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| 1. REPORT DATE (DD-MM-YYYY)<br>17-11-2014                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                   | 2. REPORT TYPE<br>Final Report |                                                      | 3. DATES COVERED (From - To)<br>1-Mar-2011 - 31-May-2014 |                                       |
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| 14. ABSTRACT<br>The purpose of the proposed work is to continue the promising anti-hemorrhagic fever virus (HFV) drug discovery and lead optimization efforts previously initiated by Prosetta through significant advancement of at least two chemical series through the first critical steps toward filing a Investigational New Drug (IND) application: efficacy, safety and mechanism of action. This project will launch from the advanced platform of lead of optimization of five Prosetta-identified compounds and will progress through target identification and efficacy assessment in rodents. Two of these advanced compounds have very strong nanomolar to low micromolar activity against all four                                                                                                                                                                                                                |                   |                                |                                                      |                                                          |                                       |
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## Report Title

Final Report: Advanced Development of Antiviral Prophylactics and Therapeutics (ADAPT) - Research Area 10

### ABSTRACT

The purpose of the proposed work is to continue the promising anti-hemorrhagic fever virus (HFV) drug discovery and lead optimization efforts previously initiated by Prosetta through significant advancement of at least two chemical series through the first critical steps toward filing a Investigational New Drug (IND) application: efficacy, safety and mechanism of action. This project will launch from the advanced platform of lead of optimization of five Prosetta-identified compounds and will progress through target identification and efficacy assessment in rodents. Two of these advanced compounds have very strong nanomolar-to-low micromolar activity against all four HFV families studied - Arenaviridae, Bunyaviridae, Filoviridae and Flaviviridae.

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**Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:**

**(a) Papers published in peer-reviewed journals (N/A for none)**

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**Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

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Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

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Number of Manuscripts:

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TOTAL:

Received      Book Chapter

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Patents Awarded

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NAME

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NAME

**Total Number:**

### Names of other research staff

NAME

PERCENT SUPPORTED

Jean Francis 0.07

Michael Corpuz 0.01

Deben Dey 0.03

Ian Brown 0.01

Marissa Baker-Wagner 0.02

Olayemi Akintunde 0.01

**FTE Equivalent: 0.15**

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**Sub Contractors (DD882)**

**Inventions (DD882)**

**Scientific Progress**

See Attachment

**Technology Transfer**

See Attachment

## **Adapt Final Contract Report**

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**ADAPT FINAL RESEARCH CONTRACT REPORT 8/1/2011-7/31/2014**  
**Update of Progress by Tasks (see Summary and Conclusions below):**

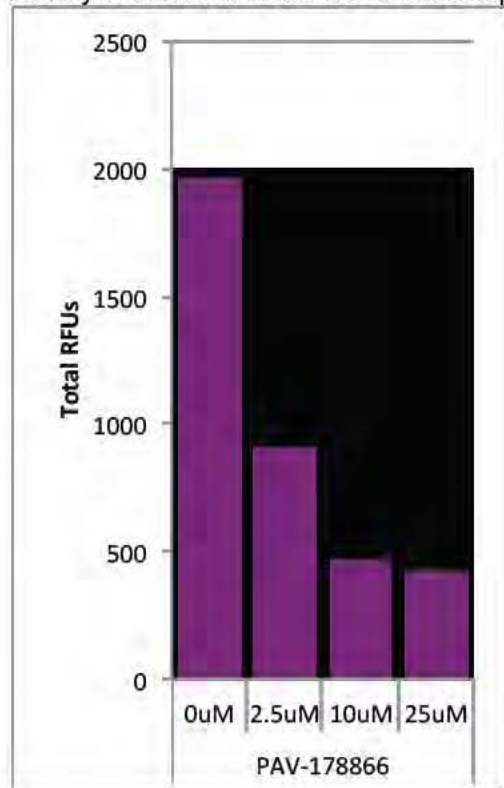
**Contract Performance Overview:**

All Aim deliverables for contract W911NF-11-C-0059 were either completely fulfilled or exceeded. Extensive screening was completed far above the requirement for Aim 1. All lead candidate molecules were assessed for pharmacokinetic and toxicity characteristics under Aim 2. Extensive *in vitro* screening was completed and one molecule was advanced to an *in vivo* animal study under Aim 3. Detailed and complex target identification and mechanism of action studies were designed and carried out under Aim 4, leading to significant knowledge-base advances both for the lead anti-Ebola pharmacophore of interest and drug characterization in general. Although the *in vivo* animal study did not show efficacy with the first lead candidate compound tested, BSL hood space scheduling and government furlough delays did not allow for time to test additional promising molecules. It is Prosetta's intention to continue testing these molecules in self-funded studies, especially in light of the growing worldwide health crisis where Ebola virus is concerned.

**Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

Over the entire contract length, Prosetta screened 268 chemical analogs designed for optimized capsid assembly inhibition in its proprietary VEEV cell-free synthesis screen. From these 268 analogs, the analogs that showed initial promising inhibition profiles in the cell-free screening were advanced for both assessment against infectious virus in cell culture (studies conducted by Pamela Glass, Ph.D. at USAMRIID on VEEV, LASV, RVFV) and for toxicity and PK profile characterization (done both in-house at Prosetta and by our collaborators at Bionees). Multiple examples of in-house screening results were reported during the contract period and can be found below in the attached quarterly and annual reports. Screening efforts, coupled with additional studies detailed in further aims below, lead to the nomination of compound PAV-866 as the primary compound for *in vivo* animal validation studies. PAV-866's behavior in the cell-free system can be seen directly below in Figure 1. All deliverables for this Aim were successfully met.

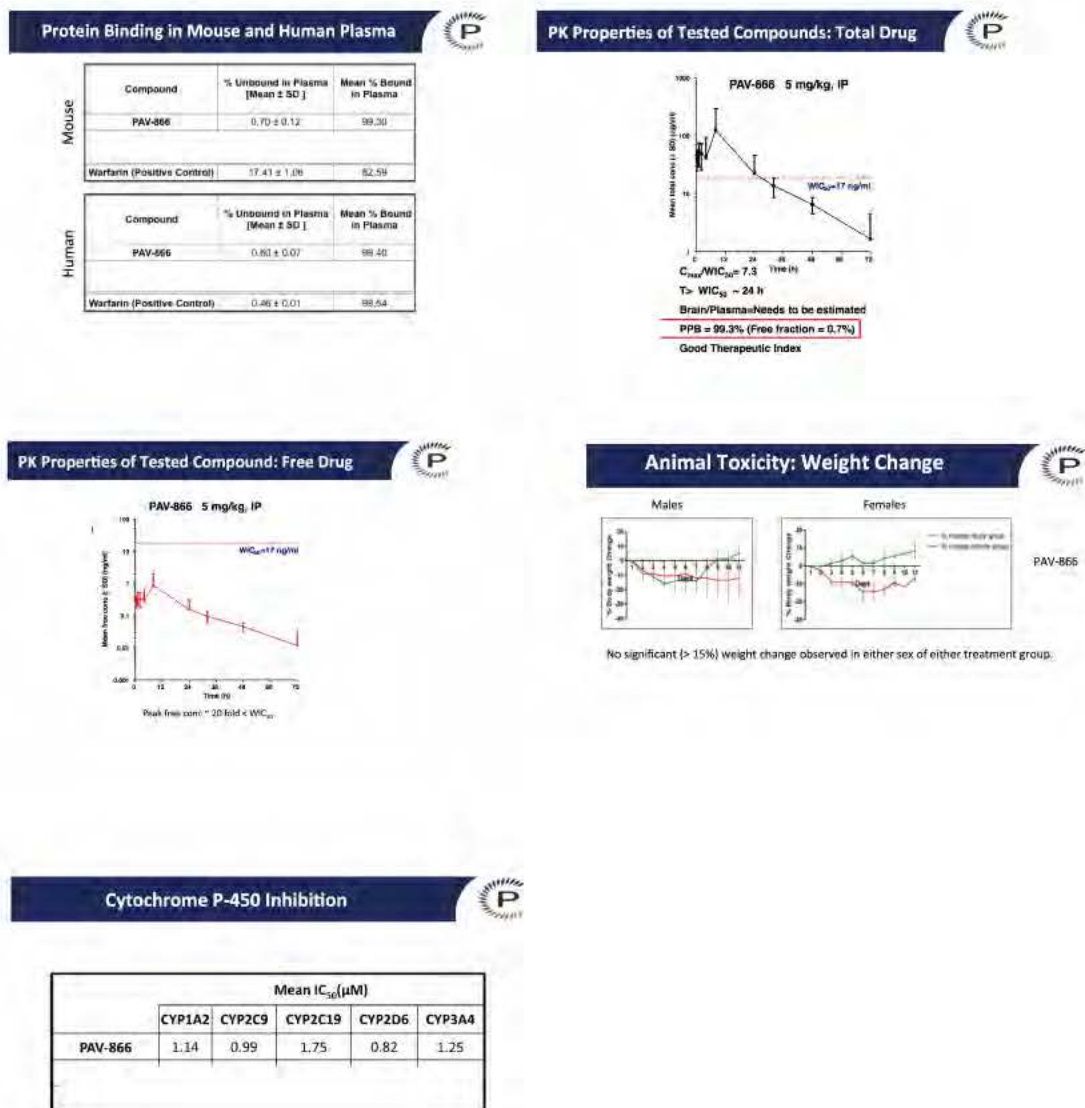
**Figure 1:**  
Activity of PAV-866 in the VEEV cell-free protein-synthesizing assay



## Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.

Over the entire contract length, greater than 100 compounds that showed initial promise in Prosetta's cell-free synthesis capsid assembly screen were assessed for *in vitro* safety and pharmacokinetic properties. Of these compounds, a smaller subset underwent *in vivo* assessment, leading to a final compound nomination for *in vivo* animal validation studies. Examples of this data were reported during the contract period and can be found below in the attached quarterly and annual reports. The pk properties of lead nominated compound PAV-866 are shown directly below in Figure 2. All deliverables for this Aim were successfully met.

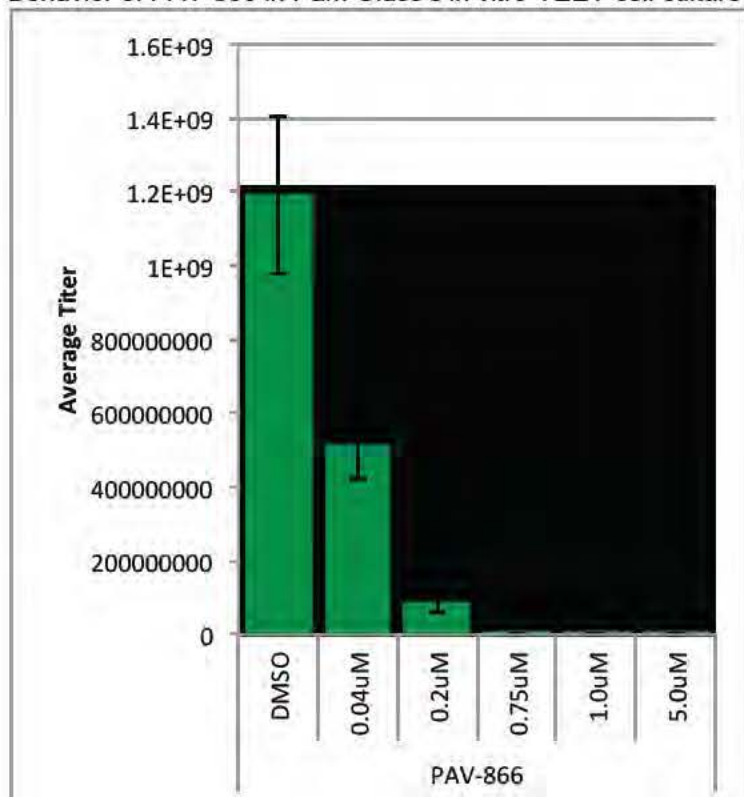
**Figure 2:**  
Pharmacokinetic and Safety Data for PAV-866



**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

Working with Pam Glass at USAMRIID, the most promising chemical analogs based on both *in vitro* cell-free synthesis testing and pk/toxicity profiling were assessed by infectious virus in cell culture testing. This *in vitro* data was reported during the contract period and can be found below in the attached quarterly and annual reports. Additionally, the *in vitro* infectious virus in cell culture data for nominated compound PAV-866 can be seen directly below in Figure 3.

**Figure 3:**  
Behavior of PAV-866 in Pam Glass's *in vitro* VEEV cell culture assay



The culmination of the data streams covered by Aims 1, 2, and 3 resulted in Prosetta nominating compound PAV-866 as our lead candidate for *in vivo* animal efficacy validation testing. Due to time constraints and BSL hoodspace scheduling difficulties, only *in vivo* studies against animals infected with VEEV were conducted, with the understanding that should PAV-866 show promising results against VEEV, further extension studies against additional viruses would be planned. Unfortunately, at the end of the contract, Prosetta has yet to achieve a convincing animal success with PAV-866, thus suspending any hopes for further animal studies at this time.

The details of the animal study conducted by Pam Glass against VEEV are as follows, in Figure 4:

**Figure 4:**  
**Study Design**  
Protocol Number: AP-13-034  
Challenge: VEEV Trinidad DVC AIMS 12856  
Back Titer: 1070 pfu/mouse

| Group | Compound                  | # of mice | Dose (mg/kg) | Regimen                            | ROA          |
|-------|---------------------------|-----------|--------------|------------------------------------|--------------|
| 1a    | PAV-866                   | 10        | 10           | 1hr post exp.; 1x daily for 5 days | IP injection |
| 2a    | 5% DMSO/35% PEG/60% water | 10        | -            | 1hr post exp.; 1x daily for 5 days | IP injection |
| 3a    | Untreated                 | 10        | -            | -                                  | -            |
| 1b    | PAV-866                   | 3         | 10           | 1hr post exp.; 1x daily for 5 days | IP injection |
| 2b    | 5% DMSO/35% PEG/60% water | 3         | -            | 1hr post exp.; 1x daily for 5 days | IP injection |
| 3b    | Untreated                 | 3         | -            | -                                  | -            |

\*For groups 1b, 2b, and 3b, tissues (kidney/lung/liver/brain/spleen) and serum were harvested on day 4 of the challenge

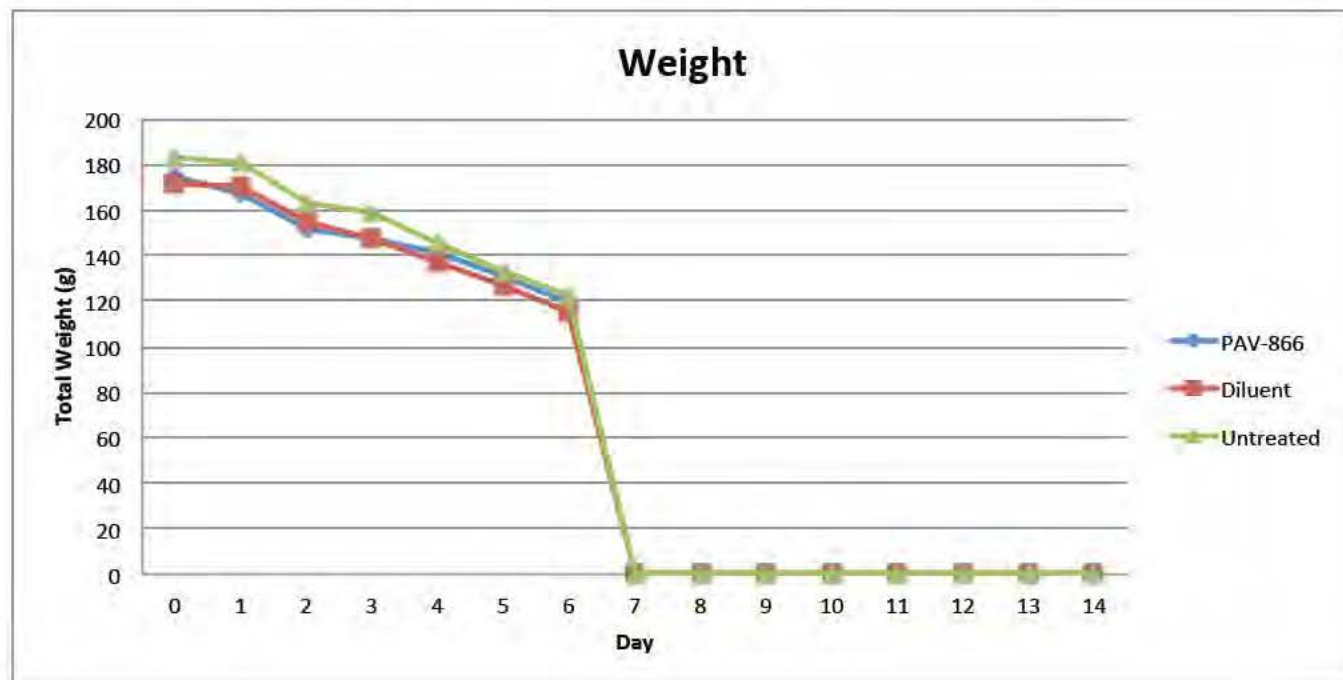
## Results

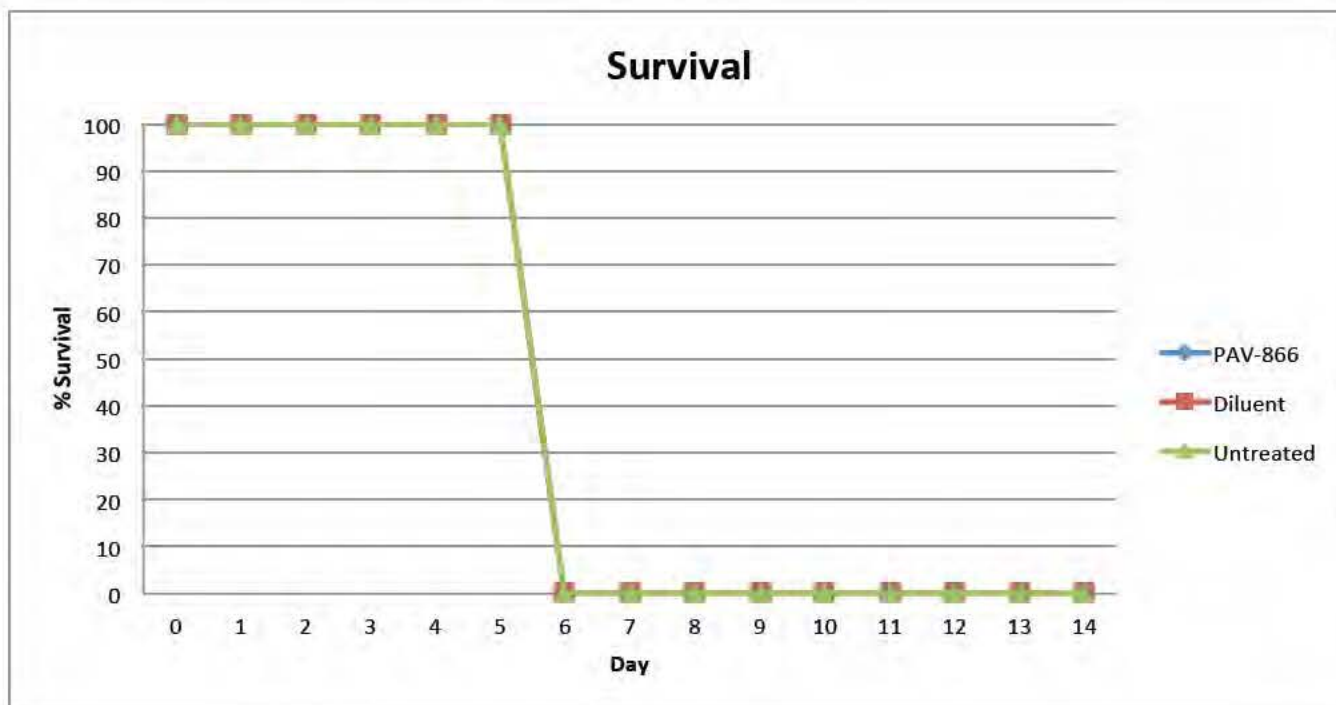
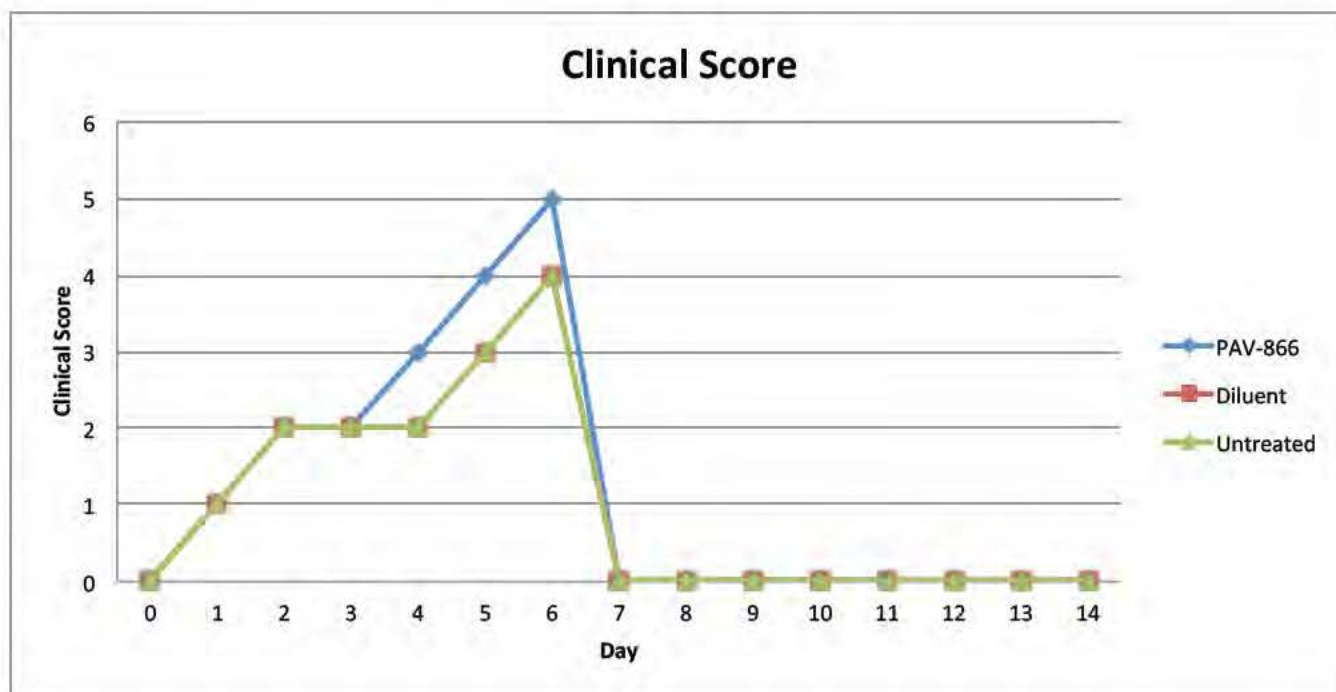
| Weight   | Compound  | Day | 0     | 1     | 2     | 3     | 4     | 5     | 6     | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|----------|-----------|-----|-------|-------|-------|-------|-------|-------|-------|---|---|---|----|----|----|----|----|
| Group 1a | PAV-866   |     | 174.2 | 167.1 | 151.8 | 147.5 | 141   | 131   | 119.8 | / | / | / | /  | /  | /  | /  | /  |
| Group 2a | Diluent   |     | 171.5 | 170.9 | 154.4 | 147.6 | 137.1 | 126.9 | 115.4 | / | / | / | /  | /  | /  | /  | /  |
| Group 3a | Untreated |     | 182.6 | 181   | 162.7 | 158.6 | 145.2 | 132.4 | 122.6 | / | / | / | /  | /  | /  | /  | /  |
| Group 1b | PAV-866   |     | 52.4  | 50    | 44.4  | 43    | /     | /     | /     | / | / | / | /  | /  | /  | /  | /  |
| Group 2b | Diluent   |     | 48.9  | 48.4  | 43.6  | 43.5  | /     | /     | /     | / | / | / | /  | /  | /  | /  | /  |
| Group 3b | Untreated |     | 52.8  | 51.7  | 47.2  | 44.9  | /     | /     | /     | / | / | / | /  | /  | /  | /  | /  |

| Clinical Score | Compound  | Day | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|----------------|-----------|-----|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|
| Group 1a       | PAV-866   |     | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Group 2a       | Diluent   |     | 0 | 1 | 2 | 2 | 2 | 3 | 4 | / | / | / | /  | /  | /  | /  | /  |
| Group 3a       | Untreated |     | 0 | 1 | 2 | 2 | 2 | 3 | 4 | / | / | / | /  | /  | /  | /  | /  |
| Group 1b       | PAV-866   |     | 0 | 0 | 2 | 2 | / | / | / | / | / | / | /  | /  | /  | /  | /  |
| Group 2b       | Diluent   |     | 0 | 0 | 2 | 2 | / | / | / | / | / | / | /  | /  | /  | /  | /  |
| Group 3b       | Untreated |     | 0 | 1 | 2 | 2 | / | / | / | / | / | / | /  | /  | /  | /  | /  |

| Survival | Compound  | Day | 0   | 1   | 2   | 3   | 4   | 5   | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|----------|-----------|-----|-----|-----|-----|-----|-----|-----|---|---|---|---|----|----|----|----|----|
| Group 1a | PAV-866   |     | 100 | 100 | 100 | 100 | 100 | 100 | 0 | / | / | / | /  | /  | /  | /  | /  |
| Group 2a | Diluent   |     | 100 | 100 | 100 | 100 | 100 | 100 | 0 | / | / | / | /  | /  | /  | /  | /  |
| Group 3a | Untreated |     | 100 | 100 | 100 | 100 | 100 | 100 | 0 | / | / | / | /  | /  | /  | /  | /  |
| Group 1b | PAV-866   |     | 100 | 100 | 100 | 100 | /   | /   | / | / | / | / | /  | /  | /  | /  | /  |
| Group 2b | Diluent   |     | 100 | 100 | 100 | 100 | /   | /   | / | / | / | / | /  | /  | /  | /  | /  |
| Group 3b | Untreated |     | 100 | 100 | 100 | 100 | /   | /   | / | / | / | / | /  | /  | /  | /  | /  |

\*All mice were euthanized on Day 6 between AM and PM checks due to clinical scores.





While there was indication, as indicated by clinical score, of a slight improvement in condition for animals dosed with PAV-866 on days 3-6, the improvement was not enough to allow the study to continue past day 6 and all animals were euthanized prematurely. As such, no therapeutic value of PAV-866 was seen against *in vivo* animals dosed with the compound and compared to control vehicle/non-treatment. While unsuccessful, all deliverables for this Aim were met.

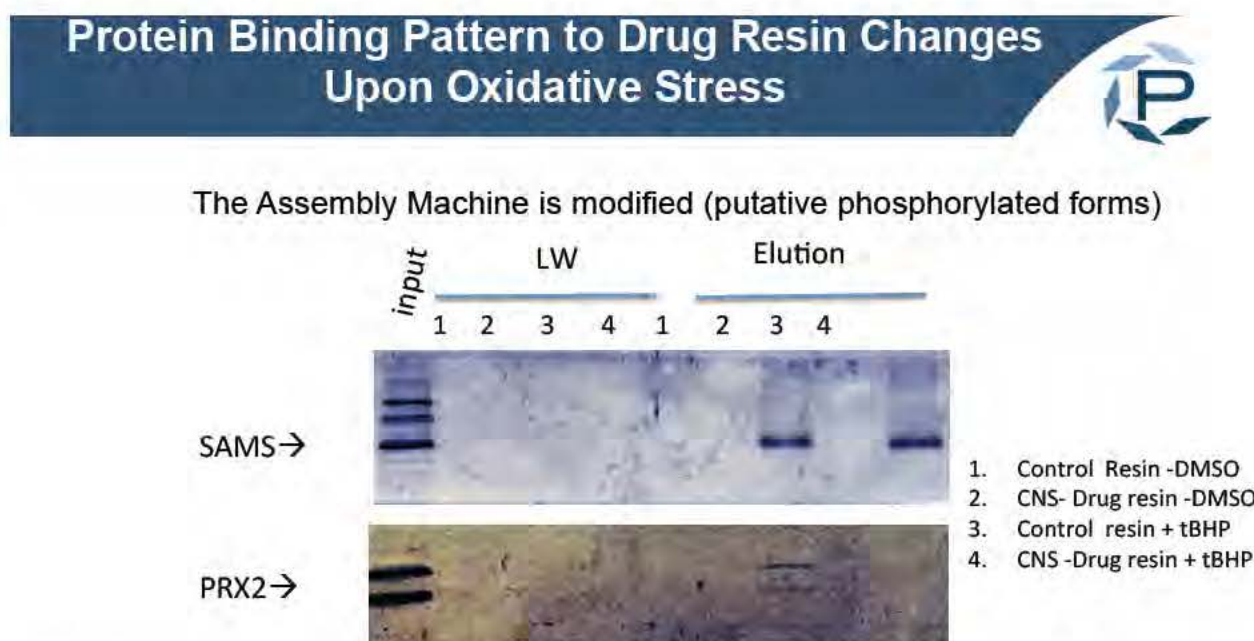
**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

Over the contract duration, much progress was made in the characterization and understanding of the host protein targets and mechanism of actions of several lead antiviral compound analogs. In order to facilitate these studies, a new approach to target protein identification was developed in house at Prosetta. This new approach allowed Prosetta to explore the interaction between specific host proteins known to be involved in capsid assembly, allowing for the resultant effect of this interaction to be assessed. Using several analogs to the active lead chemical compound, Prosetta showed that the analog compounds were binding allosteric sites as opposed to directly binding the active sites involved in the capsid assembly process. This was an exciting breakthrough by according with current thinking found in the literature that allosteric sites may prove to be the most druggable targets for protein-protein interactions.

Building on this insight, Prosetta also showed that the time of action of active anti-EBOV analog compounds points to early and discrete substrate recognition times by the capsid-assembly machine. Prosetta was able to pinpoint this early recognition timepoint to the time when the nascent chain is still bound to the ribosome, and no longer associated with t-RNA.

Prosetta also showed, as seen in figure 5 below, that the putative assembly machines under study are discrete complexes, leading to appreciation of the diversity of assembly machines relevant for capsid assembly. With this insight into the heterogeneity of said targets, a method for using the lead antiviral compound analogs to characterize said heterogeneity was initiated by Prosetta.

Figure 5:



Published literature has demonstrated a critical role for oxidative stress in the pathogenesis of Ebola virus. Cells were treated with 25  $\mu$ M tBHP (Tert-Butyl Hydroperoxide) for 4 hrs, washed with ice cold PBS, Cell extract (post mitochondrial supernatant) was prepared and loaded onto Control and drug resin, incubated at 4 C for 1 hr, washed and eluted with free drug. Western blot with SAM Synthetase and Peroxiredoxin 2 antibodies. SAMS shows some increased binding whereas PRX2 binding pattern is completely changed. Thus, the assembly machines respond to oxidative stress by reorganizing their composition. This change could mimic either defensive or pathological consequences of viral takeover of cellular machinery. Both could be, in principle, worthy antiviral targets.

Proprietary to Prosetta Inc

Lastly, to address the conundrum that several identified host factors appear to be used in multiple steps of the capsid assembly pathway, Prosetta piloted an addition approach to Target ID involving expressing the viral nucleoprotein(s) in the cell-free synthesis systems and then applying the expressed proteins to drug resins. This would allow for a means to identify which assembly intermediates contain host factors bound to newly synthesized (and radiolabeled) viral glycoprotein.

All deliverables for this Aim were successfully met.

#### **Final Summary and Conclusions:**

The entire run of this contract, including two no-cost extensions, demonstrated significant and meaningful progress by Prosetta towards identification and characterization of a novel antiviral compound efficacious against EBOV, RVFV, LASV, and CCFV. Working closely with Pam Glass and her team at USAMRIID and using her *in vitro* VEEV cell culture assay as a surrogate assay, hundreds of compounds were assessed and investigated towards the ultimate contract goal of a successful molecule. Along the way, impressive amounts of insight and method development were gained for the purpose of target identification and method of action characterization purposes, allowing Prosetta to amass a broad understanding of the lead anti-EBOV pharmacophore studied for this contract. Hampered by time and scheduling difficulties, Prosetta was only able to conduct one initial *in vivo* animal study with a single nominated lead compound. While there was a hint of improvement in clinical condition with animals dosed with PAV-866, the animal study was ultimately unsuccessful due to early termination of animals. Yet Prosetta remains confident in the science and methodologies used to nominate the initial lead compound and hopeful that further studies conducted with more time using additional potential lead compound analogs will yield more fruitful antiviral results.

**ADAPT ANNUAL RESEARCH STATUS UPDATE 8/1/2012-7/31/2013**  
**Update of Progress by Tasks (see Summary and Conclusions below):**

**Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

70 new analogs designed for optimized capsid assembly inhibition were synthesized and screened during the last year of the contract. Analogs showing promising inhibition profiles in initial screening were advanced for assessment against infectious virus in cell culture and toxicity characterization. (See quarterly reports below for representative data)

**Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.**

*In vitro* safety and pharmacokinetics studies have essentially been on hold during the last three quarters of this contract since compounds have been advanced to the stage that they are ready for testing in animals. This requires scheduling of the BSL-4 hoods at USAMRIID. In order to do this, we are awaiting the decision of the 9-month contract extension requested last month.

**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

While awaiting the results of our 9-month contract extension request, additional infectious virus in cell culture testing has been underway at USAMRIID. See quarterly reports below for representative data. As soon as a decision is given on the contract extension, lead compounds for animal testing will be nominated based on this data.

**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

During Quarter 7, a new approach to Target ID was developed through which the interaction between specific host proteins involved in capsid assembly, and the resultant effect of this on said host proteins, can be assessed. This allowed us to show disruption of a specific protein-protein interaction using analogs of compounds active against EBOV. We also showed that these analog compounds bind allosteric sites, as opposed to active sites, involved in the capsid assembly process. This is in accordance with current thinking in the literature that allosteric sites may prove to be the most-druggable targets where host protein-protein interactions are concerned.

During Quarter 8, new insight was gained into time of action of active anti-EBOV compound analogs. Results have pointed to an early and discrete time of substrate recognition by the capsid-assembly machine, specifically when the nascent chains are still ribosome bound, but no longer t-RNA associated. This specific observation has led to additional experiments to determine whether said observation is one of several possible binding events or if it is the general timing event for this studied capsid-assembly machine substrate binding.

Additionally, in Quarter 8 we showed that the putative assembly machines being studied are discrete complexes. This gives insight into the diversity of assembly machines relevant for capsid assembly (specifically EBOV capsid assembly) and provides a path forward for using anti-EBOV analogs to characterize said heterogeneity of targets.

During Quarter 9, we piloted a new approach to Target ID, which is to express the viral nucleoprotein(s) in the cell-free system and *then* apply them to the drug resins, as a means to identifying which assembly intermediates contain host-factor bound to (radiolabeled) newly synthesized viral glycoprotein. Initial studies suggest this to be a promising method for addressing the dilemma that some host factors appear to be used at multiple steps in the capsid assembly pathway.

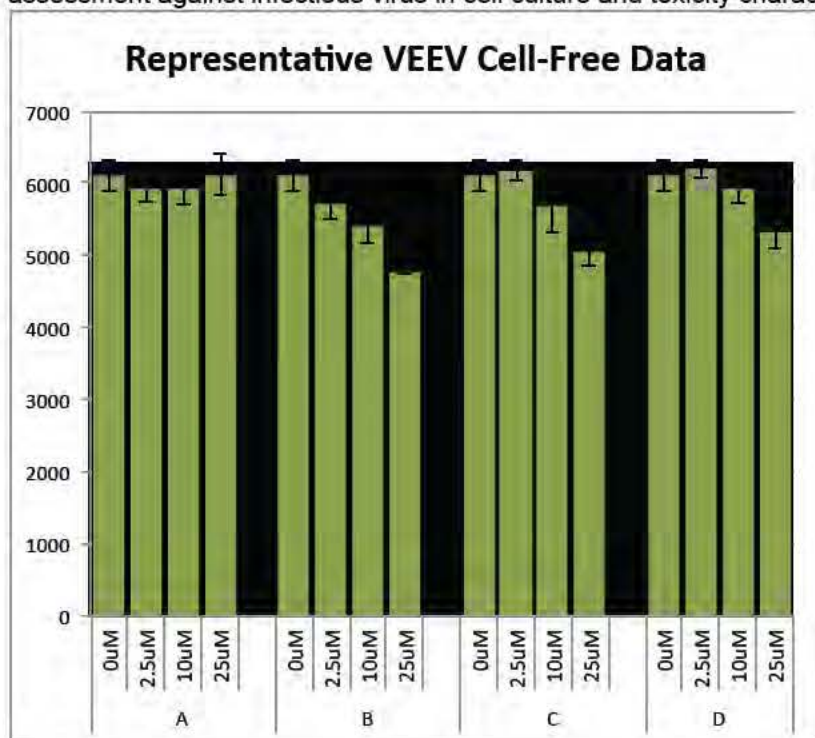
Further Target ID studies will commence as soon as our chemists are finished synthesizing new drug resins.

**Annual Summary and Conclusions:** The past year of this contract has shown significant progress towards identification and characterization of the targets of the lead series compounds we are working with. The bulk of the remaining work involves animal validation of these compounds at the USAMRIID labs. This continues to be reliant on the decision of the proposed additional 9-month contract extension. Unfortunately it has proven difficult to finish the animal validation studies in our initially proposed timeline, but we remain confident in the potency and effectiveness of our lead compounds and are committed to finishing the animal studies to fulfill the aims of this contract.

### **ADAPT QUARTER 9 RESEARCH STATUS UPDATE** **Update of Progress by Tasks (see Summary and Conclusions below):**

**Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

17 new analogs designed for optimized capsid assembly inhibition were synthesized and screened during the latest quarter of the contract. Analogs showing promising inhibition profiles in initial screening were advanced for assessment against infectious virus in cell culture and toxicity characterization.

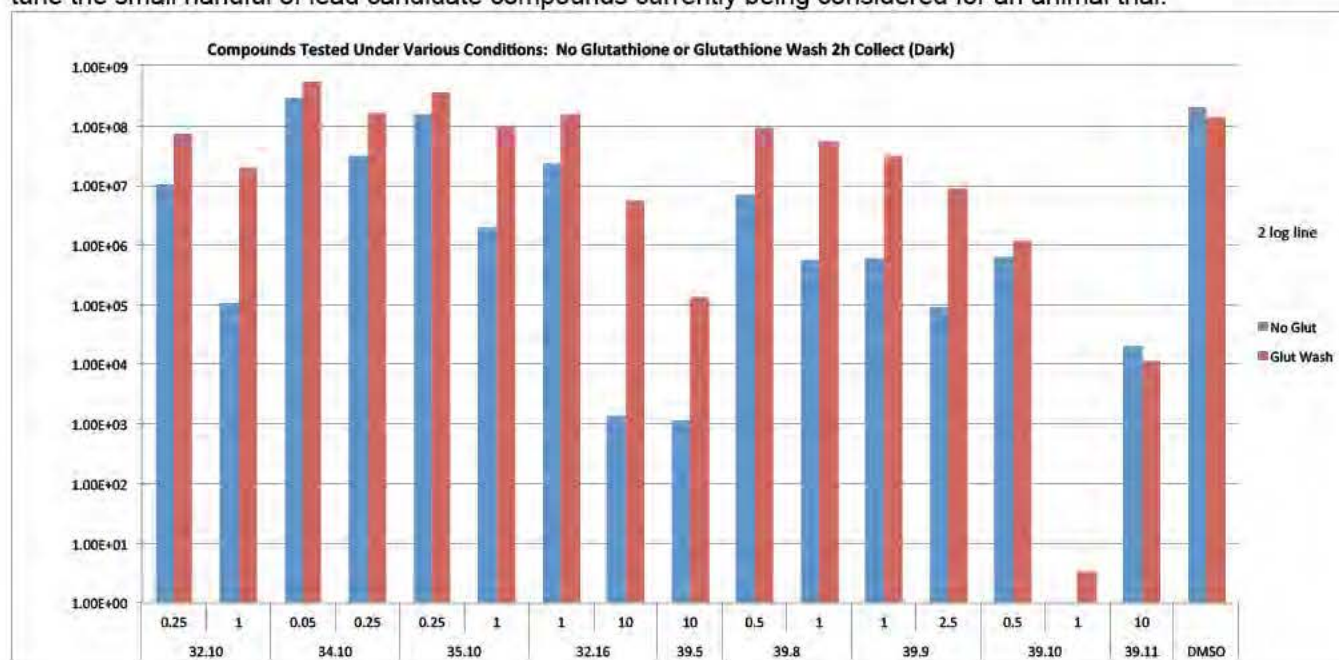


**Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.**

*In vitro* and *in vivo* safety and pharmacokinetics have been initiated during the latest quarter of the contract. Results are pending and will be reported in next month's update.

**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

Consultation with USAMRIID is continuing regarding planned animal studies once BSL-4 hood space becomes available. In the meantime, additional rounds of infectious virus in cell culture testing are being conducted to fine-tune the small handful of lead candidate compounds currently being considered for an animal trial.



**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

During this latest quarter, we have piloted a new approach to Target ID, which is to express the viral nucleoprotein(s) in the cell-free system and *then* apply them to the drug resins, as a means to identifying which assembly intermediates contain host-factor bound to (radiolabeled) newly synthesized viral glycoprotein. Initial studies suggest this to be a promising method for addressing the dilemma that some host factors appear to be used at multiple steps in the capsid assembly pathway.

Further work using the novel approach described above has provided new insight into assembly machine heterogeneity in uninfected and infected cells. We now have evidence that host-multiprotein-complex

heterogeneity is far greater than we or other had previously appreciated, and that the Prosetta compounds target allosteric sites. Our estimate is between 1,000-10,000 variants of host-assembly machines existing in uninfected cells. An essential event in infection is the virus's identification of the one machine in this diversity that can be adapted to assembly of its capsid. With SAR resulting in analogs of greater and greater potency, we bind assembly machines not only more tightly, but also more selectively. Thus, extension of this approach should result in the most-potent, least-toxic compound being most selective for the specific aberrant assembly machine commandeered by the virus. In other words, viral disease can be viewed as disordered homeostasis set right by the Prosetta compounds.

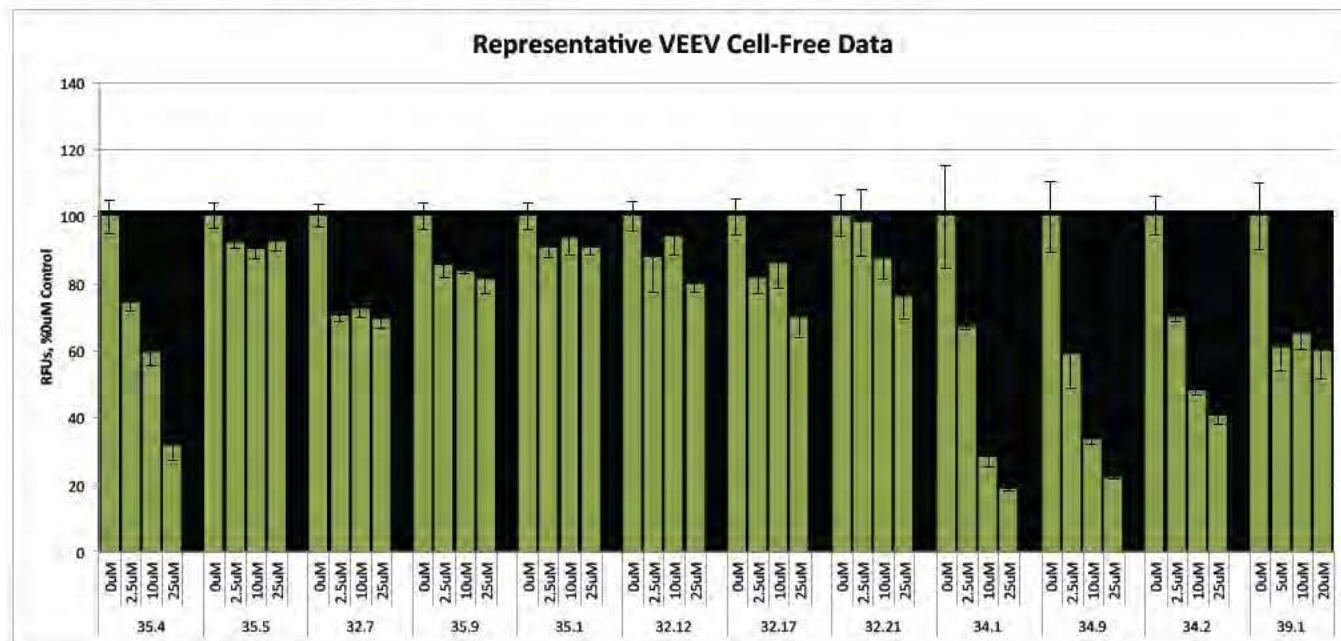
We have also reproducibly shown that advanced compounds preferentially elute viral capsid protein containing machines as compared to earlier analogs. In addition to corroborating our working hypothesis, this finding makes possible new approaches to quantitative SAR optimization.

### ADAPT QUARTER 8 RESEARCH STATUS UPDATE

#### Update of Progress by Tasks (see Summary and Conclusions below):

#### **Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

19 new analogs designed for optimized capsid assembly inhibition were synthesized and screened during the latest quarter of the contract. Analogs showing promising inhibition profiles in initial screening were advanced for assessment against infectious virus in cell culture and toxicity characterization.



#### **Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.**

No additional studies for *in vitro* and *in vivo* safety and pharmacokinetics have been conducted during the last quarter of the contract. At this stage in the contract, compounds are undergoing final rounds of infectious virus testing in cell culture in preparation for testing in animals. Animal testing is awaiting scheduling of BSL-4 hood space at USAMRIID to initiate.

**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

Consultation with USAMRIID is continuing regarding planned animal studies once BSL-4 hood space becomes available. In the meantime, additional rounds of infectious virus in cell culture testing is being conducted to ensure that compounds chosen as candidates for animal studies have the best possible selectivity index based on their EC<sub>50</sub> and CC<sub>50</sub> profiles.



**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

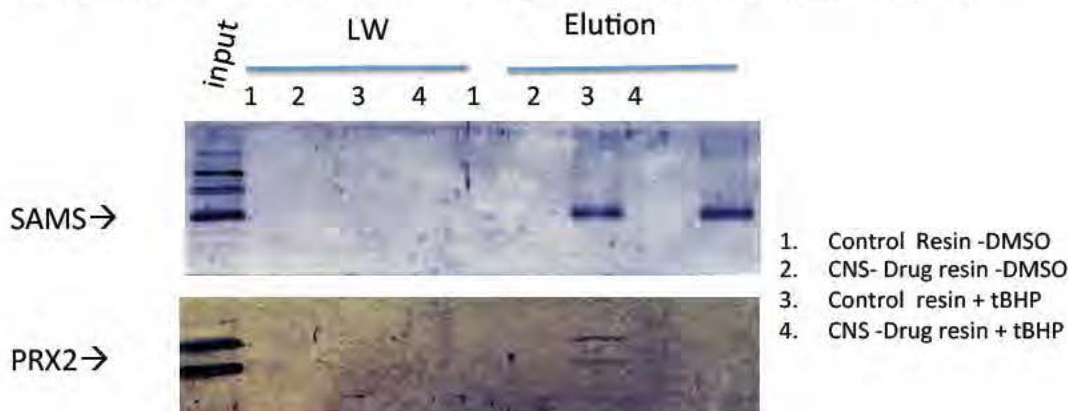
During Quarter 8, new insight was gained into time of action of active anti-EBOV compound analogs. Results have pointed to an early and discrete time of substrate recognition by the capsid-assembly machine, specifically when the nascent chains are still ribosome bound, but no longer t-RNA associated. This specific observation has lead to additional experiments to determine whether said observation is one of several possible binding events or if it is the general timing event for this studied capsid-assembly machine substrate binding.

Additionally, in Quarter 8 we have shown that the putative assembly machines being studied are discrete complexes. This gives insight into the diversity of assembly machines relevant for capsid assembly (specifically EBOV capsid assembly) and provides a path forward for using anti-EBOV analogs to characterize said heterogeneity of targets.

## Protein Binding Pattern to Drug Resin Changes Upon Oxidative Stress



The Assembly Machine is modified (putative phosphorylated forms)



Published literature has demonstrated a critical role for oxidative stress in the pathogenesis of Ebola virus. Cells were treated with 25  $\mu$ M tBHP (Tert-Butyl Hydroperoxide) for 4 hrs, washed with ice cold PBS, Cell extract (post mitochondrial supernatant) was prepared and loaded onto Control and drug resin, incubated at 4 C for 1 hr, washed and eluted with free drug. Western blot with SAM Synthetase and Peroxiredoxin 2 antibodies. SAMS shows some increased binding whereas PRX2 binding pattern is completely changed. Thus, the assembly machines respond to oxidative stress by reorganizing their composition. This change could mimic either defensive or pathological consequences of viral takeover of cellular machinery. Both could be, in principle, worthy antiviral targets.

Proprietary to Prosetta Inc

### ADAPT QUARTER 7 RESEARCH STATUS UPDATE

#### Update of Progress by Tasks (see Summary and Conclusions below):

**Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

16 new analogs designed for optimized capsid assembly inhibition were synthesized and screened during the last quarter of the contract. Analogs showing promising inhibition profiles in initial screening were advanced for assessment against infectious virus in cell culture and toxicity characterization. (See quarterly reports below for representative data)

**Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.**

No additional studies for *in vitro* and *in vivo* safety and pharmacokinetics have been conducted during the last quarter of the contract. At this stage in the contract, compounds are ready to be tested in animals and are awaiting scheduling of BSL-4 hood space at USAMRIID to initiate.

**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and –CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

Consultation with USAMRIID is continuing regarding planned animal studies once BSL-4 hood space becomes available.

**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

During Quarter 7, a new approach to Target ID was developed through which the interaction between specific host proteins involved in capsid assembly, and the resultant effect of this on said host proteins, can be assessed. This allowed us to show disruption of a specific protein-protein interaction using analogs of compounds active against EBOV. We also showed that these analog compounds bind allosteric sites, as opposed to active sites, involved in the capsid assembly process. This is in accordance with current thinking in the literature that allosteric sites may prove to be the most-druggable targets where host protein-protein interactions are concerned.

**ADAPT ANNUAL RESEARCH STATUS UPDATE****Update of Progress by Tasks (see Summary and Conclusions below):****Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

168 new analogs designed for optimized capsid assembly inhibition were synthesized and screened during the last year of the contract. Analogs showing promising inhibition profiles in initial screening were advanced for assessment against infectious virus in cell culture and toxicity characterization. (See quarterly reports below for representative data)

**Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.**

Over 85 analogs have been assessed for *in vitro* safety and pharmacokinetics during the past year of the contract. Studies included testing for PK and TK properties, PBS stability, plasma stability, RLM half-life, ADME, and rat liver microsome stability. These studies have been halted for the time being until final compound nominations for the animal trials at USAMRIID are made. (See quarterly reports below for representative data)

**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and –CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

Over 25 analogs to compounds showing anti-Ebola efficacy were assessed against infectious virus in cell culture during the past year of this contract. Since the bulk of the remaining work on the contract involves BSL-4 studies at USAMRIID, the critical next step is scheduling of time in the BSL-4 suite. With the approval of the 12-month no cost extensions, discussions with USAMRIID staff in this regards have been initiated.

**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

During the past year of this contract, significant progress has been made on target identification. Three new target ID protocols were developed, corroboration of the drug targets identified in the cell-free system using cultured mammalian cells was achieved, further supporting the validity and applicability of our cell-free target

identification approach, previously generated host factor columns were used to further identify putative targets, giving insight into mechanism of action of analog compounds, and three proteins implicated in the viral life cycle were identified using drug-affinity chromatography as part of the multi-protein complex being studied.

**Annual Summary and Conclusions:** The past year of this contract has shown continued progress towards identification and optimization of viable compound analogs with respect to both safety and efficacy. The bulk of the remaining work involved animal validation of these viable compounds at the USAMRIID labs. A 12-month no-cost extension was granted for this contract in order to achieve these animal studies. As soon as scheduling issues with the USAMRIID BSL-4 laboratory space is sorted out, these animal studies will commence.

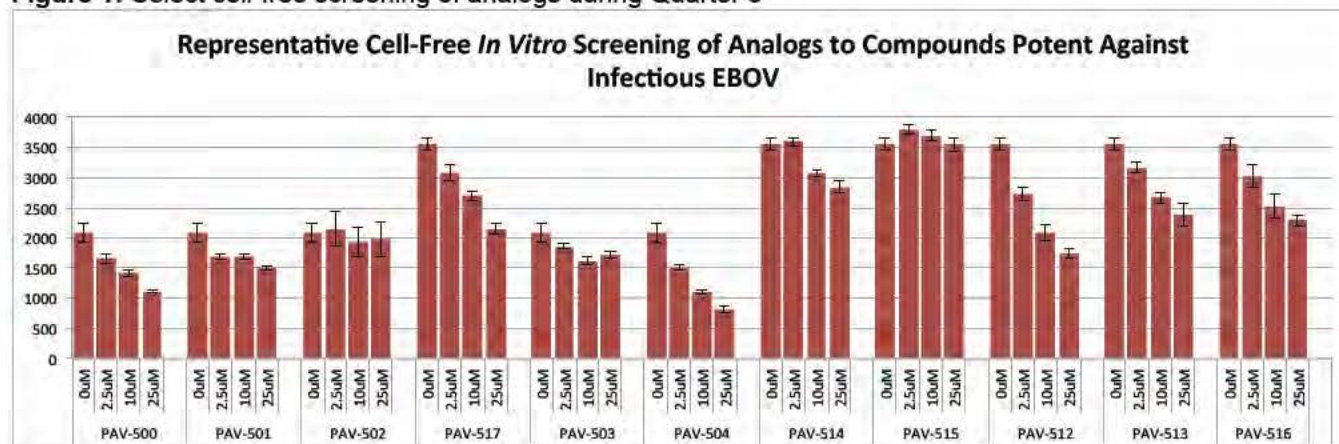
## ADAPT Q5 RESEARCH STATUS UPDATE

### Update of Progress by Tasks (see Summary and Conclusions below):

**Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

Forty-six new analogs designed for optimized capsid assembly inhibition were synthesized and screened during Quarter 5. Analogs showing promising inhibition profiles in initial screening were advanced for assessment against infectious virus in cell culture.

**Figure 1: Select cell-free screening of analogs during Quarter 5**



**Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.**

Over 25 analogs have been assessed for *in vitro* safety and pharmacokinetics during Quarter 5. Studies included testing for PK and TK properties, PBS stability, plasma stability, RLM half-life, ADME, and rat liver microsome stability. Analogs are continuously being scaled-up to sufficient quantities to enable *in vivo* safety and PK assessment in small rodents.

**Figure 2: Representative PK Properties of PAV-343**

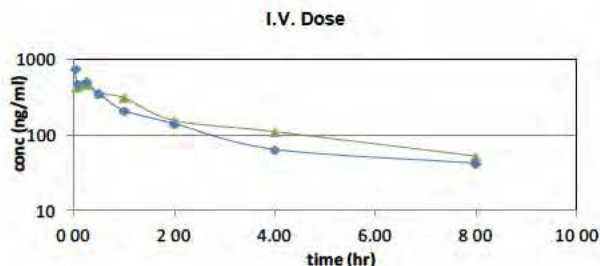


Table 1: Representative PK Overview Table PAV-343

| Route | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub> (hr) | 1/2-Life (hr) | CL<br>(mL/min/kg) | AUC <sub>last</sub><br>(hr*ng/mL) | BioAvail.<br>(P%) |
|-------|-----------------------------|-----------------------|---------------|-------------------|-----------------------------------|-------------------|
| IV    | 594                         | na                    | 3.1           | 13                | 1095                              | na                |
| PO    | 0                           | 0.0                   | 0.0           | 0                 | 0                                 | 0                 |

Figure 3: Representative PK Properties of PAV-442

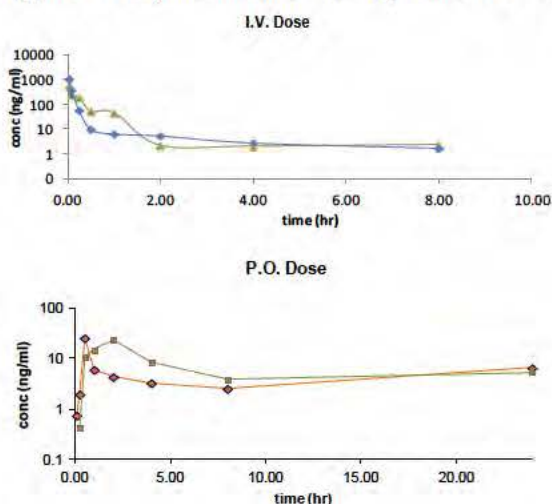


Table 2: Representative PK Overview Table PAV-442

| Route | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub> (hr) | 1/2-Life (hr) | CL<br>(mL/min/kg) | AUC <sub>last</sub><br>(hr*ng/mL) | BioAvail.<br>(P%) |
|-------|-----------------------------|-----------------------|---------------|-------------------|-----------------------------------|-------------------|
| IV    | 851                         | na                    | 5.6           | 91                | 169                               | na                |
| PO    | 18.2                        | 0                     | 0             | 0                 | 135                               | 18.1              |

**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

Twelve analogs to compounds showing anti-Ebola efficacy were assessed against infectious virus in cell culture during Quarter 5. Based on efficacy results from these tests, further small animal model challenges will be designed and implemented.

**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

During Quarter 5 of this contract, significant progress has been made on target identification. Three new target ID protocols were developed, allowing us insight into a) how the compounds we are developing can both target the host and still show improvements in potency with diminution of toxicity concurrently, b) distinguishing allosteric and active target binding sites, and c) the possibility of pinpointing the time of action of our compounds. We have also achieved functional reconstitution using the cell-free ELISA screen with target-enriched glycerol gradient fractions, significant insight into the lability of our unconventional drug targets, and further insight into the energy dependence of these complexes in that addition of energy substrates substantially stimulates the complexes and induces post-translational modifications of some of their components. Finally, we have achieved corroboration of the drug targets identified in the cell-free system using cultured mammalian cells, further supporting the validity and applicability of our cell-free target identification approach.

**Quarter 5 Summary and Conclusions:** The fifth quarter has shown continued progress towards identification and optimization of viable compound analogs with respect to both safety and efficacy. Progress has also been made in the identification of binding proteins and their validation in cell-free and cellular systems. In addition, progress has been made in understanding the mechanism of action of these unconventional targets.

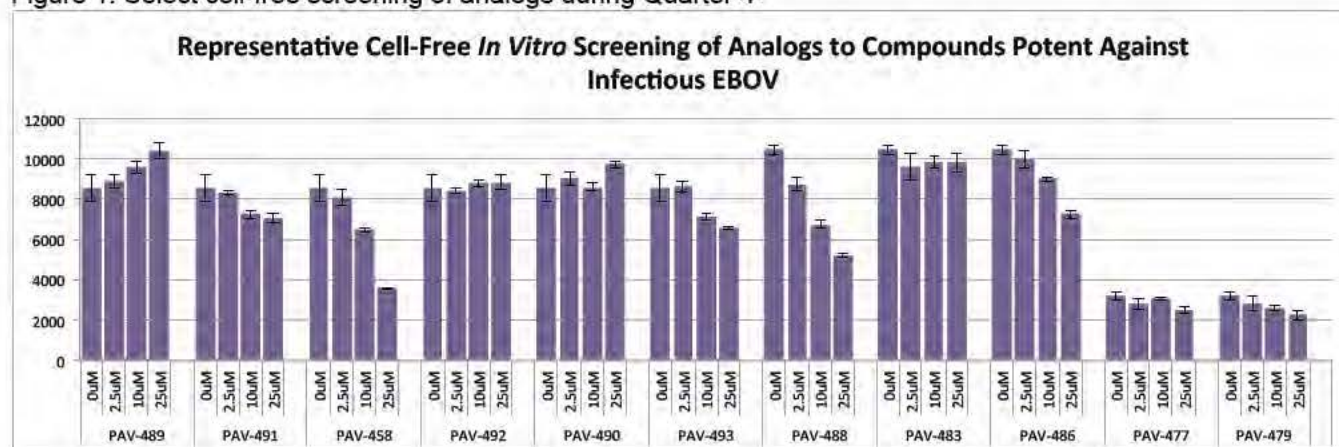
## ADAPT Q4 RESEARCH STATUS UPDATE

**Update of Progress by Tasks (see Summary and Conclusions below):**

**Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

39 new analogs designed for optimized capsid assembly inhibition were synthesized and screened. Analogs showing promising inhibition profiles in initial screening were advanced for assessment against infectious virus in cell culture.

Figure 1: Select cell-free screening of analogs during Quarter 4



**Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.**

Over 30 analogs have been assessed for *in vitro* safety and pharmacokinetics during quarter four. Studies included testing for PK and TK properties, PBS stability, plasma stability, RLM half-life, ADME, and rat liver microsome stability. Analogs are continuously being scaled-up to sufficient quantities to enable *in vivo* safety and PK assessment in small rodents.

**Representative *in vitro* and *in vivo* data for Aim 2 in Quarter 4:**

W911NF-11-C-0059

Table 1: PAV-772 PK Summary Table

| Route | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub><br>(hr) | 1/2-Life<br>(hr) | CL<br>(mL/min/kg) | AUC <sub>last</sub><br>(hr*ng/mL) | BioAvail<br>(%) |
|-------|-----------------------------|--------------------------|------------------|-------------------|-----------------------------------|-----------------|
| IV    | 947                         | na                       | 1.5              | 40                | 410                               | na              |
| PO    | 441                         | 0.1                      | 1.0              | 256               | 322                               | 16              |
| IP    | 625                         | 0.1                      | 1.4              | 143               | 579                               | 27              |

Figure 2: Plots of PAV-772 PK Properties

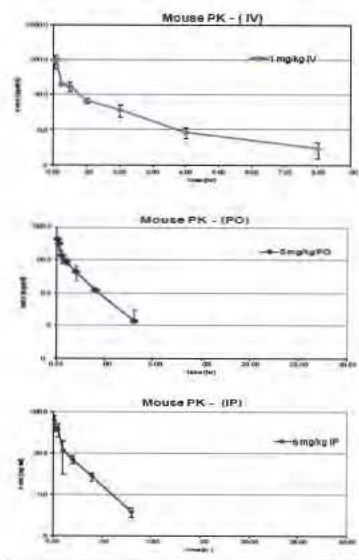


Table 2: PAV-772 TK Summary Table

| Study Day | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub><br>(hr) | T 1/2<br>(hr) | Cl<br>(mL/min/kg) | AUC <sub>last</sub><br>(hr*ng/mL) |
|-----------|-----------------------------|--------------------------|---------------|-------------------|-----------------------------------|
| 1         | 1917                        | 0.3                      | 1.3           | 108               | 1525                              |
| 10        | 921                         | 0.3                      | 1.8           | 136               | 1163                              |

Table 3: PAV-337 PK Summary Table

| Route | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub><br>(hr) | 1/2-Life<br>(hr) | CL<br>(mL/min/kg) | AUC <sub>last</sub><br>(hr*ng/mL) | BioAvail<br>(%) |
|-------|-----------------------------|--------------------------|------------------|-------------------|-----------------------------------|-----------------|
| IV    | 596                         | na                       | 2.5              | 62                | 218                               | na              |
| PO    | 31                          | 0.5                      | 5.9              | 536               | 144                               | 12              |
| IP    | 137                         | 1.0                      | 3.0              | 64                | 1136                              | 25              |

Table 4: PAV-337 TK Summary Table

| Study Day | C <sub>max</sub><br>(ng/mL) | T <sub>max</sub><br>(hr) | T 1/2<br>(hr) | Cl<br>(mL/min/kg) | AUC <sub>last</sub><br>(hr*ng/mL) |
|-----------|-----------------------------|--------------------------|---------------|-------------------|-----------------------------------|
| 1         | 173                         | 1.0                      | 18.5          | 34                | 974                               |
| 10        | 49                          | 0.3                      | 15.7          | 215               | 216                               |

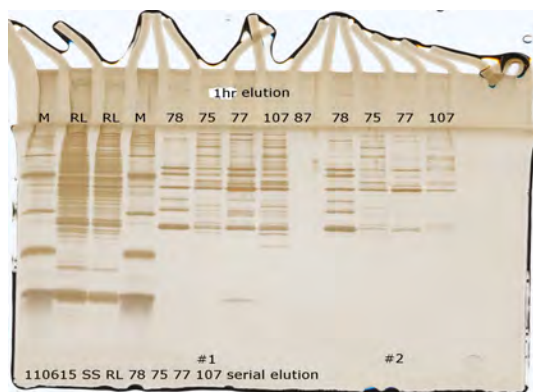
**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

Eleven analogs to compounds showing anti-Ebola efficacy were assessed against infectious virus in cell culture during Quarter 4. Based on efficacy results from these tests, further small animal model challenges will be designed and implemented.

**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

During Quarter 4, previously generated host factor columns were used to further identify putative targets, giving insight into mechanism of action of analog compounds. Three proteins implicated in the viral life cycle were identified using drug-affinity chromatography as part of the multi-protein complex being studied. Identification of these proteins could provide immense value to current and future EBOV viral research and help in gaining a much fuller understanding of the pathogenesis of infection.

Figure 3: Serial Elutions using Analogs to Compounds Showing anti-EBOV Efficacy



Legend to Figure 3: Each column represents a drug affinity ligand used to identify the functional host protein complexes found in rat lung that play a key role in viral capsid assembly. The 'control direct binding' reveals the protein pattern when rat lung extract is loaded directly onto each column and eluted with 100ug/mL of respective free compound after one hour. On the 'flow through (FT) serial binding', the rat liver extract was first loaded onto column 78, then passed serially to columns 75, then 77 and lastly 102. The columns were then eluted with 100ug/mL of respective free compound for one hour and the pattern shown illustrates the binding affinity of the different columns.

Col 78: PAV-8697

Col 75: PAV-8703

Col 77: PAV-8688

Col 107: 8702

Col 87: Control

**Annual Summary and Conclusions:** The fourth quarter has shown continued progress towards identification and optimization of viable compound analogs with respect to both safety and efficacy. Significant progress has been made in the identification of three proteins implicated in the ebola viral life cycle. Upon gaining additional data from our collaborators on-going studies regarding infectious virus in cell culture studies, further animal work will be planned for the coming quarters.

### ADAPT Q3 RESEARCH STATUS UPDATE

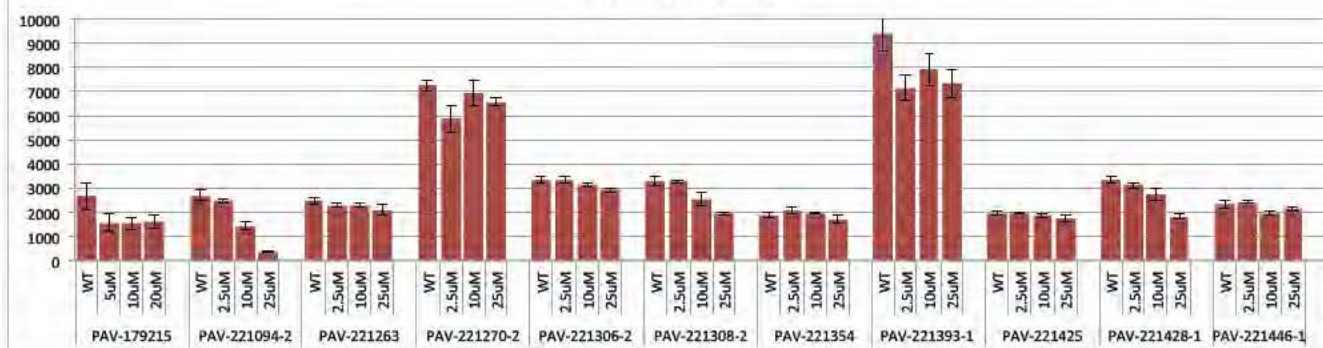
#### Update of Progress by Tasks (see Summary and Conclusions below):

**Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

46 new analogs designed for optimized capsid assembly inhibition were synthesized and screened. Analogs showing promising inhibition profiles in initial screening were advanced for assessment against infectious virus in cell culture.

Figure 1: Select cell-free screening of analogs during Quarter 3

### Representative Cell-Free *In Vitro* Screening of Analogs to Compounds Potent Against Infectious EBOV



### Aim 2: Optimize Lead Chemical Series for *in vitro* and *in vivo* safety and pharmacokinetics.

Over 20 analogs have been assessed for *in vitro* safety and pharmacokinetics during quarter three. Studies included testing for PK and TK properties, PBS stability, plasma stability, RLM half-life, and ADME. Summary of these data lead to key observations including:

1. Analogs of PAV-866 were less stable in the *in vitro* PK assays (plasma and PBS stability) due to substitutions in the 3 position of the molecule
2. The *in vivo* data supports the *in vitro* results for metabolic stability. A metabolic soft spot was identified.
3. Additional modifications in the 3-position of the molecule are underway to increase the metabolic stability of this soft spot and optimize the ADME profile

Analogs are continuously being scaled-up to sufficient quantities to enable *in vivo* safety and PK assessment in small rodents.

**Table 1 : Compound Stability**

| PAV#         | PBS Buffer<br>(pH=7.4)<br>% Remaining<br>@37C 30 min | 1mM NADPH<br>% Remaining<br>@37C 5 min | Human Plasma<br>% Remaining<br>@37C 60 min | Rat Liver<br>Microsomes<br>Thalf min |
|--------------|------------------------------------------------------|----------------------------------------|--------------------------------------------|--------------------------------------|
| PAV-221142-1 | 82.5                                                 | 78.2                                   | 98.2                                       | 1.5                                  |
| PAV-221143-1 | 67.9                                                 | 75.3                                   | 100                                        | 3.5                                  |
| PAV-221226-1 | 52.0                                                 | 59.9                                   | 100                                        | 1.5                                  |
| PAV-221340-1 | 8.3                                                  | 68.6                                   | 100                                        |                                      |
| PAV-221341-1 | 33.1                                                 | 50.0                                   | 100                                        |                                      |
| PAV-221342-1 | 88.8                                                 | 30.3                                   | 100                                        |                                      |
| PAV-221343-1 | 69.4                                                 | 61.3                                   | 100                                        | 33.7                                 |
| PAV-221358-1 | 63.5                                                 | 65.3                                   | 99.0                                       | 4.5                                  |
| PAV-221361-1 | 68.1                                                 | 76.9                                   | 64.1                                       |                                      |
| PAV-221373-1 | 46.1                                                 | 64.0                                   | 100                                        |                                      |
| PAV-221374-1 | 78.2                                                 | 23.3                                   | 66.4                                       |                                      |

**Table 2: TK Parameter Summary**

| Compound ID | Group ID       | Study Animal<br>Survival after 10<br>Days (n= 3) | Study Animal<br>Ave. Trough<br>Plasma Level<br>(ng/mL) | t <sup>1/2</sup> (hr) | Tmax (hr) | Cmax (ng/mL) | AUC<br>(hr*ng/mL) |
|-------------|----------------|--------------------------------------------------|--------------------------------------------------------|-----------------------|-----------|--------------|-------------------|
| PAV-178866  | TK (Day1)      | NA                                               | NA                                                     | 46.3                  | 8         | 65           | 449               |
|             | TK (Day10)     | NA                                               | NA                                                     | 54.2                  | 1         | 114          | 778               |
|             | Study (male)   | 3                                                | 67                                                     | NA                    | NA        | NA           | NA                |
|             | Study (female) | 3                                                | 86                                                     | NA                    | NA        | NA           | NA                |
| PAV-221063  | TK (Day1)      | NA                                               | NA                                                     | 3.0                   | 0.3       | 18           | 73                |
|             | TK (Day10)     | NA                                               | NA                                                     | -                     | -         | -            | -                 |
|             | Study (male)   | 0                                                | -                                                      | NA                    | NA        | NA           | NA                |
|             | Study (female) | 0                                                | -                                                      | NA                    | NA        | NA           | NA                |
| PAV-178896  | TK (Day1)      | NA                                               | NA                                                     | 29.8                  | 0.3       | 494          | 1997              |
|             | TK (Day10)     | NA                                               | NA                                                     | 8                     | 0.5       | 190          | 635               |
|             | Study (male)   | 3                                                | 80                                                     | NA                    | NA        | NA           | NA                |
|             | Study (female) | 3                                                | 10                                                     | NA                    | NA        | NA           | NA                |

Table 3 : PK Parameter Summary

| PAV#       | Route | AUC (ng*hr/mL) | C initial | Cl (mL/min/kg) | Cmax (ng/mL) | Thalf (hr) | Tmax (hr) | Vss (L/kg) | Dose (mg/kg) | F (%) | MRT (hr) | Gender | Species | Strain |
|------------|-------|----------------|-----------|----------------|--------------|------------|-----------|------------|--------------|-------|----------|--------|---------|--------|
| PAV-221063 | IV    | 41             | 9.3       | 261            | 9            | 5.8        | -         | 125        | 1            | -     | 3.3      | Male   | Mouse   | Balb/C |
|            | PO    | 47             | -         | -              | 3            | 94         | 24        | -          | 5            | 130   | 14.5     | Male   | Mouse   | Balb/C |
|            | IP    | 31             | -         | -              | 8            | 7.6        | 0.08      | -          | 5            | 25    | 3.1      | Male   | Mouse   | Balb/C |
| PAV-178866 | IV    | -              | 9.8       | -              | 10           | -          | -         | -          | 1            | -     | -        | Male   | Mouse   | Balb/C |
|            | PO    | -              | -         | -              | -            | -          | -         | -          | 5            | -     | -        | Male   | Mouse   | Balb/C |
|            | IP    | 550            | -         | -              | 81           | 81.4       | 24        | -          | 5            | -     | 4.4      | Male   | Mouse   | Balb/C |
| PAV-178896 | IV    | 216            | 201.1     | 63             | 201          | 3.6        | -         | 17         | 1            | -     | 2.3      | Male   | Mouse   | Balb/C |
|            | PO    | 287            | -         | -              | 65           | 6          | 1         | -          | 5            | 41    | 3.4      | Male   | Mouse   | Balb/C |
|            | IP    | 811            | -         | -              | 290          | 290        | 0.5       | -          | 5            | 80    | 2.9      | Male   | Mouse   | Balb/C |
| PAV-178802 | IV    | 2              | 11.4      | 7787           | 11           | 0.2        | -         | 165        | 1            | -     | 0.2      | Male   | Mouse   | Balb/C |
|            | PO    | -              | -         | -              | 1            | -          | 0.08      | -          | 5            | -     | -        | Male   | Mouse   | Balb/C |
|            | IP    | 29             | -         | -              | 22           | 1.1        | 0.25      | -          | 5            | 3     | 1.3      | Male   | Mouse   | Balb/C |

**Aim 3: Validate anti-EBOV, -RVFV, -LASV, and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

Analogous to compounds showing anti-Ebola efficacy are continuously being assessed against infectious virus in cell culture. We are awaiting definitive results from our collaborators. Based on efficacy results from these shipments, further small animal model challenges will be designed and implemented.

**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

During quarter three, we have identified nine host proteins known to be involved in hemorrhagic fever virus life cycles using our drug columns. This provides strong corroboration of the validity of our target identification approach; such a correlation is not likely to occur at random.

**Summary and Conclusions:** Significant progress toward lead optimization based on *in vitro* data was made during first quarter of this contract. The second quarter was marked by progress toward optimizing the lead series for *in vivo* studies. The third quarter has shown great improvement of understanding of the lead series, both with respects to PK and safety profiles as well as target and mechanism of the lead candidates. Upon gaining data from our collaborators on-going studies regarding infectious virus in cell culture studies, further animal work will be planned.

## ADAPT Q2 RESEARCH STATUS UPDATE

**Update of progress by Tasks (see Summary and Conclusions below):**

**Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).**

The results of 'live' virus studies conducted during the previous quarter (data presented in Figure 1, Q1 report) was analyzed and prioritized for animal studies in light of relevant factors, including cell and animal safety, efficacy and bioavailability. See Aim 3, below.

**Aim 2: Optimize lead chemical series for *in vitro* and *in vivo* safety and pharmacokinetics.**

Based on the results of ongoing studies, Prosetta continues to design lead series analogs to optimize antiviral activity, as well as to enhance safety and pharmacokinetics (PK). Based on results of the recent animal studies, see Aim 3, below, additional compound analogue are being synthesized.

**Aim 3: Validate anti-EBOV, -RVFV, -LASV and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

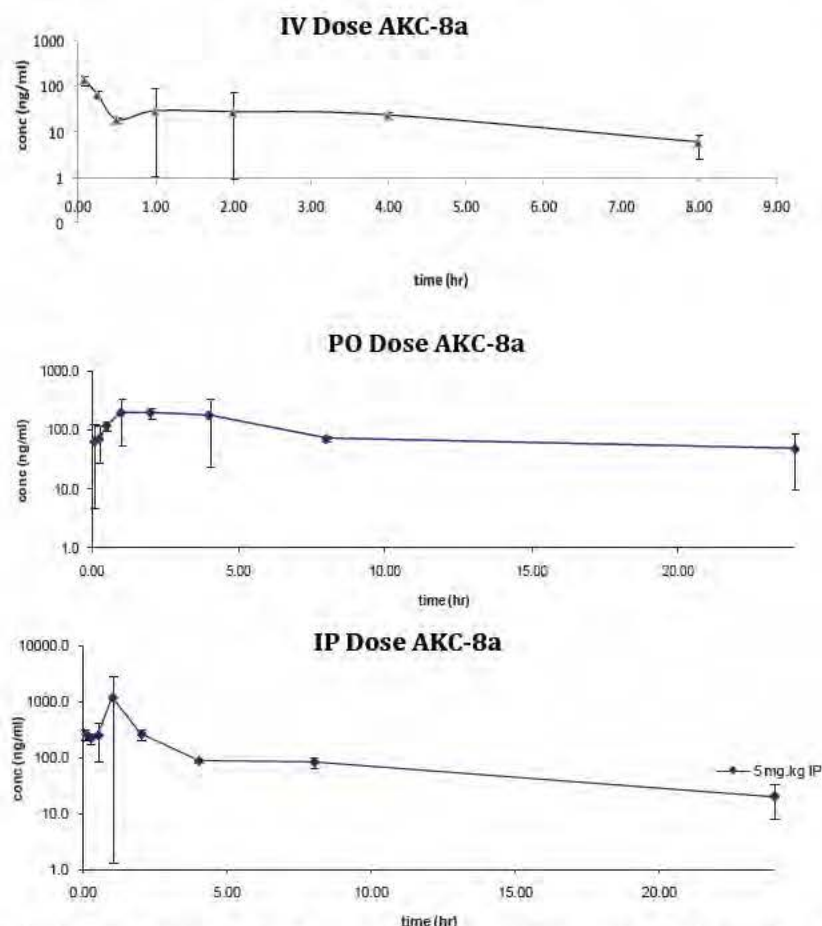
*In vivo* efficacy assessment of a putative pan-HFV compounds has been performed based on compound prioritization resulting from *in vitro* efficacy and *in vivo* safety and pharmacokinetic (PK) studies. Table 1 presents the bioavailability of compound AKC-8a in non-challenged mice via three different routes of administration – intravenous (IV), oral (PO) and intraperitoneal (IP) – and Figure 1 shows the PK curves for these routes.

**Test Article AKC-8a**

| Route | Gender | Observed C <sub>max</sub> (ng/mL) | Observed T <sub>max</sub> (hr) | Half Life (hr) | CL (mL/hr/kg) | AUC last (hr*ng/mL) | Bioavailability (%) |
|-------|--------|-----------------------------------|--------------------------------|----------------|---------------|---------------------|---------------------|
| IV    | male   | 135                               |                                | 3.0            | 78.6          | 212                 |                     |
| PO    | male   | 202                               | 2.0                            | 16.3           |               | 3326                | 314                 |
| IP    | male   | 1225                              | 1.0                            | 9.0            |               | 3018                |                     |

**Table 1: Mouse PK Summary of typical averaged PK parameters**

**Figure 1**



**Legend to Figure 1:** Each graph represents one dose route pharmacokinetic curve based on AKC-8a concentration levels extracted from plasma. The error bars are the standard deviation for each time point (n=3).

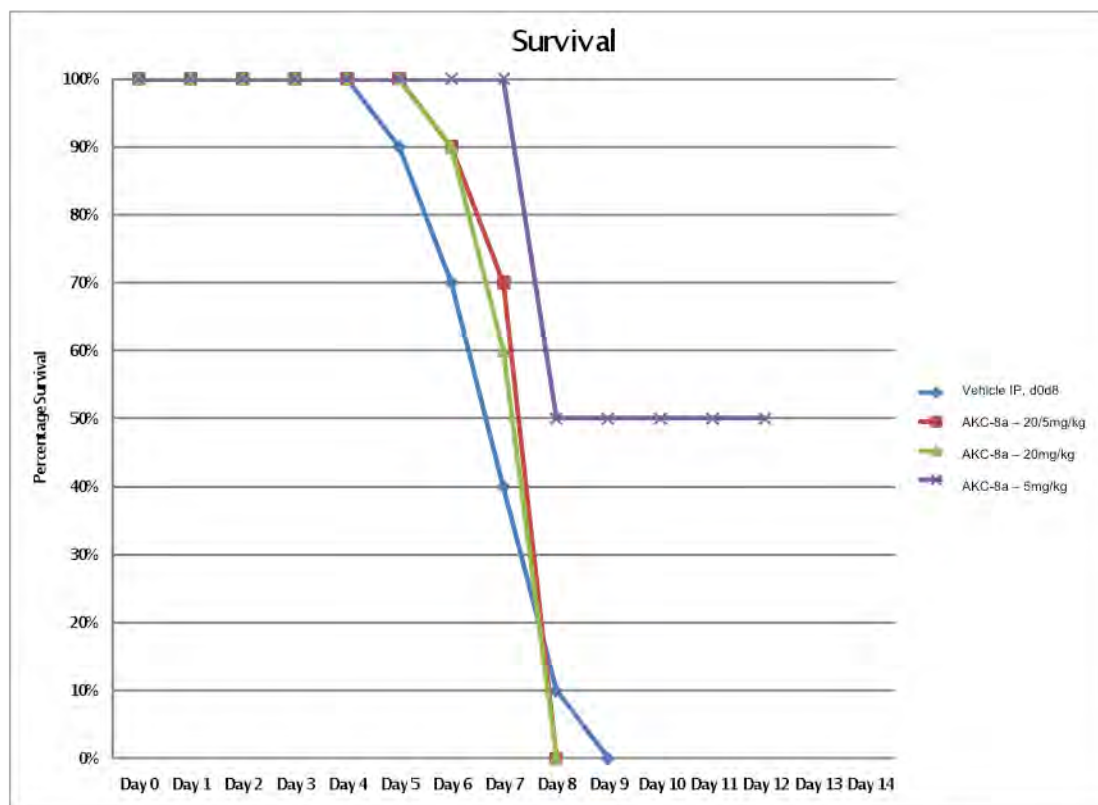
Nine animals per dose route were used – three time points per animal were sampled. The oral bioavailability (F%) is greater than 100%. This suggests that enterohepatic recirculation could be occurring. Clearance (CL) is close to apparent blood liver flow, though there is high variability in the PK points in this data set. Because of the high concentration exposure levels when dosed via PO and IP routes, the data suggests that it is possible to achieve fairly good therapeutic levels in a mouse model using these routes of administration.

Table 2, below, outlines a mouse adapted EBOV- Zaire challenged (1000pfu/mL) pilot study efficacy trial that was performed at USAMRIID by T. Warren, Ph.D. The animals were 8-12 weeks old – C57BL/6 mouse strain. Compound was dosed SID via intraperitoneal (IP) injection at 100uL per animal. After dose regimen, observations with an endpoint at day 14 were recorded. The results for group 4 showed a 50% protection of the infected animals (a previous study had similar results). The other groups did not show any significant viral protection.

| Study Group | Treatment | Dose       | Route     | Regimen                             | n= | Challenge |
|-------------|-----------|------------|-----------|-------------------------------------|----|-----------|
| 1           | Vehicle   | -          | IP 100 ul | Once Daily: d0-d8                   | 10 | EBOV      |
| 2           | AKC-8a    | 20/5 mg/kg | IP 100 ul | Once Daily: d0 (20MPK), d5-8 (5MPK) | 10 | EBOV      |
| 3           | AKC-8a    | 20 mg/kg   | IP 100 ul | Once Daily: d0, d3, d6, d9          | 10 | EBOV      |
| 4           | AKC-8a    | 5 mg/kg    | IP 100 ul | Once Daily: d0-d3                   | 10 | EBOV      |

**Table 2: EBOV mouse pilot study design (USAMRIID)**

**Figure 2: USAMRIID EBOV mouse pilot study – Survival Chart**



The above chart illustrates mouse survival in the recent EBOV challenge study. AKC-8a in dose group 4 shows 50% protection from viral death. The other groups in this study showed little or no protection compared to the vehicle control group. Prosetta and USAMRIID observe that virus-compromised animals appear to experience AKC-8a toxicity at levels deemed safe in safety studies performed on healthy animals. Future studies will include the dosing of more potent and better-tolerated compounds to gain full protection from EBOV.

Figure 3, below, shows the PK profile for AKC-8a, as well as IP plasma levels at or above  $EC_{50}$  *in vitro* concentrations for Marburg virus (MARV). From the kinetics plot, one observes that the plasma concentration of AKC-8a is above  $EC_{50}$  and almost 20 hours above  $EC_{99}$  predicted concentrations. One can conclude that effective compound concentrations are on-board in the mouse model. Potential study liabilities may include off-target toxicity as well as compound potency issues. More live virus as well as safety and bioavailability data will be necessary to identify future lead candidates.

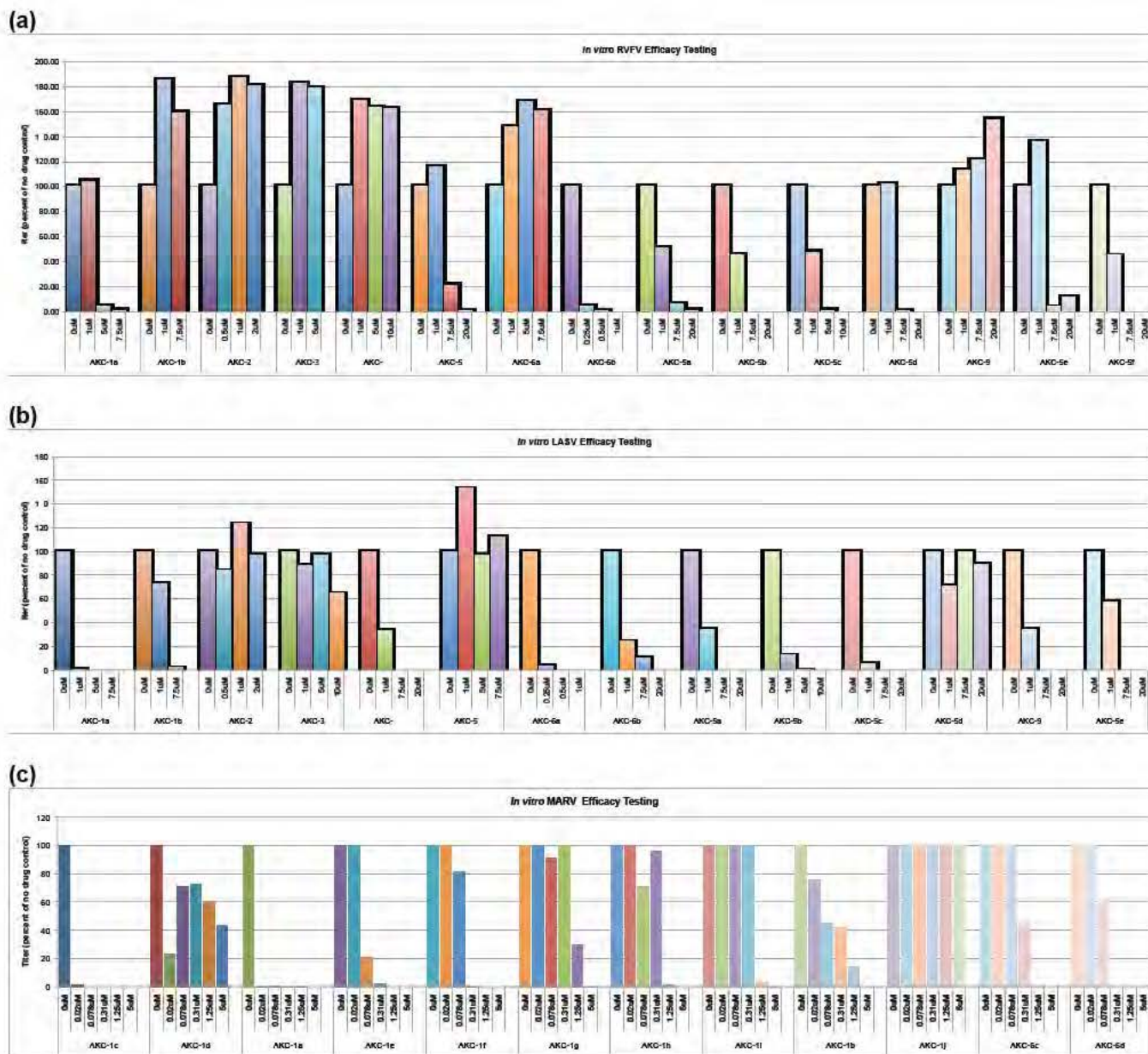
## ADAPT Q1 RESEARCH STATUS UPDATE

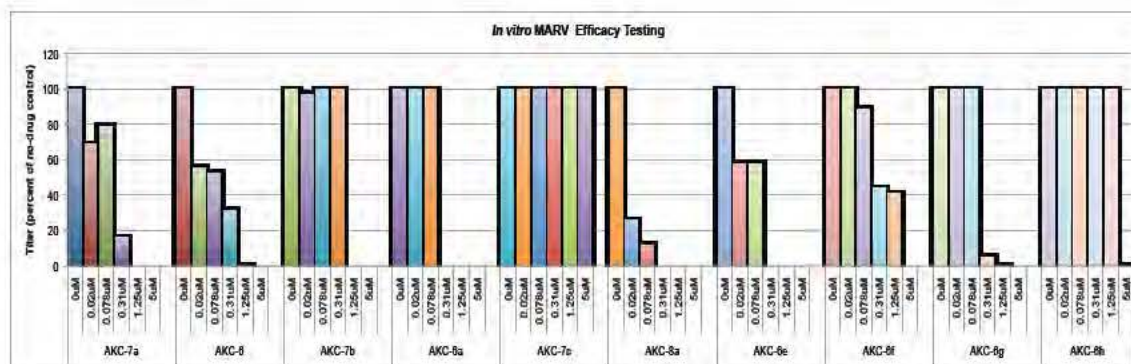
### Update of progress by Tasks (see Summary and Conclusions below):

#### Aim 1: Optimize lead chemical series for *in vitro* efficacy against multiple hemorrhagic fever viruses (HFVs).

Chemical analogs to the lead series designed in March were subsequently synthesized and screened for capsid assembly inhibition in an HFV *in vitro* plate assay throughout the first quarter of the contract. Ongoing synthesis and screening of newly designed analogs to optimize capsid assembly inhibition is based in large part on the activity profiles of analogs that have already been screened against one or more viruses of interest under this contract. See Figure 1.

Figure 1





(d)

| ADAPT Compound | Cytotoxicity (CC <sub>50</sub> ) |
|----------------|----------------------------------|
| AKC-1a         | 2.5-10 μM                        |
| AKC-1b         | >25 μM                           |
| AKC-1c         | 10 μM                            |
| AKC-1d         | 10-25 μM                         |
| AKC-1e         | 10-25 μM                         |
| AKC-1f         | 10-25 μM                         |
| AKC-1g         | 25 μM                            |
| AKC-1h         | 25 μM                            |
| AKC-1i         | 10-25 μM                         |
| AKC-1j         | 25 μM                            |
| AKC-6          | 1.6 μM                           |
| AKC-6a         | 2.5-10 μM                        |
| AKC-6c         | 1 μM                             |
| AKC-6d         | 10 μM                            |
| AKC-6e         | 1.4 μM                           |
| AKC-6f         | 25 μM                            |
| AKC-6g         | 1 μM                             |
| AKC-6h         | 4.5 μM                           |
| AKC-6j         | 1 μM                             |
| AKC-6j         | 10-25 μM                         |
| AKC-6k         | 2.5-10 μM                        |
| AKC-7a         | <1 μM                            |
| AKC-7b         | <0.4 μM                          |
| AKC-7c         | 25 μM                            |
| AKC-8a         | 25 μM                            |
| AKC-8b         | 10-25 μM                         |

**Legend to Figure 1:** Panels (a), (b) and (c) show the activity profiles of various Prosetta compounds against Rift Valley Fever Virus (RVFV), Lassa virus (LASV) and Marburg virus (MARV), respectively. Activity is demonstrated as a reduction in viral titer compared to the untreated (no drug) control. Panel (d) shows the cytotoxicity profiles of each compound. CC<sub>50</sub> is the concentration of drug that kills 50% of treated, cultured cells.

In May, two sets of analogs were sent to the laboratories of Drs. Hensley and Bavari at USAMRIID for *in vitro* efficacy testing against various hemorrhagic fever viruses, including Ebola (EBOV), Marburg (MARV), Lassa (LASV), Rift Valley Fever (RVFV) and/or Crimean Congo (CCFV) virus. Figure 2 lists these compounds, as well as their respective *in vitro* safety profiles.

**Figure 2**

| ADAPT Compound | Cytotoxicity (CC <sub>50</sub> ) | Action                                    |
|----------------|----------------------------------|-------------------------------------------|
| AKC-1e         | 10-25 μM                         | Sent to Dr. Hensley in May for LV testing |
| AKC-6j         | 10-25 μM                         | Sent to Dr. Hensley in May for LV testing |
| AKC-11a        | 10 μM                            | Sent to Dr. Hensley in May for LV testing |
| AKC-8a         | 25 μM                            | Sent to Dr. Hensley in May for LV testing |

|         |                |                                           |
|---------|----------------|-------------------------------------------|
| AKC-8b  | 10-25 $\mu$ M  | Sent to Dr. Hensley in May for LV testing |
| AKC-8c  | 10-25 $\mu$ M  | Sent to Dr. Hensley in May for LV testing |
| AKC-6c  | 1 $\mu$ M      | Sent to Dr. Hensley in May for LV testing |
| AKC-10  | 2.5 $\mu$ M    | Sent to Dr. Hensley in May for LV testing |
| AKC-6f  | 25 $\mu$ M     | Sent to Dr. Hensley in May for LV testing |
| AKC-7b  | <0.4 $\mu$ M   | Sent to Dr. Hensley in May for LV testing |
| AKC-6k  | 2.5-10 $\mu$ M | Sent to Dr. Hensley in May for LV testing |
| AKC-12a | >25 $\mu$ M    | Sent to Dr. Hensley in May for LV testing |
| AKC-6j  | 10-25 $\mu$ M  | Sent to Dr. Bavari in May for LV testing  |
| AKC-11a | 10 $\mu$ M     | Sent to Dr. Bavari in May for LV testing  |
| AKC-8b  | 10-25 $\mu$ M  | Sent to Dr. Bavari in May for LV testing  |
| AKC-8c  | 10-25 $\mu$ M  | Sent to Dr. Bavari in May for LV testing  |
| AKC-10  | 2.5 $\mu$ M    | Sent to Dr. Bavari in May for LV testing  |
| AKC-5c  | 25 $\mu$ M     | Sent to Dr. Bavari in May for LV testing  |
| AKC-5e  | >25 $\mu$ M    | Sent to Dr. Bavari in May for LV testing  |
| AKC-5d  | >25 $\mu$ M    | Sent to Dr. Bavari in May for LV testing  |
| AKC-5b  | >25 $\mu$ M    | Sent to Dr. Bavari in May for LV testing  |
| AKC-6k  | 2.5-10 $\mu$ M | Sent to Dr. Bavari in May for LV testing  |
| AKC-12a | >25 $\mu$ M    | Sent to Dr. Bavari in May for LV testing  |

## **Aim 2: Optimize lead chemical series for *in vitro* and *in vivo* safety and pharmacokinetics.**

In addition to designing lead series analogs to optimize antiviral activity, Prosetta has initiated design of analogs based on the structural properties believed or observed to enhance safety and pharmacokinetics (PK). *In vitro* safety and PK profiling of new analogs is underway.

## **Aim 3: Validate anti-EBOV, -RVFV, -LASV and -CCFV compounds demonstrating *in vitro* activity in *in vivo* small animal models of infection.**

*In vivo* efficacy assessment of pan-HFV compounds will be performed based on compound prioritization resulting from *in vitro* efficacy and *in vivo* safety and pharmacokinetic studies.

## **Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

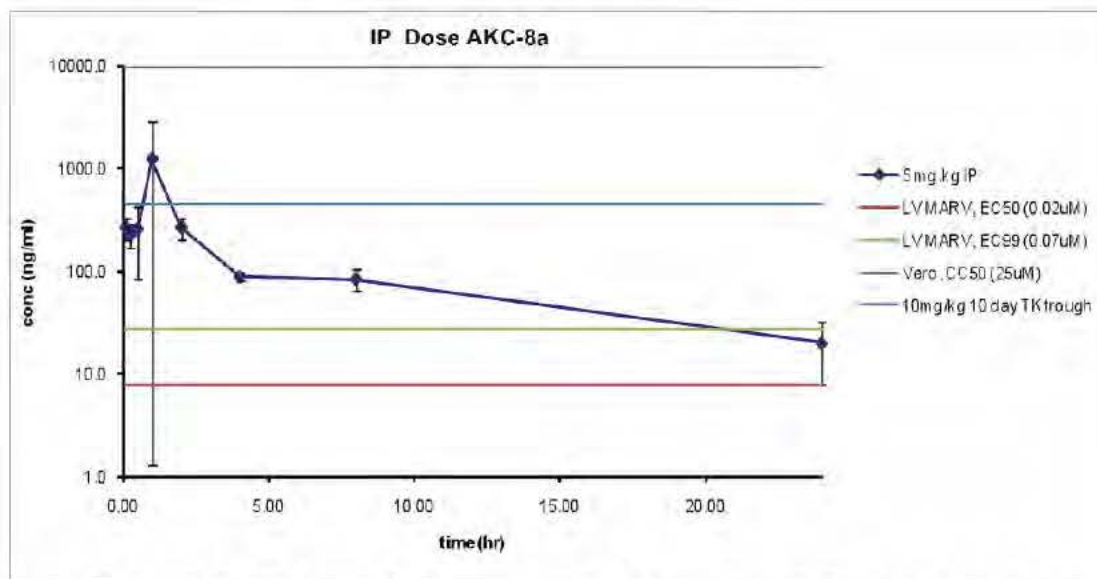
Exploration of the host proteins targeted by the lead anti-HFV chemical series was initiated in March. Column preparation was performed using photolabile activation of chemical crosslinkers to immobilize the test compound within the column matrix. During April, the column has been used to extract putative host targets of the test compound, and experiments to corroborate the activity of these putative targets are underway.

**Management and Oversight.** Prosetta hosted the ADAPT kickoff meeting at its headquarters in San Francisco, CA, attended by Stephanie McElhinny, Gary Qiao, Michael Farmer, and Vishwanath Lingappa. Additionally, Prosetta conducted telephonic kickoff meetings with the USAMRIID collaborators under ADAPT, Drs. Bavari and Hensley.

## **Summary and Conclusions:**

Significant progress was made toward ADAPT program goals in the first quarter of this contract. More than 20 new analogs of lead anti-HFV chemical series previously identified by Prosetta were generated – a process critical to discovering an antiviral that is not only active but also non-toxic and biologically available. Two sets of these analogs were sent to USAMRIID for efficacy assessment against live MARV, RVFV and LASV. Further, initial experiments in furtherance of anti-HFV drug target identification have been conducted.

**Figure 3: Mouse IP, PK and *in vitro* data plots for AKC-8a**



**Legend to Figure 3:** The Figure 3 PK profile for AKC-8a, below, shows exposure for IP plasma levels at or above  $EC_{50}$  *in vitro* concentrations for Marburg virus (*in vitro* live virus data provided by T. Warren, Ph.D., USAMRIID). Also graphed are the plasma levels for cell cytotoxicity at 50% ( $CC_{50}$ ) in Verda Reno (Vero) cell lines and the average plasma level for the 10-day PK trough samples.

**Aim 4: Perform studies to characterize host protein target(s) and/or other aspects of mechanism of action of at least two lead antiviral compounds active against one or more viruses of interest following lead optimization of existing chemical series.**

Exploration of the host proteins targeted by the lead anti-HFV chemical series was initiated in March and continues as new, active analogs are identified. Putative target data will be compiled, summarized and presented in the next contract report.

**Summary and Conclusions:** Significant progress toward lead optimization based on *in vitro* data was made during first quarter of this contract. The second quarter was marked by progress toward optimizing the lead series for *in vivo* studies. The *in vivo* data generated over the past three months strongly suggest that molecular modifications (new analogues) based on our current chemical scaffold should lead to improving the achieved 50% survival rate, hopefully eventually achieving full protection.