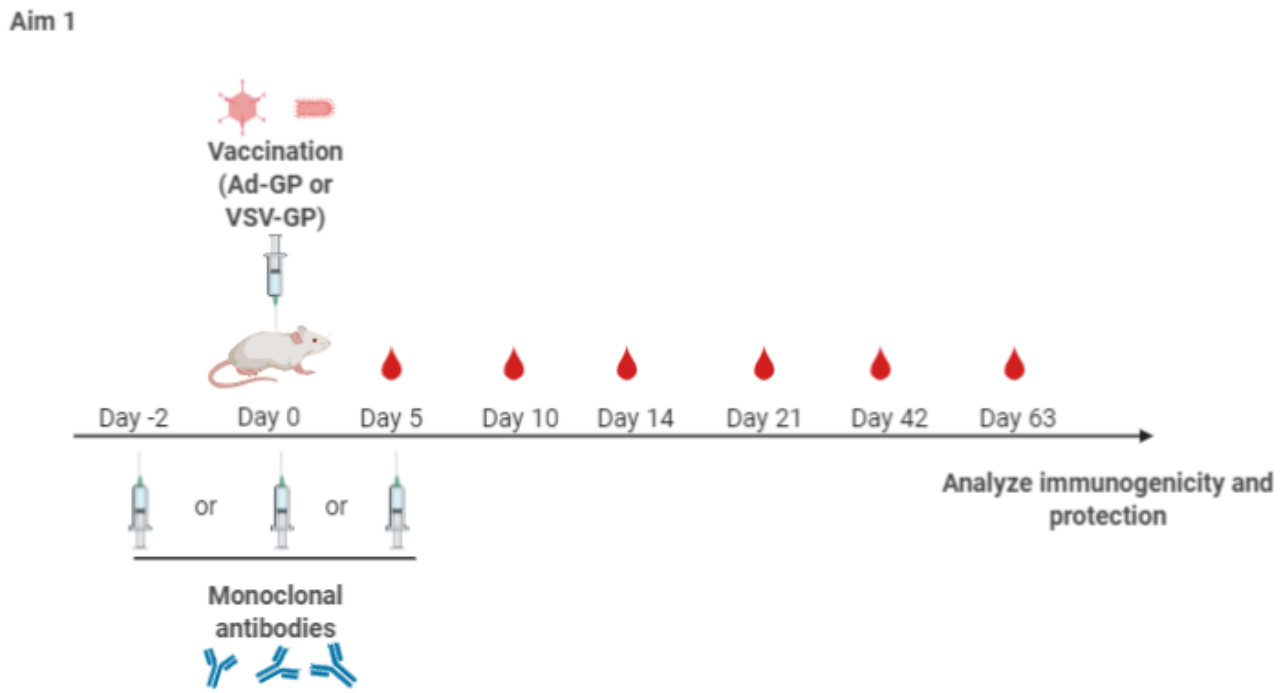



Figure 2. Experimental design for Task 1, Year 1. BALB/c mice (n=5 per group) were vaccinated with AdV-GP or VSV-GP on day 0. On day -2, 0, or 5, mice were injected with 100 ug of MIL77 or mAb114 or left untreated. Mice were bled on days 5, 10, 14, 21, 42, and 63. Mouse antibodies and human antibodies (MIL77 or mAb114) against Ebola virus GP were measured at these time points. Created with BioRender.com

Methods. BALB/c mice (n=5 per group) were vaccinated intramuscularly with either 2 x $10^4$ pfu of VSV-GP, 1 x $10^9$ viral particles of Ad-GP, or mock vaccinated (as a negative control) on day 0. The MIL77 cocktail or mAb114 was injected intravenously with 100 ug of each antibody treatment on days -2, 0, and +5 (as a control, for each vaccine one group was vaccinated but not injected with antibody). Mice were bled via the submandibular route on days 5, 10, 14, 21, and 42 to assay for vaccine-induced antibody responses. ELISAs were conducted on a 1:40 dilution of sera for anti-Ebola GP IgG and IgM mouse antibody for days 5, 10, and 14, and anti-Ebola GP IgG mouse antibody on days 21 and 42 using protocols established by the Bradfute laboratory. Anti-mouse antibodies were cross-adsorbed against human antibodies and vice-versa, allowing us to specifically differentiate between vaccine-induced mouse antibodies and passively-administered human monoclonal antibodies (MIL77 and mAb114). Neutralizing antibody titers were measured against Ebola virus using VSV expressing luciferase and carrying Ebola GP in place of VSV-G. Figure 2 depicts the experimental design.

Statistical analysis. Antibody levels were compared to vaccine-only groups with vaccine+antibody groups using a one-way Anova with Dunnett’s multiple comparisons test. A p value of $<0.05$ was considered to be significant.

Results.

*VSV-GP vaccination*

Mice receiving VSV-GP on day 0 and treated with MIL77 or mAb114 on days -2, 0, or 5 were bled on days 5, 10, 14, 21, and 42. For days 5, 10, and 14, we used an anti-mouse IgM/IgG antibody to detect both IgG and IgM, as IgM has been shown to be a major contributor to VSV-GP-generated functional antibodies (3). As shown in Figure 3, on day 5 there was a mouse anti-Ebola GP response detected by ELISA in the VSV-GP vaccinated mice. Administration of mAb114 on days -2, 0, or 5 did not significantly diminish the mouse anti-GP responses. However, administration of MIL77 on day -2, but not day 0 or day 5, significantly decreased mouse IgM/IgG responses to VSV-GP vaccination.

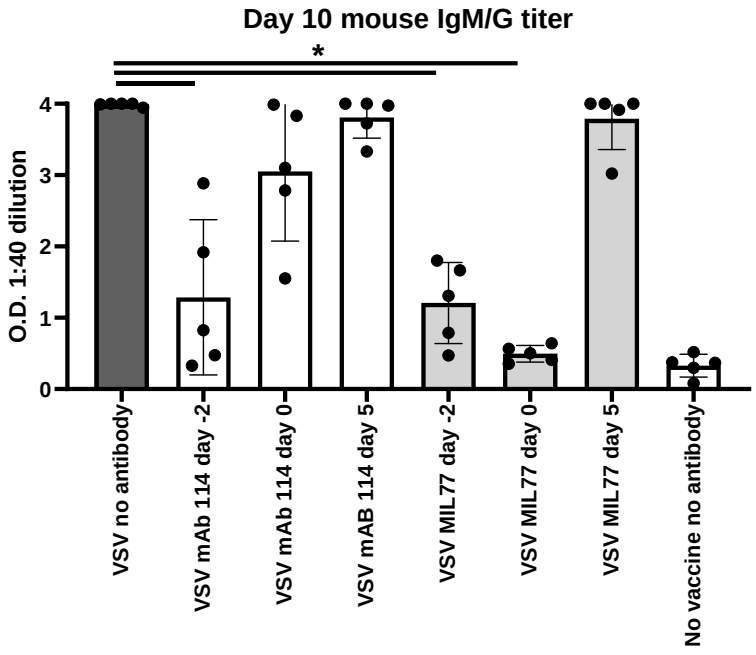



Figure 4. Day 10 mouse IgM and IgG antibody responses after VSV-GP vaccination and mAb114 or MIL77 treatment. *p<0.05

On day 10, mouse anti-GP responses were decreased when mAb114 was given on day -2 or when MIL77 was given on day -2 or day 0 (Figure 4).

On day 14, mouse IgM/G responses against Ebola virus GP was negatively affected when mAb114 was injected on day -2 and when MIL77 was injected on day -2 or day 0 (Figure 5).

On day 21, we utilized an anti-mouse IgG secondary antibody since IgM was likely to be low or undetectable. As shown in Figure 6, mAb114 given on days -2, 0, or 5 reduced mouse vaccine-induced IgG responses, as did MIL77 given on days -2 or 0. However, MIL77 administered on day 5 did not decrease mouse IgG responses against Ebola GP.

Importantly, we did not detect any anti-human IgG reactivity with our anti-mouse antibody, as unvaccinated mice treated with monoclonal human antibodies did not show any signal above background (Figure 6, right side).

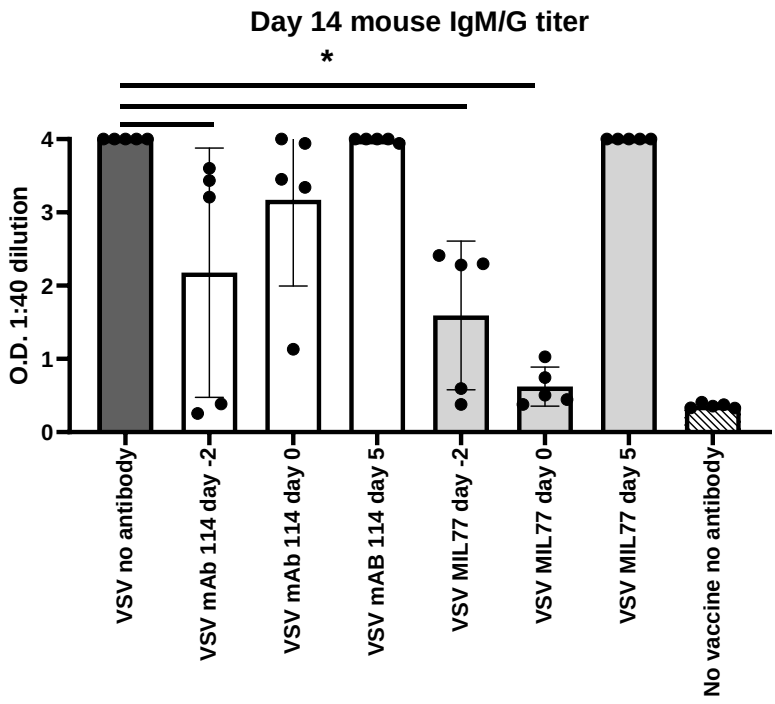



Figure 5. Day 14 mouse IgM and IgG antibody responses after VSV-GP vaccination and mAb114 or MIL77 treatment. *P<0.05

The day 42 analysis revealed no significant difference between mouse anti-Ebola GP IgG responses between the VSV-GP-vaccinated group and the VSV-GP-vaccinated+monoclonal antibody treated groups, although there was a broad range of antibody levels in many of the groups. The day 63 bleeds have occurred but the samples are in the process of being analyzed.

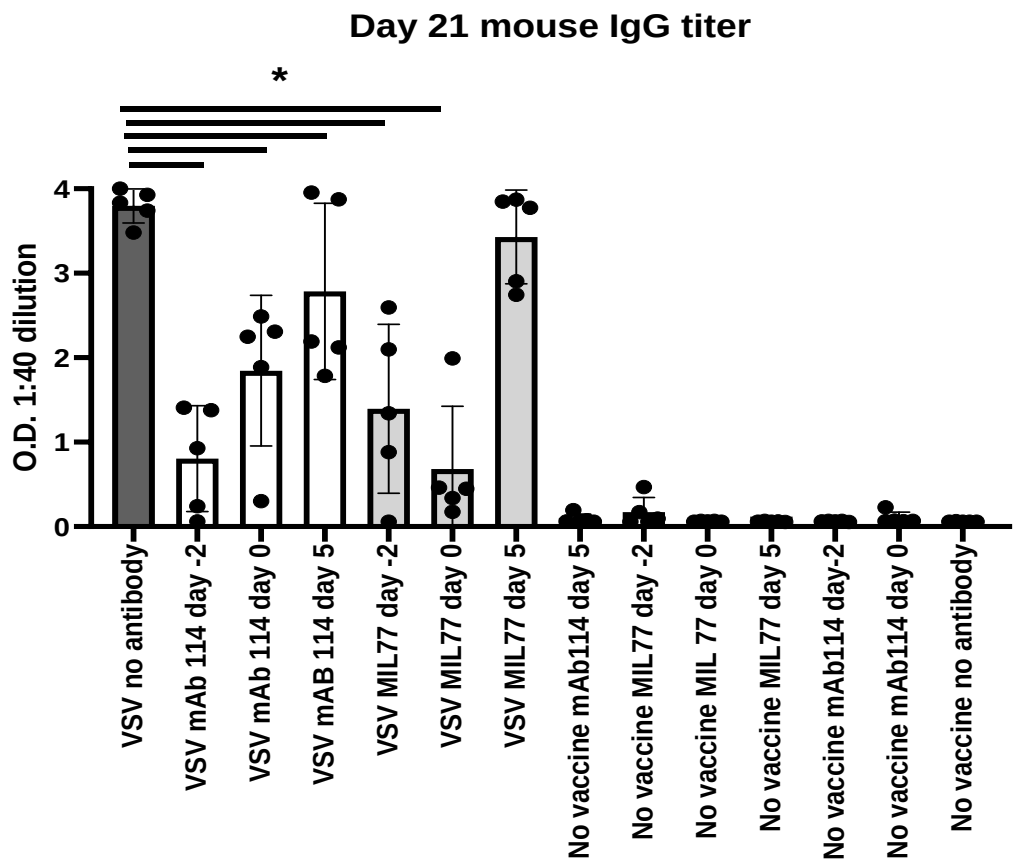



Figure 6. Day 21 mouse IgG antibody responses after VSV-GP vaccination and mAb114 or MIL77 treatment. *p<0.05

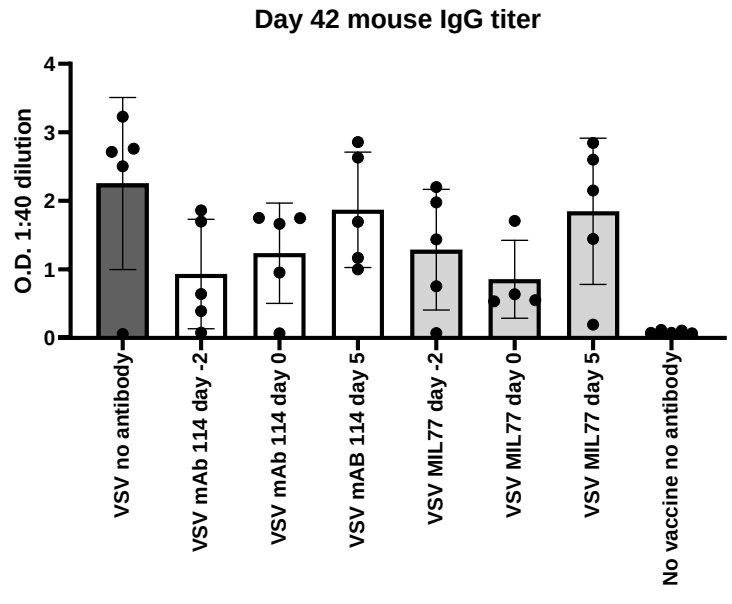



Figure 7. Day 42 mouse IgG antibody responses after VSV-GP vaccination and mAb114 or MIL77 treatment. *P<0.05

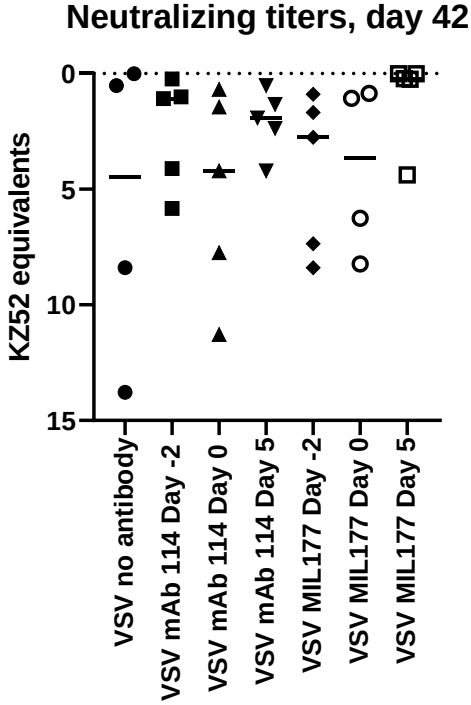



Figure 8. Day 42 mouse IgG antibody responses after VSV-GP vaccination and mAb114 or MIL77 treatment. *p<0.05

Neutralizing antibody titers were performed from mice on day 42. As shown in Figure 8, we did not detect significant differences in neutralizing antibody levels. Although the variation in neutralizing titers is wide in this experiment, the results match the ELISA data as shown above.

*AdV-GP vaccination*

Mice vaccinated with AdV on day 0 were treated with mAb114 or MIL77 on days -2, 0, or 5, or left untreated as listed above.

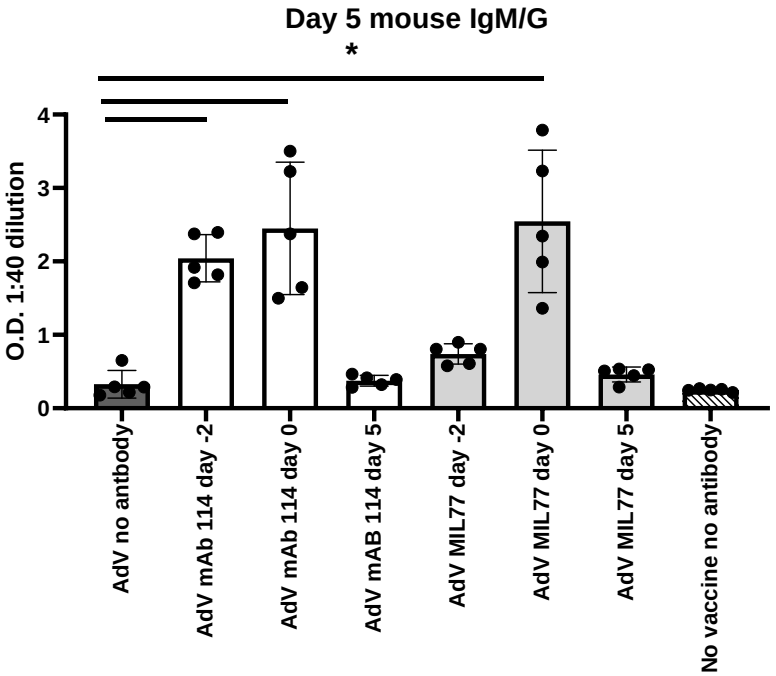



Figure 9. Day 5 mouse IgM and IgG antibody responses after AdV-GP vaccination and mAb114 or MIL77 treatment. *p<0.05

On day 5 (Figure 9), mouse IgM/G against Ebola GP was not significantly higher in the AdV only group compared to unvaccinated mice. Therefore, on day 5 AdV-GP vaccination did not induce IgM or IgG antibodies against Ebola virus GP.

However, on day 5 there was an increase in mouse IgM/IgG in AdV-GP vaccinated mice treated with mAb114 on day -2 and day 0 and in AdV-GP vaccinated mice treated with MIL77 on day 0. These data suggest that human monoclonal antibody administration can actually increase mouse antibody responses in these regimens.

To alleviate concerns that this rise in antibody titer could be due to cross-detection of the anti-mouse IgG/IgM secondary antibody to the human monoclonal antibodies, we compared the rise in mouse antibody levels in AdV groups versus VSV groups (Figures 3 and 9). In the VSV day 5 groups, there was not a significant rise in mouse antibody levels in passively infused groups, confirming that the anti-mouse antibody does not cross-react with human antibody. Similarly, while we were able to detect MIL77 and mAb114 in mice injected on day 5 with the anti-human IgG secondary, we did not detect these antibodies with the anti-mouse IgG secondary (data not shown). Therefore, the data strongly suggest that AdV-GP does not induce mouse anti-GP antibodies on day 5, but injection of MIL77 (day 0) or mAb114 (day -2 or day 0) enhances the immunogenicity of the vaccine at this early time point.

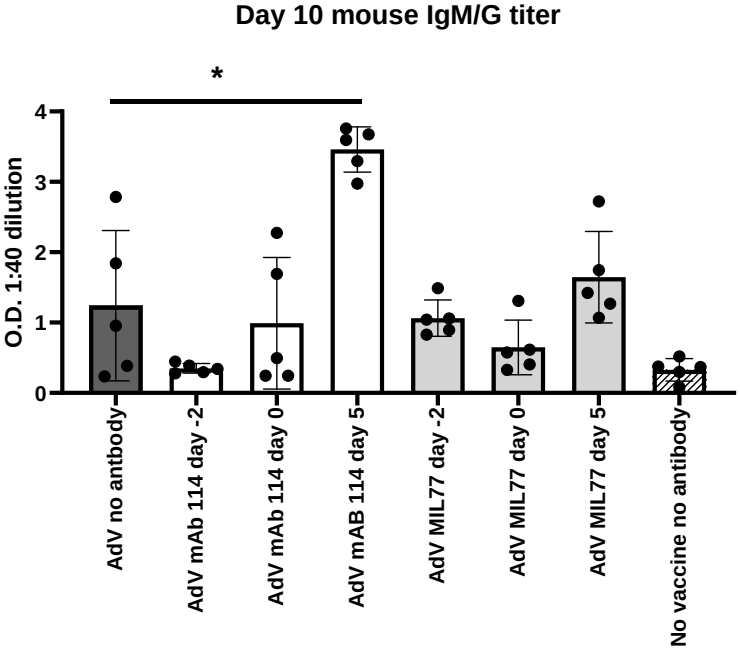



Figure 10. Day 10 mouse IgM and IgG antibody responses after AdV-GP vaccination and mAb114 or MIL77 treatment. *P<0.05

Similarly, we found that on day 10, administration of mAb114 on day 5 increased mouse anti-GP antibody responses in AdV-vaccinated mice compared to vaccination alone (Figure 10). Vaccination alone did not induce significantly higher levels of mouse IgM/G compared to mock-vaccinated groups,

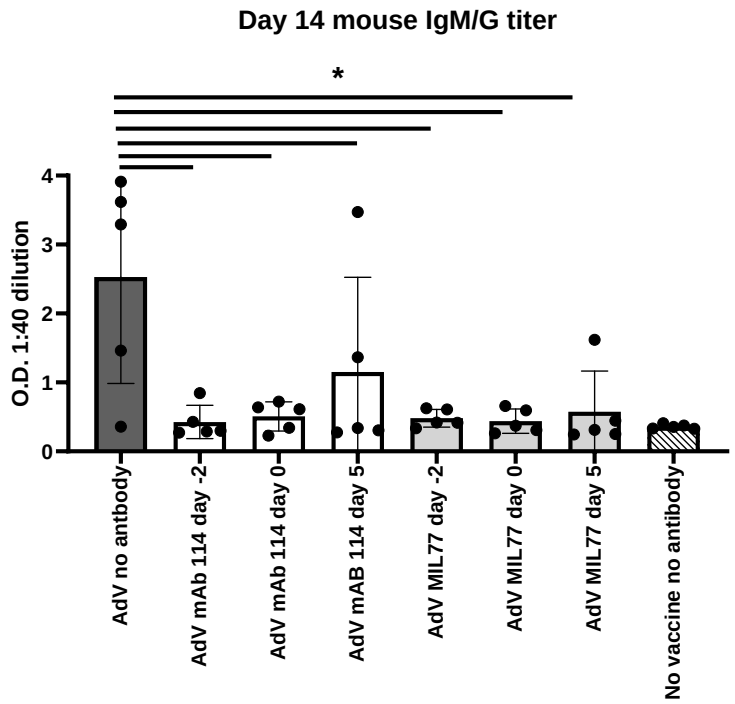



Figure 11. Day 14 mouse IgM and IgG antibody responses after AdV-GP vaccination and mAb114 or MIL77 treatment. *P<0.05

although two or three mice appeared to have responses.

On day 14, however, all MIL77 and mAb114-injected groups had significantly lower mouse antibody responses to Ebola GP, suggesting the antibody treatment abrogated the vaccine-induced immune responses at this time point (Figure 11).

Similar findings were present on day 21 (Figure 12), as the passive administration of MIL77 or mAb114 significantly decreased mouse anti-Ebola GP IgG responses.

On day 42, mice treated with MIL77 or mAb114 on days -2 or 0 had lower levels of mouse IgG against Ebola GP than vaccinated only mice, again suggesting a negative effect on vaccination-induced immunogenicity (Figure 13).

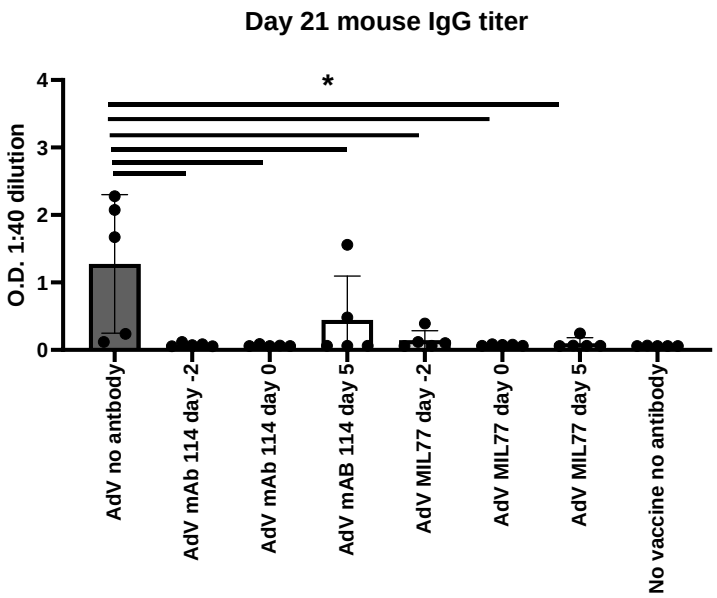



Figure 12. Day 21 mouse IgG antibody responses after AdV-GP vaccination and mAb114 or MIL77 treatment.

Neutralizing antibody titers were performed from mice on day 42. As shown in Figure 14, we did not detect significant differences in neutralizing antibody levels. However, the variation in neutralizing titers is wide in this experiment and additional assays are being conducted.

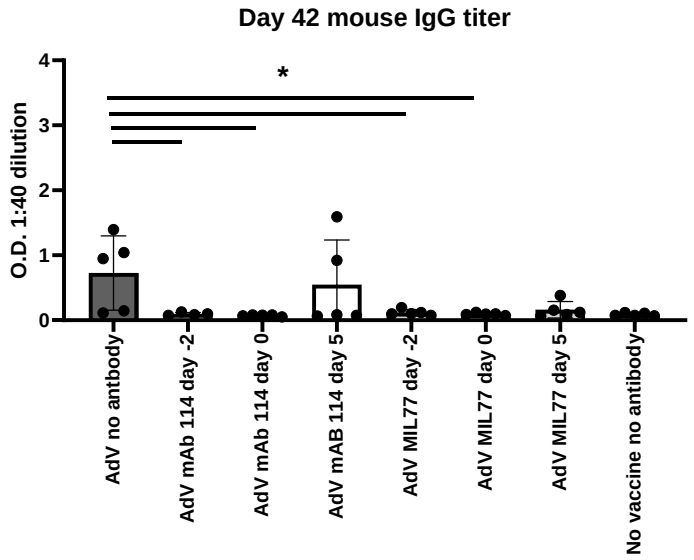



Figure 13. Day 42 mouse IgG antibody responses after AdV-GP vaccination and mAb114 or MIL77 treatment. *P<0.05

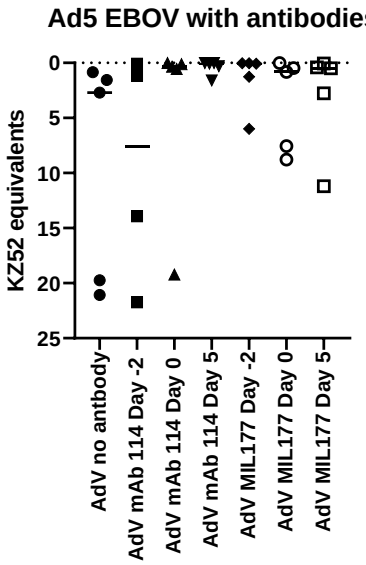



Figure 14. Day 42 mouse IgG antibody responses after VSV-GP vaccination and mAb114 or MIL77 treatment.

| | d-2 mAb114 | D0 mAb114 | D5 mAb114 | D-2 MIL77 | D0 MIL77 | D5 MIL77 |
|---|---|---|---|---|---|---|
| **Day 5** | | | | | | |
| **VSV-GP** | ↔ | ↔ | ↔ | ↓ | ↔ | ↔ |
| **AdV-GP** | ↑ | ↑ | ↔ | ↔ | ↑ | ↔ |
| | | | | | | |
| **Day 10** | | | | | | |
| **VSV-GP** | ↓ | ↔ | ↔ | ↓ | ↓ | ↔ |
| **AdV-GP** | ↔ | ↔ | ↑ | ↔ | ↔ | ↔ |
| | | | | | | |
| **Day 14** | | | | | | |
| **VSV-GP** | ↓ | ↔ | ↔ | ↓ | ↓ | ↔ |
| **AdV-GP** | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ |
| | | | | | | |
| **Day 21** | | | | | | |
| **VSV-GP** | ↓ | ↓ | ↓ | ↓ | ↓ | ↔ |
| **AdV-GP** | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ |
| | | | | | | |
| **Day 42** | | | | | | |
| **VSV-GP** | ↔ | ↔ | ↔ | ↔ | ↔ | ↔ |
| **AdV-GP** | ↓ | ↓ | ↔ | ↓ | ↓ | ↔ |

Table 1. Changes in vaccine-induced mouse anti-Ebola GP antibody responses by ELISA due to human monoclonal nantibody injection.

The results for the vaccine-induced mouse anti-Ebola GP antibody ELISA responses are summarized in Table 1.

*Subtask 3: Determine the effects of vaccine administration on longevity of passively-administered neutralizing antibody in vivo*.

Methods. BALB/c mice (n=5 per group) were vaccinated, treated with antibodies, and bled as above. ELISAs were conducted on sera for the presence of injected human monoclonal antibodies (both MIL77 and mAb114 are human IgG antibodies and can be differentiated from mouse antibody by use of cross-adsorbed secondary antibodies).

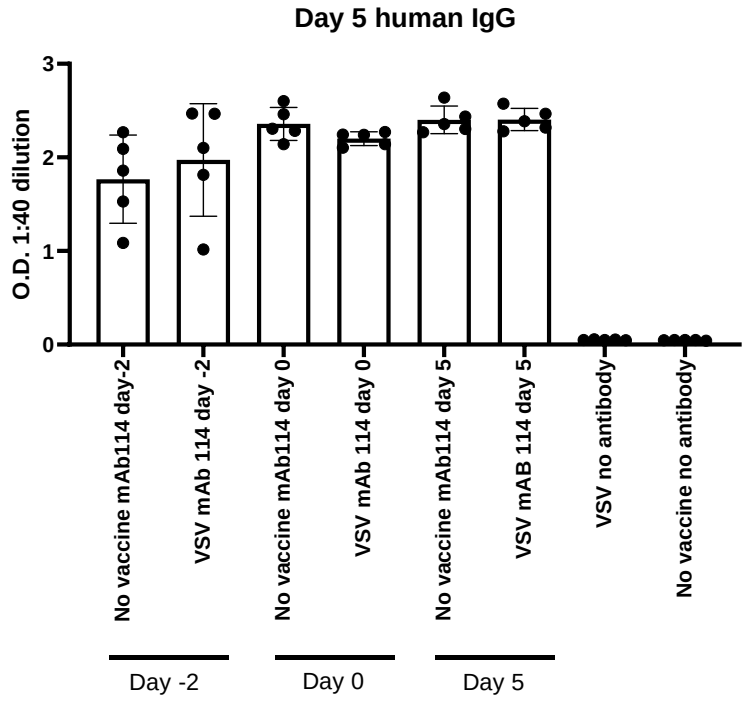



Figure 15. Day 5 human IgG antibody responses after VSV-GP vaccination and mAb114 treatment.

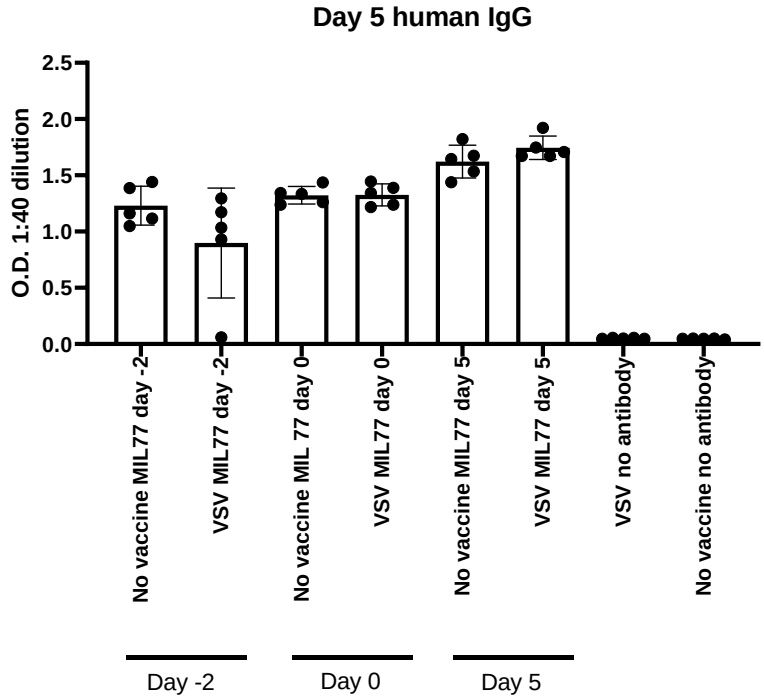



Figure 16. Day 5 human IgG antibody responses after VSV-GP vaccination and MIL77 treatment.

Results.

*VSV-GP*

Mice were vaccinated, treated, and bled as above. The sera were analyzed for the injected MIL77 or mAb114 antibodies by using an anti-human IgG antibody which did not cross-react against mouse antibody. On day 5, there was no significant difference in mAb114 levels when injected on days -2, 0, or 5 in VSV-GP vaccinated versus unvaccinated mice (Figure 15). Similarly, there was no decrease in MIL77 levels in the blood of VSV-GP-vaccinated compared with unvaccinated mice regardless of the day of injection (Figure 16).

On day 10, mAb114 antibodies were low to undetectable in the mice receiving them on day -2 and day 0 in both vaccinated and unvaccinated groups. However, the day 5 treated groups had detectable mAb114 levels, and there were no differences between vaccinated and unvaccinated mice (Figure 17).

Similar results were seen with MIL77-treated animals, with the exception of higher antibody titers in the day -2 no vaccine/MIL77 treated animals compared with the MIL77/AdV-GP group (Figure 18).

MIL77 and mAb114 were not generally detected on days 14 or 21 (data not shown), suggesting these human monoclonal antibodies had been cleared from the mouse serum at these time points.

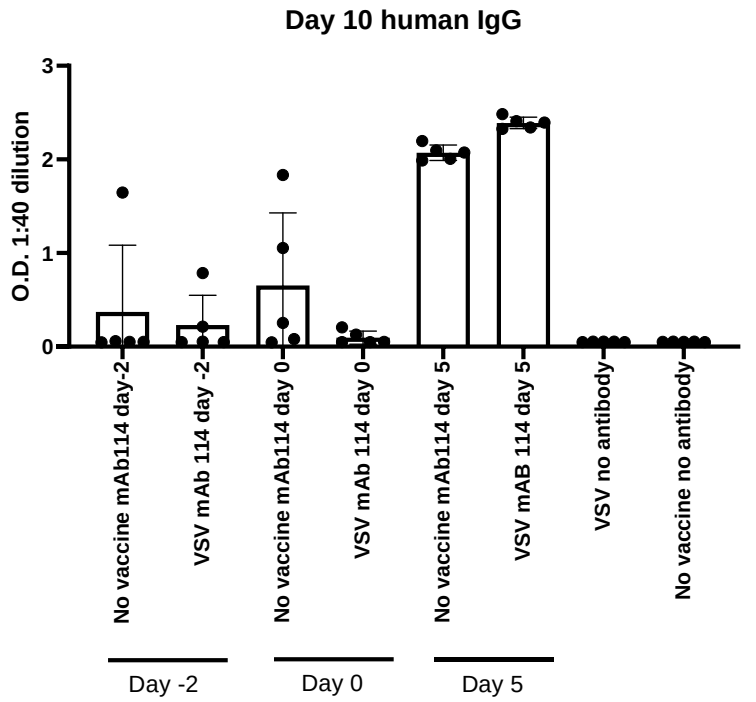



Figure 17. Day 10 human IgG antibody responses after VSV-GP vaccination and mAb114 treatment.

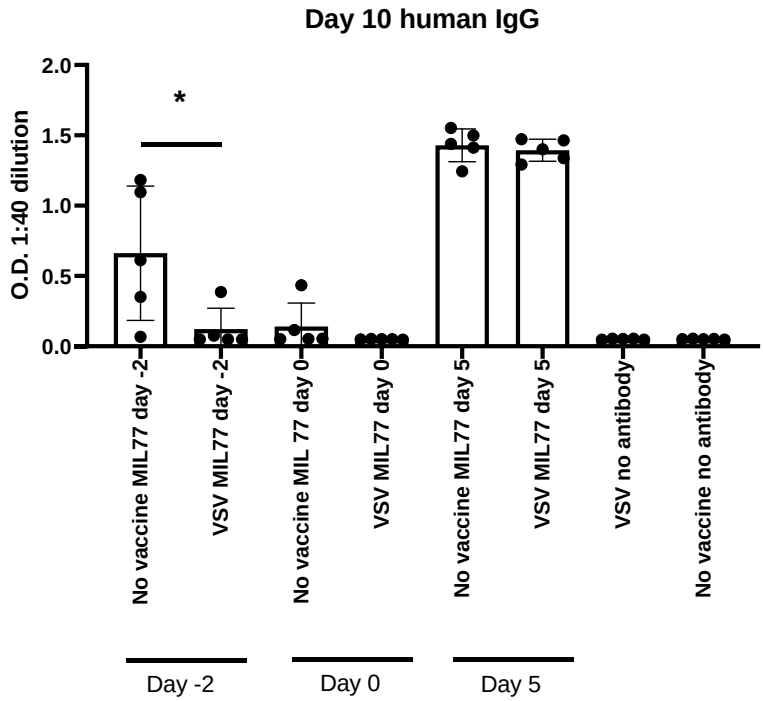



Figure 18. Day 10 human IgG antibody responses after VSV-GP vaccination and MIL77 treatment.

*AdV-GP*

Mice were vaccinated, treated, and bled as above. The sera were analyzed for the injected MIL77 or mAb114 antibodies by using an anti-human IgG antibody which did not cross-react against mouse antibody.

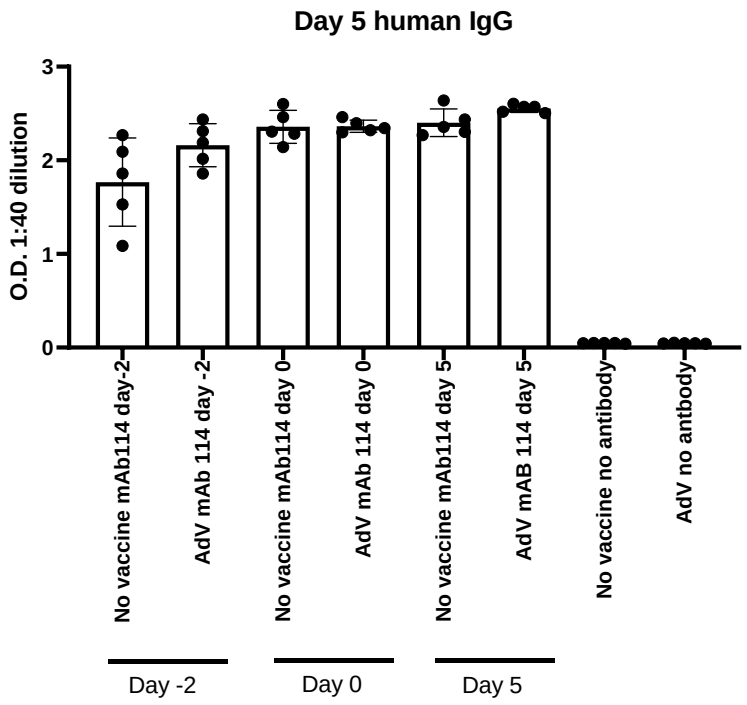



Figure 19. Day 5 human IgG antibody responses after AdV-GP vaccination and mAb114 treatment.

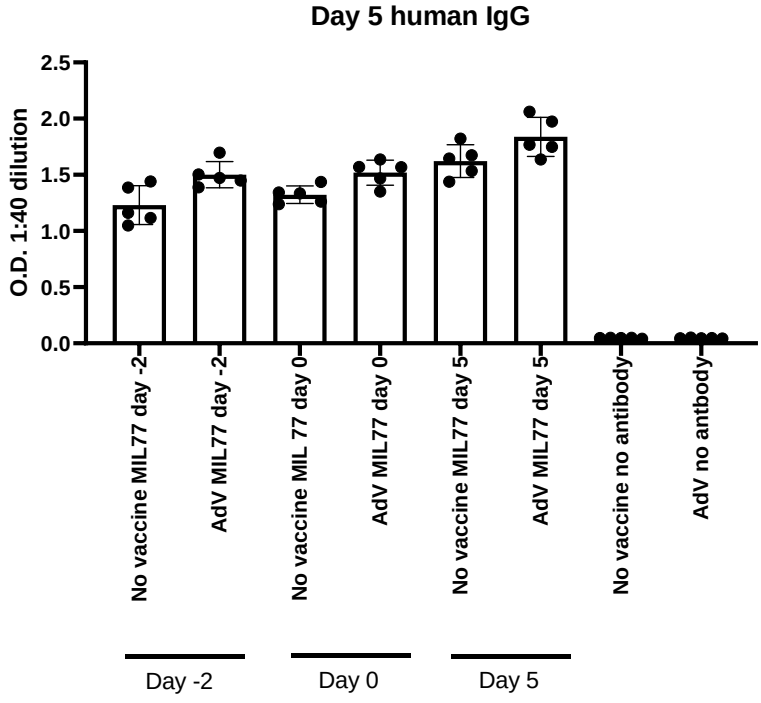



Figure 20. Day 5 human IgG antibody responses after AdV-GP vaccination and MIL77 treatment.

There was no significant difference in mAb114 or MIL77 serum levels between vaccinated and unvaccinated mice on Day 5 (Figures 19 and 20).

Similarly, on Day 10 we did not detect any significant differences between AdV-GP vaccinated and unvaccinated groups in the level of mAb114 or MIL77 (Figures 21 and 22).

MIL77 and mAb114 were not generally detected on days 14 or 21, suggesting these human monoclonal antibodies had been cleared from the serum at these time points.

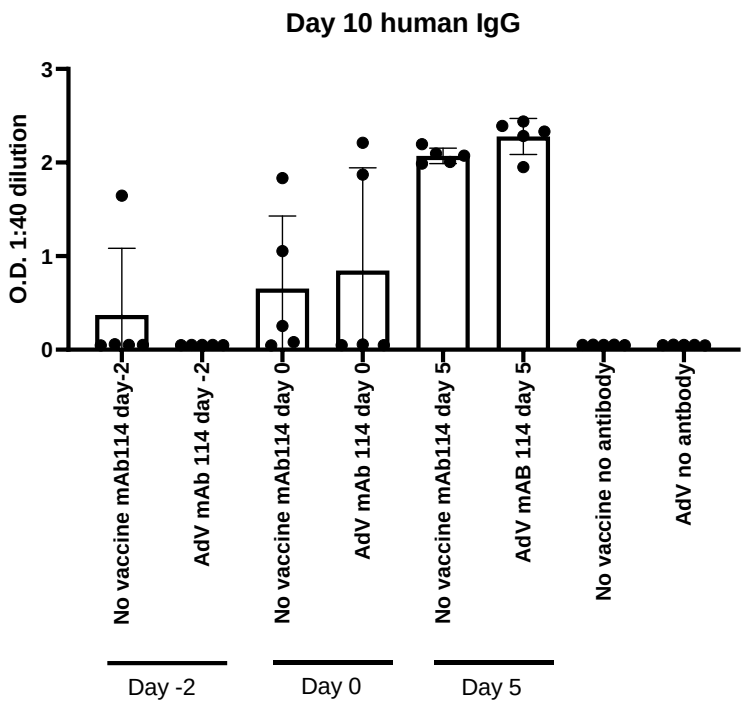



Figure 21. Day 10 human IgG antibody responses after AdV-GP vaccination and mAb114 treatment.

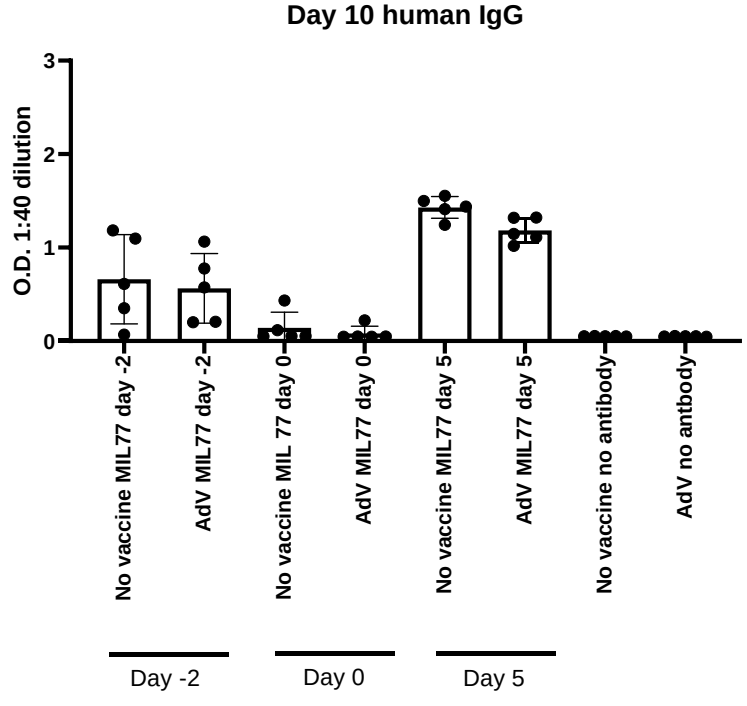



Figure 22. Day 10 human IgG antibody responses after AdV-GP vaccination and MIL77 treatment.

*Subtask 4: Assessment of protection against live virus challenge.* Due to COVID-19, the BSL-4 facility we had planned to do these experiments with (NIH/NIAID/IRF) was not able to conduct these experiments.

Major Task 1 Conclusions (both positive and negative)

1) In AdV-GP-vaccinated mice, passive administration of MIL77 or mAb114 increased mouse antibody responses against Ebola virus GP on day 5 and day 10.

2) In AdV-GP-vaccinated mice, on days 14, 21, and 42, passive administration of MIL77 or mAb114 generally caused a reduction in mouse anti-Ebola GP IgG responses.

3) In VSV-GP vaccinated mice, there was a decrease in mouse anti-Ebola virus GP antibody levels on day 10, 14, and 21 when MIL77 or mAb114 was administered on day -2 or day 0-administered

4) In VSV-GP vaccinated mice, day 5 administered MIL77 or mAb114 antibody does not generally negatively impact mouse antibody responses at any time point.

5) In VSV-GP vaccinated animal groups, day 42 mouse anti-Ebola virus GP ELISA and neutralizing titers are not significantly affected by administration of MIL77 or mAb114.

6) Neither VSV-GP nor AdV-GP vaccination appears to impact the levels of passively-infused MIL77 or mAb114.

Major Task 2: Determine whether functional non-neutralizing anti-GP1,2 antibodies, and selective anti-sGP antibodies, affect vaccine immunogenicity.

*Subtask 1: Determine effects of protective non-neutralizing antibody administration on vaccine immunogenicity*

Methods. BALB/c mice (n=5 per group) were vaccinated intramuscularly with either 2 x 10^4 pfu of VSV-GP, 1 x 10^9 viral particles of Ad-GP, or mock vaccinated (as a negative control) on day 0. The functional non-neutralizing murine antibodies 6D8 and 13c6 were injected intravenously with 100 ug of each antibody treatment on days -2, 0, and +5 (as a control, for each vaccine one group was vaccinated but not injected with antibody). Mice were bled via the submandibular route on days 5, 10, 14, 21, 42, and 63 to assay for vaccine-induced antibody responses. ELISAs were conducted on a 1:40 dilution of sera for anti-Ebola GP IgG and IgM mouse antibody for days 5, 10, 14, 21, 42 and 63 (Figure 2). The 13c6 and 6D8 are both IgG isotype IgG2a, and so levels of IgG recorded both administered murine antibody and induced IgG. IgM antibodies were assayed by ELISA to demonstrate the response solely from the vaccinated mice. ELISAs were performed using protocols established by the Bradfute laboratory.

**Statistical analysis.** Antibody levels were compared to vaccine-only groups with vaccine+antibody groups using a one-way ANOVA with Dunnett's multiple comparisons test. A p value of $<0.05$ was considered to be significant.

**Results.**

*VSV-GP vaccination*

*IgM titers after VSV-GP vaccination.*

Mice receiving VSV-GP on day 0 and treated with 13c6 or 6D8 on days -2, 0, or 5 were bled on days 5, 10, 14 and 21. As IgM has been shown to be a major contributor to VSV-GP-generated functional antibodies (Khurana et al, 2016). ELISAs were performed with the sera to assay IgM levels. The anti-mouse IgM secondary antibody was cross-absorbed to prevent binding to IgG, which should prevent the administered antibodies, which are IgG isotype, from being non-specifically detected by the anti-IgM secondary. To further control for this, the unvaccinated controls were also treated with antibodies at the same times as the vaccinated groups (the unvaccinated groups are listed in the figures as untreated, 6D8 -2, 6D8 0, 6D8 5, 13c6 -2, 13c6 0 and 13c6 5) to show there is no IgM background or non-specific binding resulting from the administration of the antibodies.

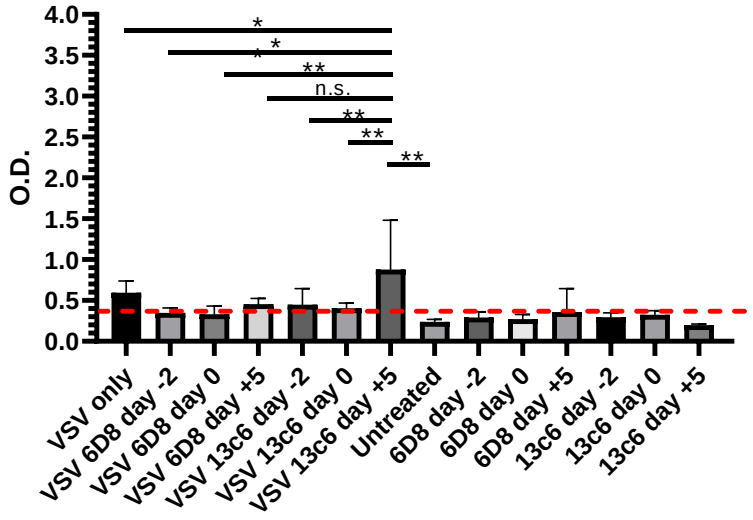



Figure 23**:** Day 5 mouse IgM antibody responses after VSV-GP vaccination and 13c6 or 6D8 treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA, the background IgM binding for this assay.

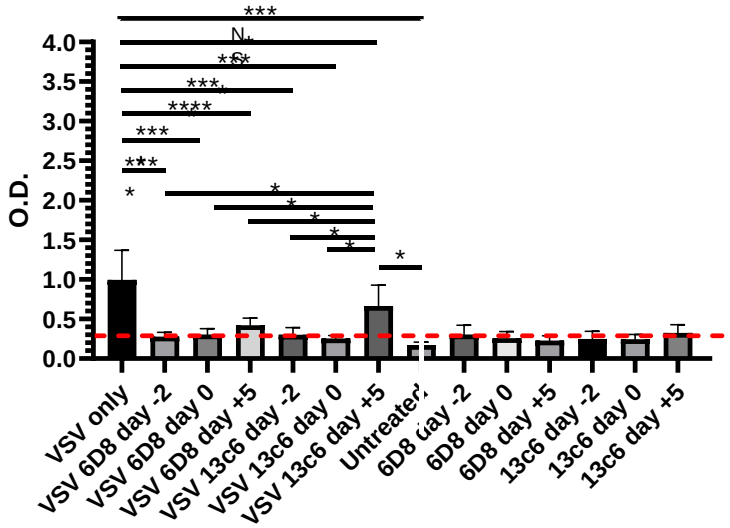



Figure 24: Day 10 mouse IgM antibody responses after VSV-GP vaccination and 13c6 or 6D8 treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA, the background

On day 5 (Figure 23), there was a mouse IgM anti-Ebola GP response detected by ELISA in the VSV-GP vaccinated group, but it was not significantly different compared to the unvaccinated. However, VSV-GP + 13c6 administered on day 5 group (VSV 13c6 d5) induced significant vaccine-elicited IgM compared to untreated/unvaccinated mice and compared to the VSV vaccine only group.

At day 10 (Figure 24), significant IgM titers were present in the VSV-GP vaccine only group, and administration of 13c6 and 6D8 on days -2 and 0 significantly diminished the mouse IgM anti-GP responses. However, administration of 13c6 on day 5 did not significantly diminish the mouse anti-GP IgM response. At day 10, there was a significant difference between the VSV-GP vaccine only and the VSV-GP vaccine with 6D8 antibody administered on day 5, but there was no difference between the VSV-GP vaccine only and the 13c6 antibody administered on day 5, suggesting the antibodies may impact the vaccination differently to one another.

On day 14 (Figure 25), the VSV only and VSV 13c6 d5 groups both had significantly higher IgM titers than the untreated/unvaccinated group, but the VSV vaccine and 13c6 or 6D8 antibodies administered on day -2 or day 0 were not significantly different to the untreated, showing the administration of the 13c6 and 6D8 suppressed the induction of IgM.

By day 21 (Figure 26), IgM titers in the VSV-GP vaccinated mice were not significantly different to untreated/unvaccinated, suggesting IgM titers wane in response to VSV vaccination by day 21. However, the VSV 13c6 d5 group have a significantly increased IgM compared to all other vaccinated groups and also the untreated/unvaccinated. This suggests that the administration of the antibody at day 5 may prolong the IgM titers compared to the vaccine only mice. Since the day 14 IgM titers were higher in the VSV 13c6 group than the VSV only, the higher IgM titers at day 21 could also be the result of higher overall IgM titer induction in the VSV 13c6 d5 group.

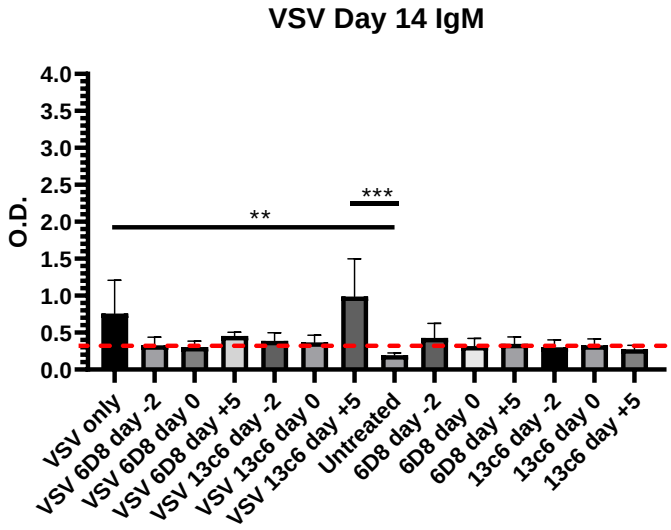



Figure 25: Day 14 mouse IgM antibody responses after VSV-GP vaccination and 13c6 or 6D8 treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA, the background IgM binding for this assay.

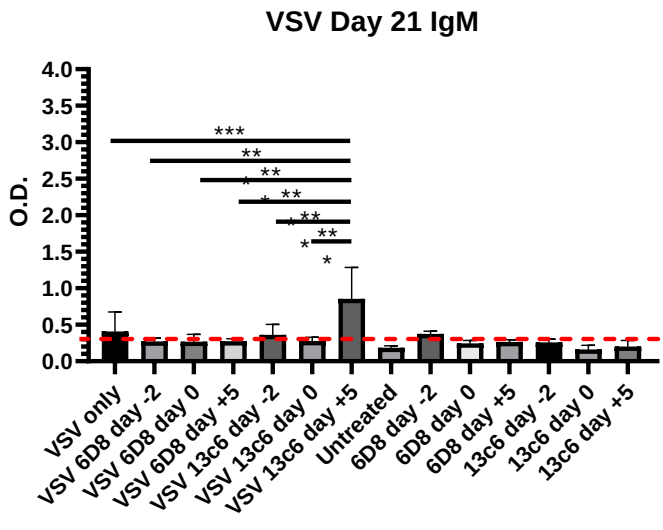


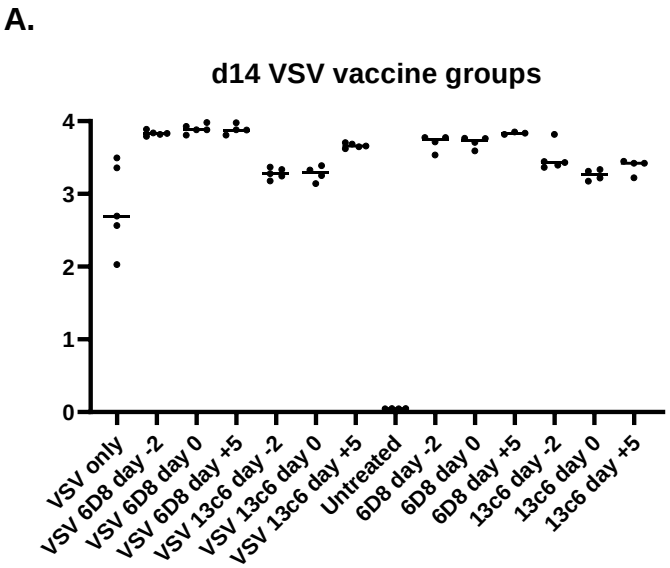

Figure 26: Day 21 mouse IgM antibody responses after VSV-GP vaccination and 13c6 or 6D8 treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA, the background IgM binding for this assay.

*IgG titers after VSV-GP vaccination.*

IgG levels were assayed at day 14, 21, 42 and day 63 after mice received VSV-GP on day 0 and were treated with 13c6 or 6D8 on days -2, 0, or 5. Administration of the antibodies 13c6 and 6D8, both murine IgG2a antibodies, in the absence of vaccination, resulted in very high IgG antibody titers in day 14 and 21, and declining but still significant IgG titers at day 42 and 63. The IgG binding had very little background and the untreated/unvaccinated group had very low background absorbance at all time-points.

At days 14 and 21 (Figure 27), there were very high levels of circulating passively administered 13c6 and 6D8 that prevent differences in any vaccinated groups with the antibodies added from being explored. Figure 27 shows the increase in concentration of IgG for the VSV-GP only group between panel A and B, at day 21 reaching a similar concentration as the circulating administered antibodies.

At day 42 (Figure 28), there are still significant levels of circulating passively administered 13c6 and 6D8, as shown by the significant difference between IgG titers in the untreated/unvaccinated group and the unvaccinated with antibodies administered on day -2, 0 and 5. Figure 28, panel A shows the day 42 VSV IgG titers. However, by day 42, the VSV-GP vaccine only has induced high IgG titers, and we can compare the IgG levels between the induced and passively administered antibody by subtracting the average antibody only group from their equivalent vaccine + antibody group i.e. IgG titer of VSV-GP with 13c6 day 0 minus the IgG titer of 13c6 day 0 only. The results of this are shown in Figure 28, panel B. It is clear from this that the VSV-GP vaccine has induced strong IgG titers, which are significantly greater than the titers from all the vaccine + antibody groups. However, VSV 13c6 d5 has significantly higher titers than the other antibody groups, suggesting that administering the antibody on day 5 still allows for strong induction of IgG responses. By contrast, the antibody levels of VSV 13c6 d-2 and VSV 13c6 d0 are equal to the antibody levels of 13c6 d-2 and 13c6 d0, suggesting the administration of 13c6 prior to or concurrent with vaccination prevents induction of strong IgG titers. This is also true for all timepoints of 6D8, suggesting the two antibodies behave differently, with 6D8 suppressing IgG responses even when administered at day 5 post-vaccination.



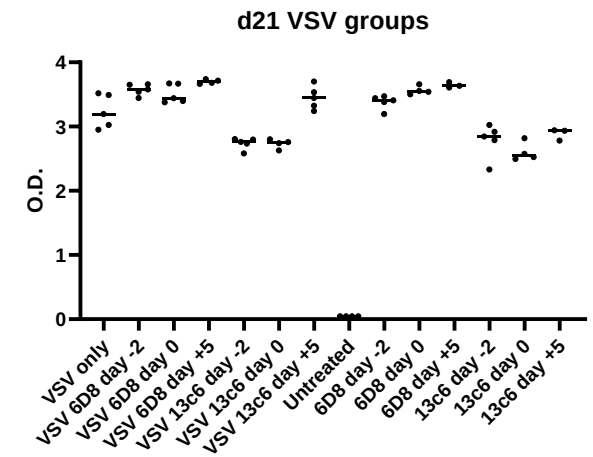
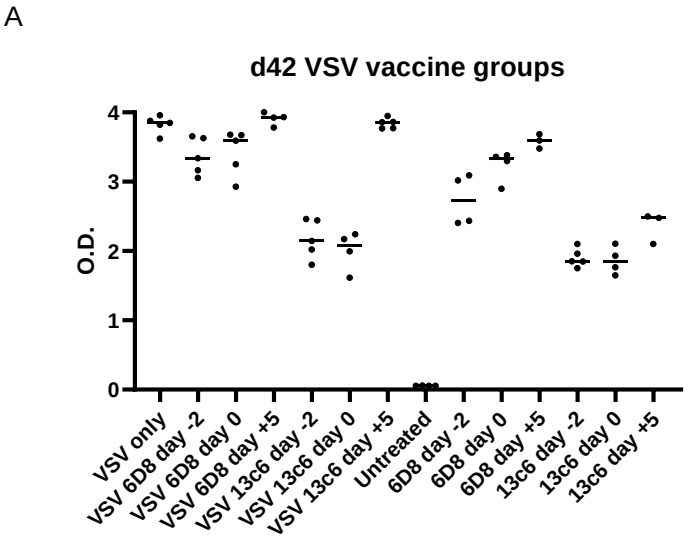




Figure 27: Day 14 (A) and Day 21 (B) mouse IgG antibody responses after VSV-GP vaccination and 13c6 or 6D8 treatment.

At day 63, administration of 13c6 or 6D8 on days -2 or 0 significantly diminished the mouse anti-GP IgG responses. However, administration of 13c6 or 6D8 on day 5 resulted in significantly higher mouse IgG responses to VSV-GP vaccination, comparable to the VSV-GP only group (Figure 29).



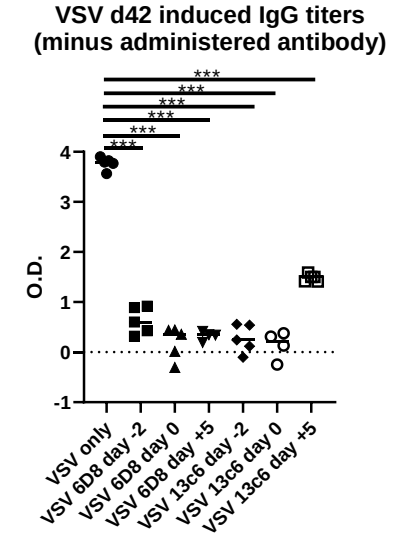




Figure 28. A) Day 42 mouse IgG antibody responses after VSV-GP vaccination and 13c6 or 6D8 treatment. Significant differences in induced IgG between the groups are obscured by high levels of circulating passively administered antibody. B) VSV-GP vaccine group IgG titers minus the average IgG titers from the antibody only groups. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

We intend to investigate IgG titers further by using IgG1a as a proxy for overall induced IgG levels to avoid confusion with the IgG antibodies that were passively transferred, which are IgG2a isotype.

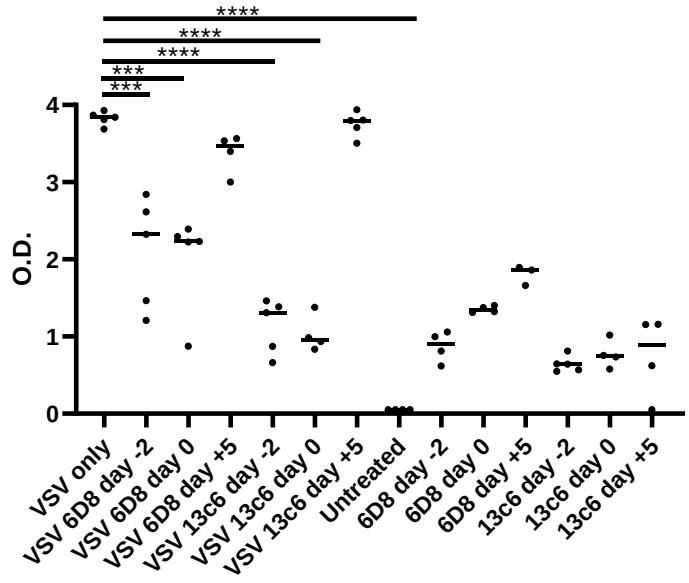



Figure 29: Day 63 mouse IgG antibody responses after VSV-GP vaccination and 13c6 or 6D8 treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

*Ad5-GP vaccination.*

*IgM titers after Ad5-GP vaccination.*

Mice receiving Ad5-GP on day 0 and treated with 13c6 or 6D8 on days -2, 0, or 5 were bled on days 5, 10, 14 and 21. Overall, Ad5-GP was weaker at inducing IgM titers than VSV-GP. On day 5, there was no induction of IgM in any of the Ad5-GP groups with or without the antibody co-administration (Figure 30).

At day 10, significant IgM titers were present in the Ad5 vaccine only group compared to the untreated/unvaccinated group. Administration of 13c6 and 6D8 on days -2 and 5 significantly diminished the mouse IgM anti-GP responses. The Ad5 6D8 d0 is not significantly different from the Ad5 only, but the overall level of induced IgM in both groups is low (Figure 31).

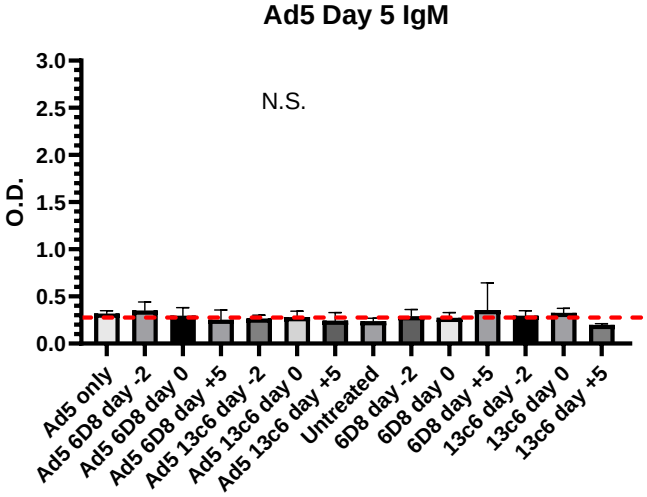



Figure 30: Day 5 mouse IgM antibody responses after Ad5-GP vaccination and 13c6 or 6D8 treatment. N.S – not significant. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA, the background level of binding.

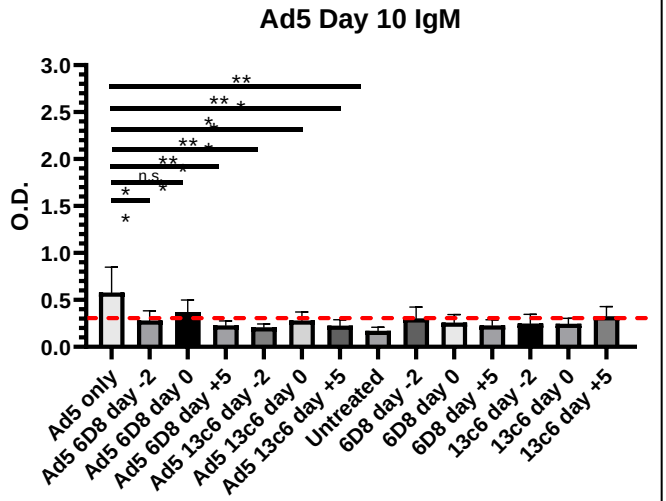



Figure 31: Day 10 mouse IgM antibody responses after Ad5-GP vaccination and 13c6 or 6D8 treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, N.S – not significant. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA, the background level of

By day 14 and day 21, IgM titers in the vaccinated mice were not significantly different to unvaccinated, suggesting IgM titers wane in response to Ad5 vaccination by day 14 (Figure 32). Since several of the VSV vaccinated groups had IgM titers at day 14, this demonstrates the Ad5 vaccine is a weaker inducer of IgM titers.

*IgG titers after Ad5-GP vaccination.*

IgG levels were assayed at day 14 (Figure 33), 21 (Figure 33), 42 (Figure 34), and day 63 (Figure 35) after mice received Ad5-GP on day 0 and treated with 13c6 or 6D8 on days -2, 0, or 5. Administration of the antibodies 13c6 and

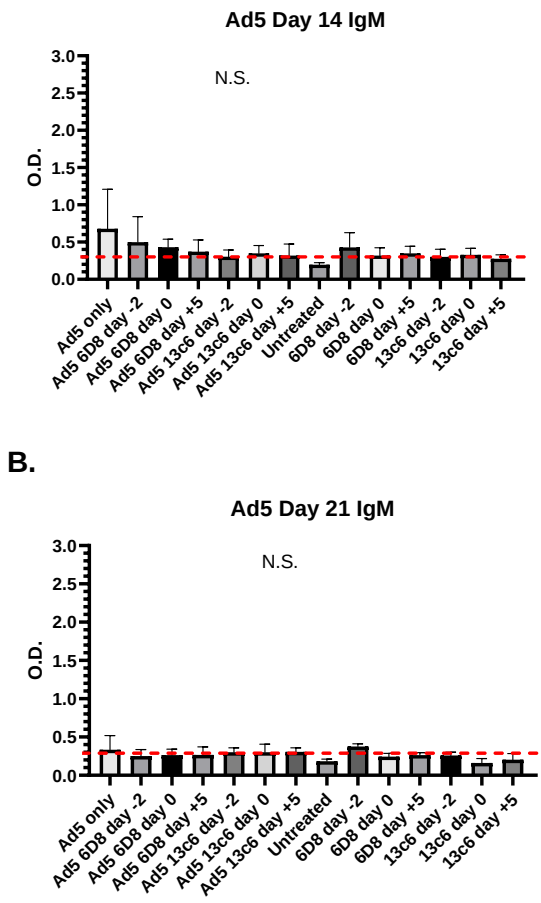



Figure 32: Day 14 (A) and Day 21 (B) mouse IgM antibody responses after Ad5-GP vaccination and 13c6 or 6D8 treatment. N.S. – not significant. The red line indicates the average IgM titer of the

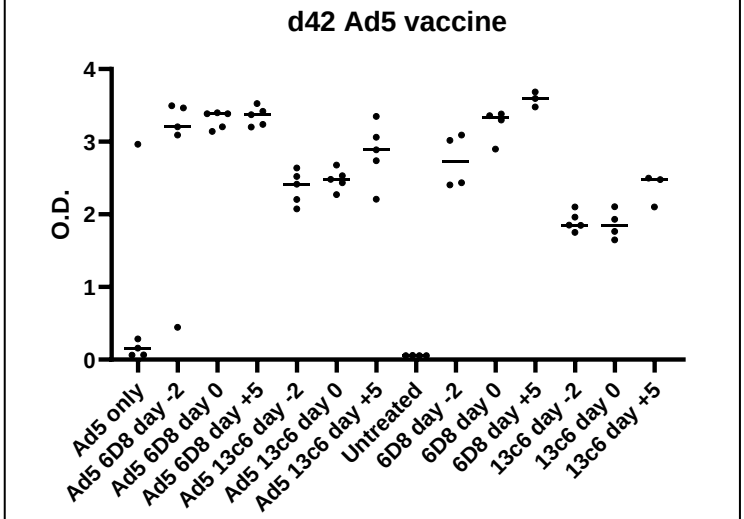
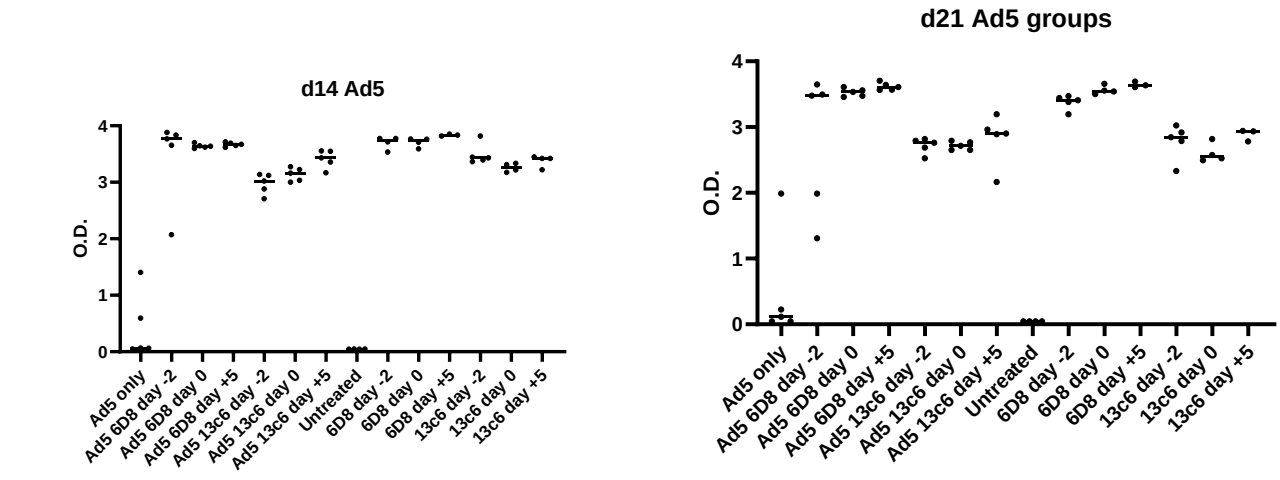



Figure 34: Day 42 Ad5-GP+antibody groups still has high levels of circulating IgG antibody, and Ad5-GP only induces poor IgG responses.




A.

B.

Figure 33: Day 14 (A) and Day 21 (B) mouse IgG antibody responses after Ad5-GP vaccination and 13c6 or 6D8 treatment. Ad5 only vaccine does not induce a strong IgG response at day 14 or day 21, and there are high levels of circulating administered antibodies.

6D8, both murine IgG2a antibodies, resulted in very high IgG antibody titers in day 14, 21 and 42, although by day 63 the administered antibodies in the Ad5 vaccine groups appeared to have decreased such that vaccine-induced antibody titers were observable. At day 14 and day 21, the IgG levels in all the vaccine+antibody groups are very high, and the IgG levels in the Ad5 only group are not significantly above the untreated/unvaccinated group. A single mouse from the Ad5 group has higher IgG antibody responses than the rest of the group at each time-point.

At day 42, there are still significant levels of circulating passively administered 13c6 and 6D8, as shown by the significant difference between IgG titers in the untreated/unvaccinated group and the unvaccinated with antibodies administered on day -2, 0 and 5 in Figure 34. As the antibody responses of the Ad5 vaccine are weaker than the VSV-GP vaccine, the difference between the Ad5 only and untreated/unvaccinated is not large enough to be able to subtract the O.Ds from the antibody only groups from the vaccine+antibody groups as we have for the VSV vaccine to compare induced IgG responses

Despite the fact that by day 63 the circulating 13c6 and 6D8 are lower, the antibody only and the vaccine+ antibody groups still have IgG at comparable concentrations, and the difference between the untreated/unvaccinated and the Ad5 only IgG is still very small. The lack of induction of IgG by Ad5 vaccine was also seen in year 1 in experiments with neutralizing antibodies. We hope that assaying for the IgG1a isotype, as discussed above, will allow for differences in IgG induction between the Ad5 and the Ad5+antibody groups to be more clearly examined.

*IgM titers against EBOV-GPdMuc.*

EBOV-neutralizing and/or protective monoclonal antibodies, such as KZ52 and the MAb cocktails ZMAb, ZMapp and MB-003, have been shown to recognize epitopes within or flanking the mucin-like domain. There is evidence that different doses of the VSV-GP vaccine in humans alter the sites of antibody epitopes, including lower (20 x 10^6 PFU) vaccination having less mucin-like domain epitopes than higher (100 x 10^6 PFU) (Khurana et al, 2016). The same paper demonstrated that IgM antibodies contribute significantly to neutralizing titers after VSV-GP vaccination in humans. To investigate whether the IgM antibodies were specific to the mucin-like domain, we used Ebola GP without the mucin-like domain protein as an ELISA target for IgM ELISAs. While the IgM ELISAs with different targets are not directly comparable without an IgM antibody standard,

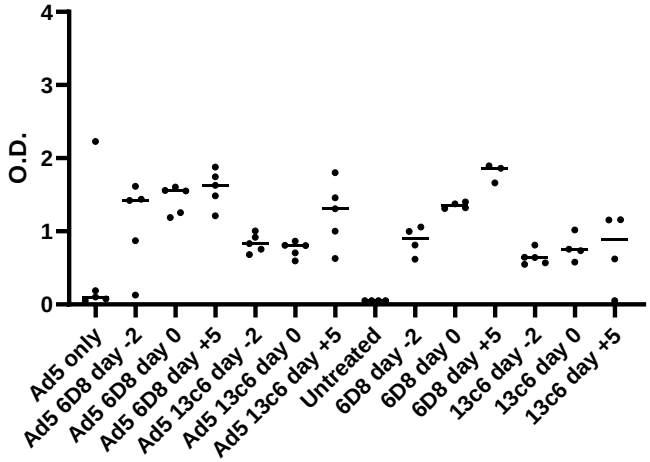



Figure 35: At day 63, the Ad5-GP+antibody groups still have higher levels of circulating IgG antibody than the IgG antibody induced by Ad5-GP only vaccination.

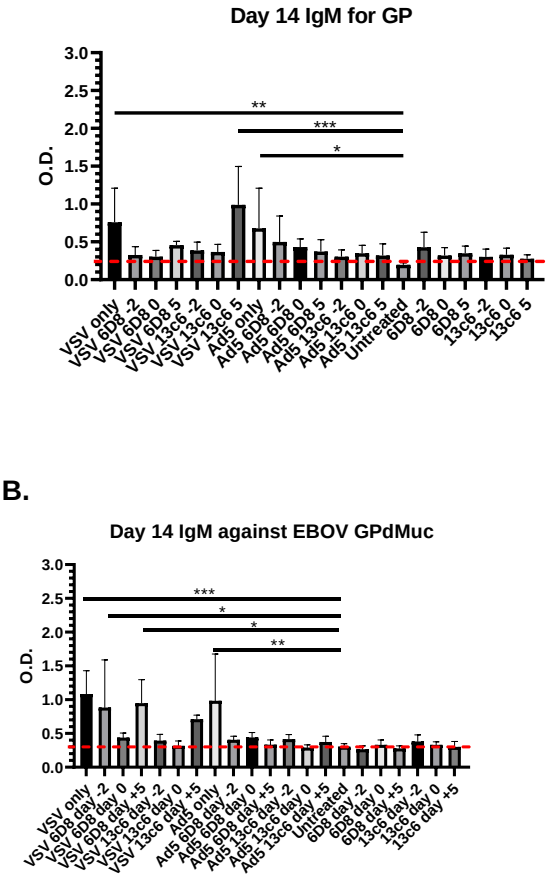



Figure 36. Day 14 IgM in all groups against EBOV GP ELISA target (A), or against EBOV GPdMuc (Ebola GP with the mucin-like domain deleted). . *p<0.05, **p<0.01, ***p<0.001. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA, the background level of binding.

it is interesting that the loss of the mucin-like domain did not result in an obvious loss of IgM binding for any of the groups (Figure 36). At day 14 in both the Ebola GP and Ebola GPdMuc ELISAs, both VSV and Ad5 vaccine-only groups are significantly different to the untreated/unvaccinated. However, VSV 13c6 day 5 is significantly increased compared to untreated/unvaccinated in ELISAs against Ebola GP but not Ebola GPdMuc, and VSV 6D8 d-2 and VSV 6D8 d5 are significantly increased compared to untreated/unvaccinated in ELISAs against Ebola GPdMuc but not Ebola GP. This suggests that the addition of the administered antibodies may alter the epitopes of induced IgM.

Conclusions (both positive and negative)

1) In VSV-GP vaccinated mice, there was a decrease in mouse anti-Ebola virus GP IgM antibody levels on day 10 and 14 when 6D8 or 13c6 was administered on day -2 or day 0. IgM levels had declined by d21 such that the VSV-GP vaccine was no longer had significant IgM titers.

2) In VSV-GP vaccinated mice, vaccine-induced IgG titers were observable at day 42 and day 63, where 6D8 or 13c6 administered on day -2 or day 0 suppressed vaccine-IgG induction compared to VSV-GP only vaccination.

3) In VSV-GP vaccinated mice, 13c6 administration at day 5 did not significantly suppress IgG induction, but 6D8 administration at day 5 did suppress IgG induction, suggesting the antibodies interact with the vaccine differently or have different kinetics.

4) In Ad5-GP vaccinated mice, significant IgM titers were present in the Ad5-only group at day 14, but not any Ad5 +antibody groups.

5) In Ad5-GP vaccinated mice, IgG titers were too low to be allow for comparison between vaccine and vaccine+ antibody groups, as the differences are obscured by the circulating IgG antibodies administered.

**Major Task 3: Determine whether antivirals effective against Ebola virus (EBOV) affect vaccine immunogenicity**

**Subtask 1: Determine effects of Remdesivir on vaccine immunogenicity**

Methods. BALB/c mice (n=5 per group) were vaccinated intramuscularly with either 2 x $10^4$ pfu of VSV-GP or mock vaccinated (as a negative control) on day 0. Remdesivir was formulated in 12% sulfobutylether-beta-cyclodextrin in water at a pH of 3.0 and injected intravenously through the ocular vein (10 mg/kg) daily for 10 days, beginning on day -2, day 0, or day +2, or injected with diluent only as a negative control. Antibody assays (both ELISA and functional assays) will be performed on sera isolated on days 5, 10, 14, 21, 42, and 63. Mice were bled via the submandibular route on days 5, 10, 14, 21, 42, and 63 to assay for vaccine-induced antibody responses. ELISAs were conducted on a 1:40 dilution of sera for anti-Ebola GP IgG and IgM mouse antibody for days 5, 10, 14 and 21 (Figure 2). In addition, Ebola virus GP1,2-specific T cell responses from spleen and mesenteric lymph nodes will be measured on day 63 as in Aim 1 to determine effects of the small molecules on vaccine-induced immune responses.

Statistical analysis. Antibody levels were compared to vaccine-only groups with vaccine+antibody groups using a one-way ANOVA with Dunnett's multiple comparisons test. A p value of $<0.05$ was considered to be significant.

Results.

*rVSV-EBOV GP vaccination with remdesivir IV treatment*

*IgG/IgM titers after VSV-GP vaccination.*

Mice receiving VSV-GP on day 0 and treated with Remdesivir on days -2, 0, or 5 were bled on days 5, 10, 14 and 21. As IgM has been shown to be a major contributor to VSV-GP-generated functional antibodies[1] ELISAs were

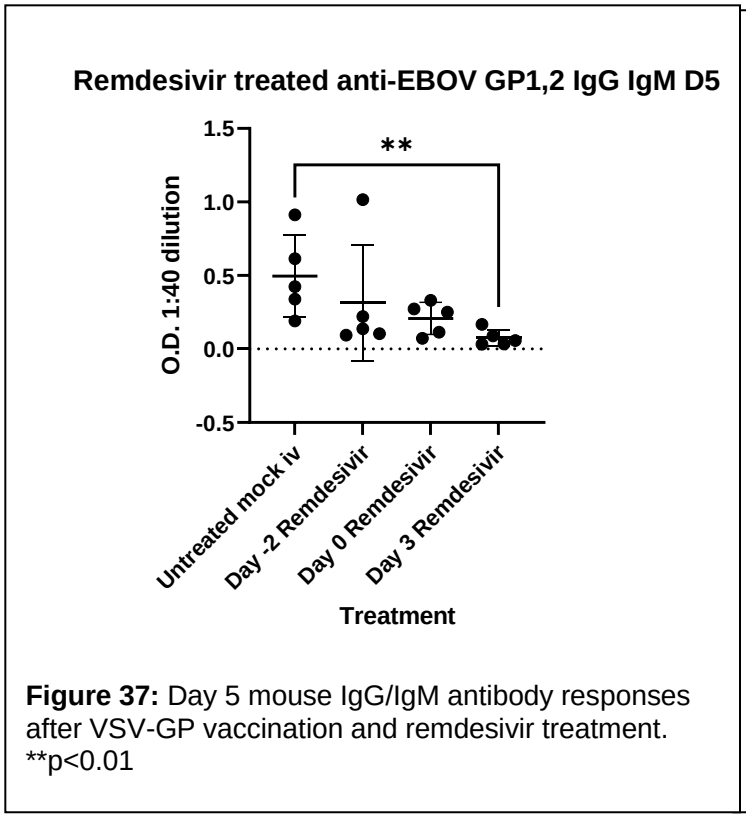



**Figure 37:** Day 5 mouse IgG/IgM antibody responses after VSV-GP vaccination and remdesivir treatment. **p<0.01

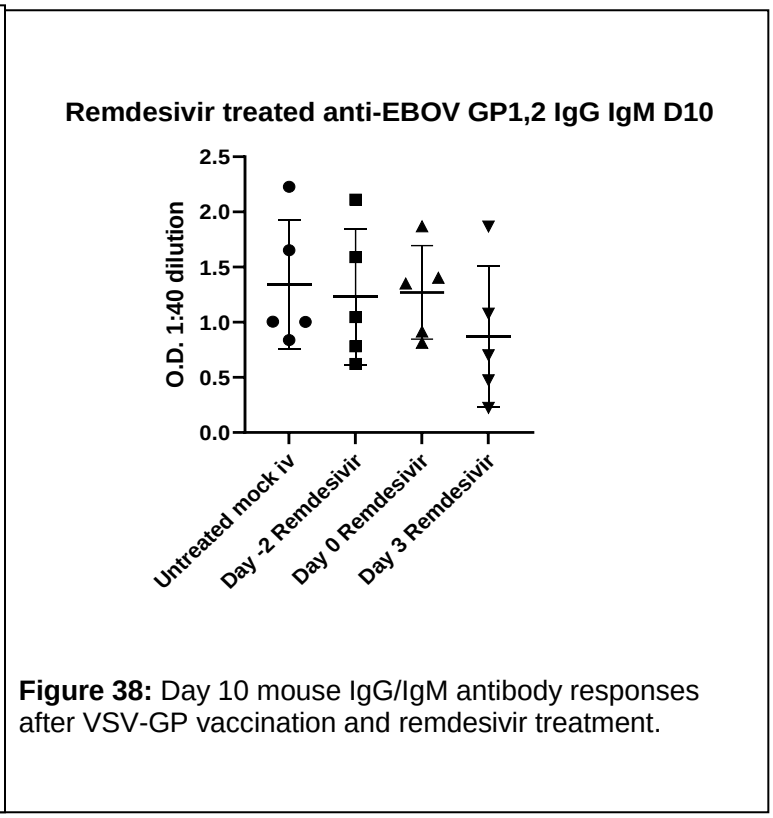



**Figure 38:** Day 10 mouse IgG/IgM antibody responses after VSV-GP vaccination and remdesivir treatment.

performed with the sera to assay both IgG and IgM levels. Early total IgG/IgM titers were assayed to determine whether the concurrent, prior or delayed antiviral treatment resulted in a delay of the establishment of antibodies against EBOV GP compared to the control vaccine-only

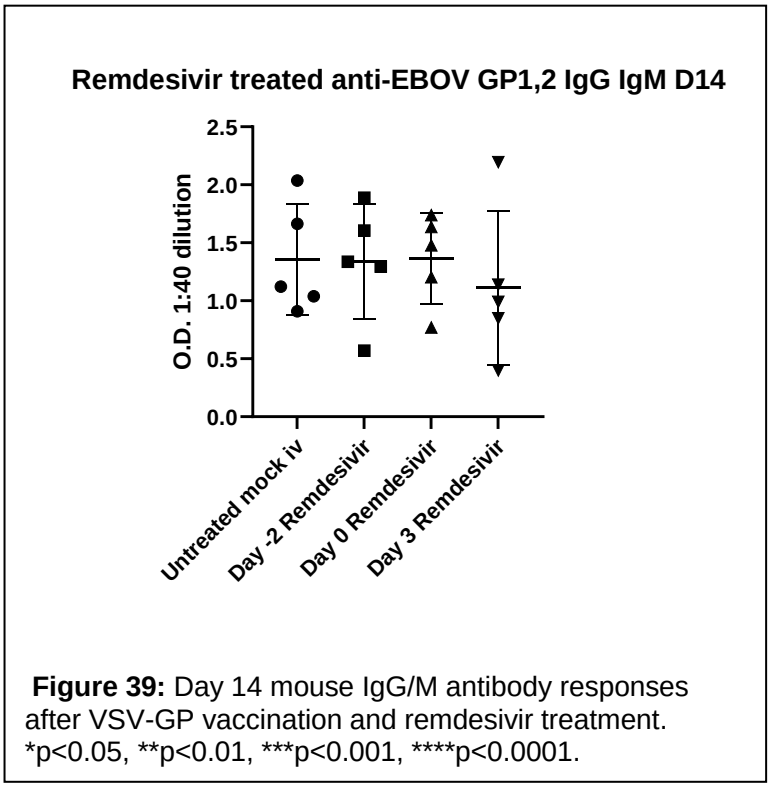



**Figure 39:** Day 14 mouse IgG/M antibody responses after VSV-GP vaccination and remdesivir treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

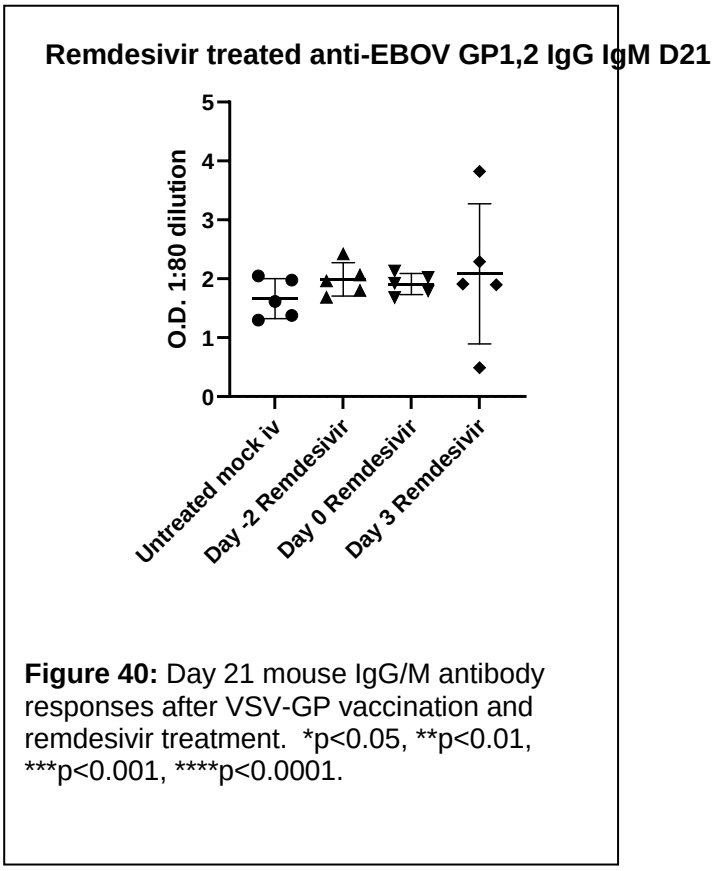



**Figure 40:** Day 21 mouse IgG/M antibody responses after VSV-GP vaccination and remdesivir treatment. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

animals.

On day 5 (Figure 37), there was a mouse IgM/IgG anti-Ebola

GP response detected by ELISA in the rVSV-EBOV GP vaccinated group, There were no significant differences in mock IV and the Day -2 and Day 0 Remdesivir treatments. However, there is a significant difference at Day 5 between the mock IV and the Day 3 Remdesivir, which did not receive any IV, vehicle or drug, prior to day 3. This may suggest there is an increase in titers in response to the IV injection itself after vaccination, rather than a suppression of the vaccine-induced titers in response to Remdesivir treatment, as no suppression is seen in the day -2 and day 0 treated groups. The mock IV group vehicle IVs were identical in timing to the Remdesivir Day -2 treated group, and so by day 5, both the Remdesivir day -2 and the mock IV group had received the same number of IV injections (6), whereas the day 3 had only received 3. In the future, a control group of mock IV mice at the same as each of the treated groups could account for this difference.

At day 10, significant IgG/IgM titers were present in the rVSV-EBOV GP vaccine only group and all three of the Remdesivir treated groups, although there are no significant differences in titer (Figure 38). On day 14, there are still no significant differences between the rVSV-EBOV GP vaccine only group and all three of the Remdesivir treated groups, but there is a trend that the Day 3 Remdesivir has lower titers than the mock IV and Day -2 Remdesivir group (Figure 39). The trend may also be accounted for by the differences in IV injections after vaccination. There do not appear to be any differences between the untreated mock IV and the Day -2 Remdesivir titers at day 10 or day 14.

By day 21, there are no significant differences between any of the Remdesivir-treated groups and the untreated group that received the mock IV (Figure 40). It appears that any trend showing a decrease in titer in the groups that received later IV has disappeared, and by Day 21 all the groups have received 10 IV injections. As expected, the titers of antibodies continue to increase from day 5 to day 21, and the total IgG/IgM antibody is higher at day 21.

**Subtask 2: Determine effects of favipiravir on vaccine immunogenicity**

The original proposal was to test favipiravir, a then promising antiviral against Ebola. However, since the original grant was summited, multiple reports suggesting the limited efficacy of favipiravir as an in vivo antiviral against Ebola virus infection have emerged, including a retrospective study that revealed that the effect on mortality of favipiravir treatment in humans was not statistically significant[2]. Due to these findings, we did not conduct experiments with this drug.

**Continuation of Major Task 1 from Year 1: Test the effects of time of administration of Ebola virus neutralizing antibody cocktails on immunogenicity of advanced Ebola virus vaccines**
**Subtask 1: Determine the effects of timing of passive neutralizing antibody administration on vaccine immunogenicity**

Methods. BALB/c mice (n=5 per group) were vaccinated intramuscularly with either 2 x $10^4$ pfu of VSV-GP or mock vaccinated (as a negative control) on day 0. The MIL77 cocktail or mAb114 was injected intravenously with 100 ug of each antibody treatment on days -2, 0, and +5 (as a control, for each vaccine one group was vaccinated but not injected with antibody). Neutralizing antibody titers were measured against Ebola virus using VSV expressing luciferase and carrying Ebola GP in place of VSV-G.

Statistical analysis. Antibody levels were compared to vaccine-only groups with vaccine+antibody groups using a one-way Anova with Dunnett's multiple comparisons test. A p value of $<0.05$ was considered to be significant.

Results.

After improving the assay to test the neutralizing titers of the serum antibodies, we were able to test day 63 serum from the co-administration of neutralizing antibody cocktails and Ebola virus vaccines. Figure 41 shows the neutralizing antibody titers in response to VSV vaccination with no antibodies, or with mAb 114 or MIL 77 at either day -2, 0 or day 5. There is a clear trend suggesting that the prior treatment with either mAb 114 or MIL77 reduces the amount of neutralization. The lower luciferase values indicate more rVSV-EBOV GP neutralization has occurred, as less rVSV-EBOV GP is able to enter the cells and produce luciferase. These results align with the previous results from day 42 ELISAs that suggested higher total IgG titers in the rVSV-EBOV GP vaccine only group, compared to the groups that received the neutralizing antibodies prior to vaccination (Figure 42). The mAb 114 delivered at day 5 also has very good neutralization with a low average and small range of results.

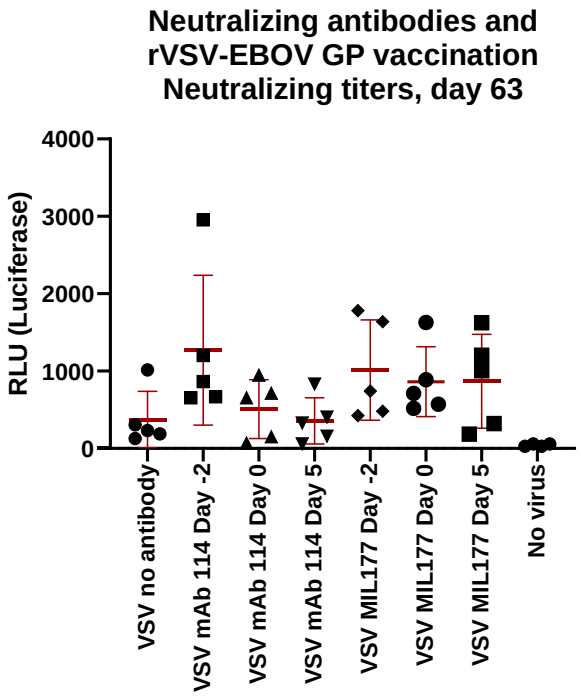



**Figure 41:** Day 63 mouse IgG antibody responses after rVSV-EBOV GP vaccination and mAb114 or MIL77 treatment. No statistically significant differences.

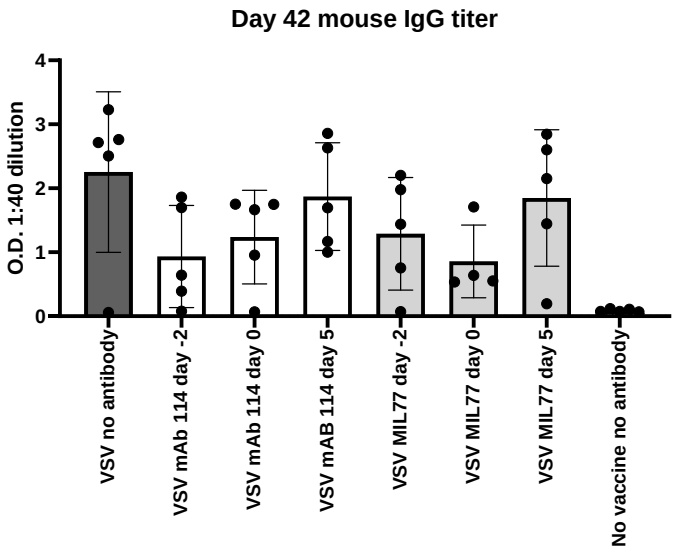



**Figure 42:** Day 42 mouse IgG antibody responses after VSV-GP vaccination and mAb114 or MIL77 treatment. No statistically significant differences.

Conclusions (both positive and negative)

1) In rVSV-EBOV GP vaccinated mice treated with remdesivir, there was an early increase in titers at day 5 in both the day -2 remdesivir treated, and the mock IV treated, compared to mice that did not receive IV injections until day 3 likely due to the effect of the IV injection itself rather than the remdesivir drug.

2) There were no significant differences in the titers of IgM/IgG in the remdesivir treated groups compared to the mock IV vaccine only group by day 21.

Overall conclusions.

1) Administration of neutralizing or non-neutralizing monoclonal anti-Ebola virus antibodies near the time of VSV or AdV-based vaccines negatively affects vaccine immunogenicity.

2) This negative effect can be ameliorated if the antibodies are administered 5 days after vaccination.

3) The administration of remdesivir at the time of VSV or AdV-based Ebola virus vaccination does not appear to negatively affect vaccine immunogenicity.

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**d) other achievements**.

- *Neutralizing assay modification.* Traditionally, we have used endpoint dilutions to quantitate neutralizing antibody activity. However, this approach requires a higher amount of sera, and with limited sera from serial submandibular bleeds which were also used for mouse and human antibody ELISAs, we needed an approach that utilized small serum amounts. To that end, we have established a single dilution-point assay that correlates neutralizing activity to a standard (the neutralizing activity of the monoclonal antibody KZ52). This approach was incorporated in this report and allowed us to test neutralizing, mouse IgG/M ELISAs, and human IgG ELISAs from a submandibular bleed at multiple serial timepoints.

- *Elisa with single dilution.* To address a similar need with ELISAs, we have developed a parallel assay using a single dilution point to compare with a dilution series of the monoclonal anti-Ebola GP antibody 13c6 with either a human or mouse constant region. This allows us to use one dilution to compare with the standard. We have established this assay more recently and will be using it moving forward in our future analysis.

**e) Discussion of stated goals not met**

- *Live virus challenge.* We were unable to conduct live Ebola virus challenge in our collaborators' BSL-4 facility (NIH/NIAID/IRF) due to this facility not being able to accommodate these requests because of the COVID-19 pandemic and personnel changes that slowed the approval process. To address this, we began discussions with UTMB-Galveston and began the paperwork for the live challenges at the facility. However, these experiments have not yet been approved. We will continue attempting to complete the live virus challenges at one of the facilities; if we are successful, we will report these data.

- *T cell analysis.* We have not yet conducted the day 63 T cell analysis. ] We have the splenocytes harvested and frozen for GP epitope responses.

- *sGP.* We were not able to acquire sufficient amounts of anti-sGP antibodies for the *in vivo* testing.

    - **What opportunities for training and professional development has the project provided?**

        - Student training: Elizabeth Clarke, Tonilynn Baranowski, Julianne Peabody, Sam Goodfellow, and Mahgol Behnia. This project has been used to fund the training of four PhD students and a postdoctoral fellow in the Bradfute laboratory. Dr. Clarke has participated in the mouse injection, harvesting, and analysis aspects of the work as well as the antibody testing. Dr. Clarke obtained her PhD in January of 2021, and stayed in the lab on a short-term postdoctoral fellowship. Dr. Clarke is still active in this project. Ms. Baranowski has helped set up the flow cytometric analysis for the T cell responses and is involved in the animal injection and handling aspects of the project. Ms. Behnia has been involved in mouse handling and tissue processing. Ms. Peabody and Mr. Goodfellow have assisted in injections, bleeds, and tissue processing. Together, this grant has significantly impacted the technical and scientific training of these four PhD students and one postdoctoral fellow.

        - Research scientist training: Chunyan Ye and Robert Nofchissey. These two individuals are senior research scientists in the Bradfute laboratory with a long history of virus research. Ms. Ye is heavily involved in the injection, tissue processing, and antibody tests reported here. Mr. Nofchissey participated in the mouse injection and tissue harvesting, as well as in the overall setup and organization of the experiments.

    - **How were the results disseminated to communities of interest?**

        - Dr. Bradfute has discussed these data at local (UNM) meetings. During the first few years of the project, there was a cancelation of multiple scientific meetings due to COVID-19 restrictions. We are writing a manuscript to publish the data presented in this report and will submit it to a peer-reviewed journal.

    - **What do you plan to do during the next reporting period to accomplish the goals?**

        - Not applicable, as this is the final report. However, we are still pursuing conducting the live virus BSL-4 experiments at an outside facility.

**4. IMPACT:**

    - **What was the impact on the development of the principal discipline(s) of the project?**

        - Our findings are relevant because they show that administration of monoclonal antibodies can negatively impact vaccine immunogenicity. This is relevant not only to Ebola virus, but also to other pathogens of DoD interest.

    - **What was the impact on other disciplines?**

        - Our approach has been a topic of discussion during the SARS-CoV-2 pandemic, which has struggled with timing of vaccination relative to monoclonal antibody administration.

    - **What was the impact on technology transfer?**

        - Nothing to report

    - **What was the impact on society beyond science and technology?**

- Nothing to report

**5. CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

  - We did not conduct experiments with favipiravir since it did not show efficacy in human trials conducted after the initial acceptance of this proposal. Remdesivir was kept in this study since initial studies suggest it may have a role as an inhibitor of persistent Ebola virus reservoir sites in human infection.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  - *ZMAPP.* Due to restrictions on the availability of ZMAPP for research purposes due to ongoing clinical trials, we had to acquire the closely related MIL77. This was accomplished with an agreement with MAPP Biopharmaceutical, but required a significant amount of paperwork, causing delays. However, we were able to acquire the material and conduct the experiments above.

  - *mAb114.* Due to restrictions on the availability of mAb114 for research purposes due to ongoing clinical trials, we took advantage of the published sequence of the antibody and contracted the production and purification of this material. This did cause a delay but we were able to acquire the antibody in time to conduct the experiments listed above.

  - *ChAd3-GP.* Again, due to restrictions on the availability of ChAd3-GP due to ongoing clinical trials, we had to generate AdV serotype 5-GP for these experiments. There was not a company or academic group we were able to find that would provide the ChAd3 background to construct ChAd3-GP; therefore, we utilized the AdV5 backbone to generate an adenovirus vaccine for these studies. AdV5 was chosen because it was the first adenovirus vaccine to protect non-human primates from Ebola virus infection (4).

  - *BSL-4 challenge studies.* Due to the SARS-CoV-2 pandemic, many experiments for this work were adversely affected. First and foremost, the planned BSL-4 live challenge experiments at NIH/NIAID/IRF were not able to be performed, as closures, personnel restrictions, and SARS-CoV-2 work took priority. We pivoted to UTMB-Galveston as a collaboration, and have submitted our paperwork to that institution. However, the paperwork has not yet been approved but we will continue to follow up on this project.

  - *COVID-19 and personnel limitations.* Due to COVID-19, our institution barred graduate students from entering the laboratories and limited the access of staff and faculty members from March 23, 2020 through June 2, 2020. During most of 2020 and 2021 our institution limited operations capacity, as staff capacity was set between 25-50% for most of that time, as New Mexico had some of the most restrictive measures taken during the pandemic. Therefore, a significant amount of time was lost due to these personnel restrictions and this caused a delay in our timeline. However, we successfully completed much of the proposed work. From the personnel and space restrictions, we found ways to expedite the large mouse studies (such as scheduling multiple personnel on bleed days, analyzing serum at one dilution measured against a monoclonal standard, and cross-training personnel for different kinds of injections).

  - *ChAd3-GP.* Due to restrictions on the availability of ChAd3-GP due to ongoing clinical trials, we had to generate AdV serotype 5-GP for these experiments. There was not a company or academic group we were able to find that would provide the ChAd3 background to construct ChAd3-GP; therefore, we utilized the AdV5 backbone to generate an adenovirus vaccine for these studies. AdV5 was chosen because it was the first adenovirus vaccine to protect non-human primates from Ebola virus infection (4).

- *Acquisition of sGP antibodies.* We were unable to acquire sufficient amounts of anti-sGP antibodies for the Year 2 experiments. We found anti-EBOV sGP antibody at a cost of $425 per 100 ug; given that the dose needed was 100 ug/mouse and we would inject 45 mice, the cost was deemed prohibitive.

- **Changes that had a significant impact on expenditures**
  - Nothing to report
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - No significant changes. A new IACUC protocol was approved from 2022-2025.
- **Significant changes in use or care of human subjects**
  - Not applicable
- **Significant changes in use or care of vertebrate animals.**
  - Nothing to report
- **Significant changes in use of biohazards and/or select agents**
  - Nothing to report

## 6. PRODUCTS:

- **Publications, conference papers, and presentations**
  - **Journal publications.** Dr. Bradfute has published an invited review entitled "Novel drug design strategies for filoviruses" (*Expert Opinion in Drug Discovery 2022 Feb;17(2):139-149. doi: 10.1080/17460441.2022.2013800, PMID: 34962451).* In it, he discusses the possibility of regimens with both antibodies and vaccines to provide short and long-term protection against EBOV. The manuscript lists this grant as support.
  - A manuscript outline is being constructed based on our data, but we are waiting on the live virus challenges prior to submitting a manuscript.
  - **Books or other non-periodical, one-time publications.** Nothing to report.
  - **Other publications, conference papers, and presentations.** Nothing to report.
- **Website(s) or other Internet site(s)**
  Nothing to report.
- **Technologies or techniques**
  Nothing to report.
- **Inventions, patent applications, and/or licenses**
  Nothing to report.
- **Other Products**
  Nothing to report.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

| | |
|---|---|
| Name: | *Steven Bradfute* |
| Project Role: | *PI* |
| Researcher Identifier (e.g. ORCID ID): | 0000-0002-1985-751X |
| Nearest person month worked: | 4 |
| Contribution to Project: | *Dr. Bradfute has overseen the project, including experimental design, acquisition of reagents, data analysis, and report generation.* |
| Funding Support: | *All of Dr. Bradfute's effort on this project came from this grant.* |

| | |
|---|---|
| Name: | *Elizabeth Clarke* |
| Project Role: | *Postdoctoral fellow* |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | *6* |
| Contribution to Project: | *Ms. Clarke has overseen the execution of the experiments and participated in injections, tissue harvesting, analysis of antibody levels, and generation of data.* |
| Funding Support: | *All of Ms. Clarke's efforts for this project have been funded by a T32 training grant.* |

| | |
|---|---|
| Name: | *Mahgol Behnia* |
| Project Role: | *Graduate Student* |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 6 |
| Contribution to Project: | *Ms. Behnia has been involved in animal handing, injections, and tissue processing.* |
| Funding Support: | *All of Ms. Behnia's efforts for this project have been funded by this grant.* |

| | |
|---|---|
| Name: | *Tonilynn Baranowski* |
| Project Role: | *Graduate Student* |

| | |
|---|---|
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | *1* |
| Contribution to Project: | *Ms. Baranowski has been involved in animal handing, injections, and tissue processing.* |
| Funding Support: | *All of Ms. Baranowski's efforts for this project have been funded by this grant.* |

| | |
|---|---|
| Name: | *Julianne Peabody* |
| Project Role: | *Graduate Student* |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | *1* |
| Contribution to Project: | *Ms. Peabody has been involved in animal handing, injections, and tissue processing.* |
| Funding Support: | *Ms. Peabody's efforts are funded by a T32 training grant.* |

| | |
|---|---|
| Name: | *Samuel Goodfellow* |
| Project Role: | *Graduate Student* |
| Researcher Identifier (e.g. ORCID ID): | 0000-0003-0471-8303 |
| Nearest person month worked: | *1* |
| Contribution to Project: | *Mr. Goodfellow has been involved in animal handing, injections, and tissue processing.* |
| Funding Support: | *Ms. Peabody's efforts are funded by a T32 training grant.* |

| | |
|---|---|
| Name: | *Chunyan Ye* |
| Project Role: | *Research Scientist* |
| Researcher Identifier (e.g. ORCID ID): | |
| Nearest person month worked: | 9 |
| Contribution to Project: | *Ms. Ye has been involved in animal handling, injections, tissue processing, antibody ELISAs, and neutralizing assays.* |
| Funding Support: | *All of Ms. Ye's efforts for this project have been funded by this grant.* |

| | |
|---|---|
| Name: | *Robert Nofchissey* |
| Project Role: | *Research Scientist* |
| Researcher Identifier (e.g. ORCID ID): | 0000-0002-7368-6758 |
| Nearest person month worked: | 2 |
| Contribution to Project: | *Mr. Nofchissey has been involved in animal handing, injections, and tissue processing.* |
| Funding Support: | *All of Mr. Nofchissey's efforts for this project have been funded by this grant.* |

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - Nothing to report
- **What other organizations were involved as partners?**
  - **Organization Name:** United States Army Medical Research Institutes of Infectious Diseases (USAMRIID)
  - **Location of Organization:** Fort Detrick, MD, USA
  - **Partner's contribution to the projec**t
    - **Other.** USAMRIID (Dye lab) supplied the monoclonal antibodies used in the Year 1 and 2 reports
  - **Organization Name:** Rocky Mountain Labs, NIH/NIAID
  - **Location of Organization:** Hamilton, MT, USA
  - **Partner's contribution to the projec**t
    - **Other.** RML (Feldmann lab) supplied the VSV-Ebola vaccine used in this Year 3 report

**8. SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** Not applicable.
- **QUAD CHARTS:** Not applicable.

**9. APPENDICES:** None.