AWARD NUMBER: W81XWH-18-1-0546

TITLE: Developing New Therapeutic Strategies for Malignant Peripheral Nerve Sheath Tumor by Interrupting Immunologic Tolerance

PRINCIPAL INVESTIGATORS: Timothy Cripe, MD, PhD and Kevin Cassady, MD

CONTRACTING ORGANIZATION: The Research Institute at Nationwide Children's Hospital

REPORT DATE: Sept 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
Sept 2019	Annual	15 August 2018 – 14 August 2019

4. TITLE AND SUBTITLE

Developing New Therapeutic Strategies for Malignant Peripheral Nerve Sheath Tumor By Interrupting Immunologic Tolerance

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0546

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Timothy Cripe, MD

E-Mail: timothy.cripe@nationwidechildrens.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

RESEARCH INSTITUTE AT NATIONWIDE CHILDRENS
700 CHILDRENS DR
COLUMBUS OH 43205-2664

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Based on recent data from us and others using human clinical MPNST specimens, we seek validation in mouse models of a novel treatment paradigm that could lead to new clinical trials. Our approach is based on stimulating antitumor immunity using oncolytic virus infections combined with inhibitors of immunosuppression, including those targeting TGFβ (both directly and indirectly, through the Janus kinase inhibitor Ruxolitinb) and T cell checkpoints.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	21	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Based on recent data from us and others using human clinical MPNST specimens, we seek validation in mouse models of a novel treatment paradigm that could lead to new clinical trials. Our approach is based on stimulating antitumor immunity using oncolytic virus infections combined with inhibitors of immunosuppression, including those targeting TGFβ (both directly and indirectly, through the Janus kinase inhibitor Ruxolitinb) and T cell checkpoints.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Virotherapy
oHSV – Oncolytic Herpes Simplex Virus
MPNST – Malignant Peripheral Nerve Sheath Tumor
TGFβ – Transforming Growth Factor Beta
Ruxolitinib
PD-1 – Programmed cell Death protein 1

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aims (specified in proposal)	Timeline	Percentage of completion
Aim 1. Compare viruses	Months	
Task 1.1 Therapeutic efficacy	1-6	50%
Task 1.2 Permissivity	1-6	100 %
Task 1.3 Immune cell modulation	3-9	16.7 %
Task 1.4 Cytokines/chemokines	3-9	50 %
Task 1.5 T cell subsets	9-12	NA
Aim 2. TGFβ and PD-1 inhibitors	Months	
Task 2.1 Antitumor efficacy	12-18	Ongoing
Task 2.2 T cell subsets	18-24	Ongoing
Task 2.3 Cytokines/chemokines	18-24	Ongoing
Task 2.4 T cell effectors	24-30	Ongoing
Task 2.5 Dependence on TGFβR2 signaling	1-36	Ongoing
Task 2.6 TGFβ and PD-1 inhibition	24-36	Ongoing

Specific Aims (continued)	Timeline	Percentage of completion
Aim 3. Ruxolitinib	Months	
Task 3.1 TGFβ and IL-10 expression	6-12	7%
Task 3.2 TGFβ and IL-10 loss of function	12-36	Ongoing
Task 3.3 Virus production/persistence	12-18	50%
Task 3.4 Antiviral T cells	18-24	Ongoing

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Task 1.1: Determine the relative therapeutic efficacy of different oHSV constructs in immunocompetent mouse models of MPNST. We set out to compare the efficacy of the oHSVs HSV1716, T-VEC, and C134 in two immunocompetent mouse MPNST models, 67C-4 and #5NPCIS. We began our efficacy studies with the 67C-4 model, originally intending to run these experiments concurrently with the objectives laid out in Task 2.1 (efficacy studies that factor in TGFβ inhibition). Figure 1A shows 67C-4 mouse survival following three intratumoral injections of each respective virus (1E8 plaque-forming units, or pfu, per dose), alongside individual tumor growth curves charted for each mouse. In order to make accurate comparisons, it is essential that equivalent dosages of each virus are delivered at each administration. Routine back-titration of our virus stocks revealed that far less C134 virus was being delivered than what we had originally calculated, and thus the data obtained from these cohorts of mice have been excluded from Figure 1A. Other issues that arose during these experiments were the unexpected latency period between injecting tumor cells and having treatable-sized tumors (>30 days) and the somewhat low success rate of tumor engraftment. Many of the animals implanted with 67C-4 also needed to be sacrificed early due to the development of significant tumor ulceration. To address these issues, we are planning to take cryopreserved sections of previously established 67C-4 tumors and implant them in the flanks of naïve mice, a process we have used in the past with other "difficult" tumor models when injections of cells proved insufficient. We intend to repeat the 67C-4 efficacy studies in the next few months.

We also conducted efficacy studies with the #5NPCIS model and found that each virus produced a slight, yet statistically significant ($p \leq 0.05$) survival benefit over the vehicle control (Figure 1B). We used a smaller number of animals in this study ($n = 5$ per group) in case we were confronted with similar challenges like those encountered with the 67C-4 model, but these experiments were completed without issue. A repeat of this study is currently underway to further increase our sample sizes, but the final results are not available as of this writing.

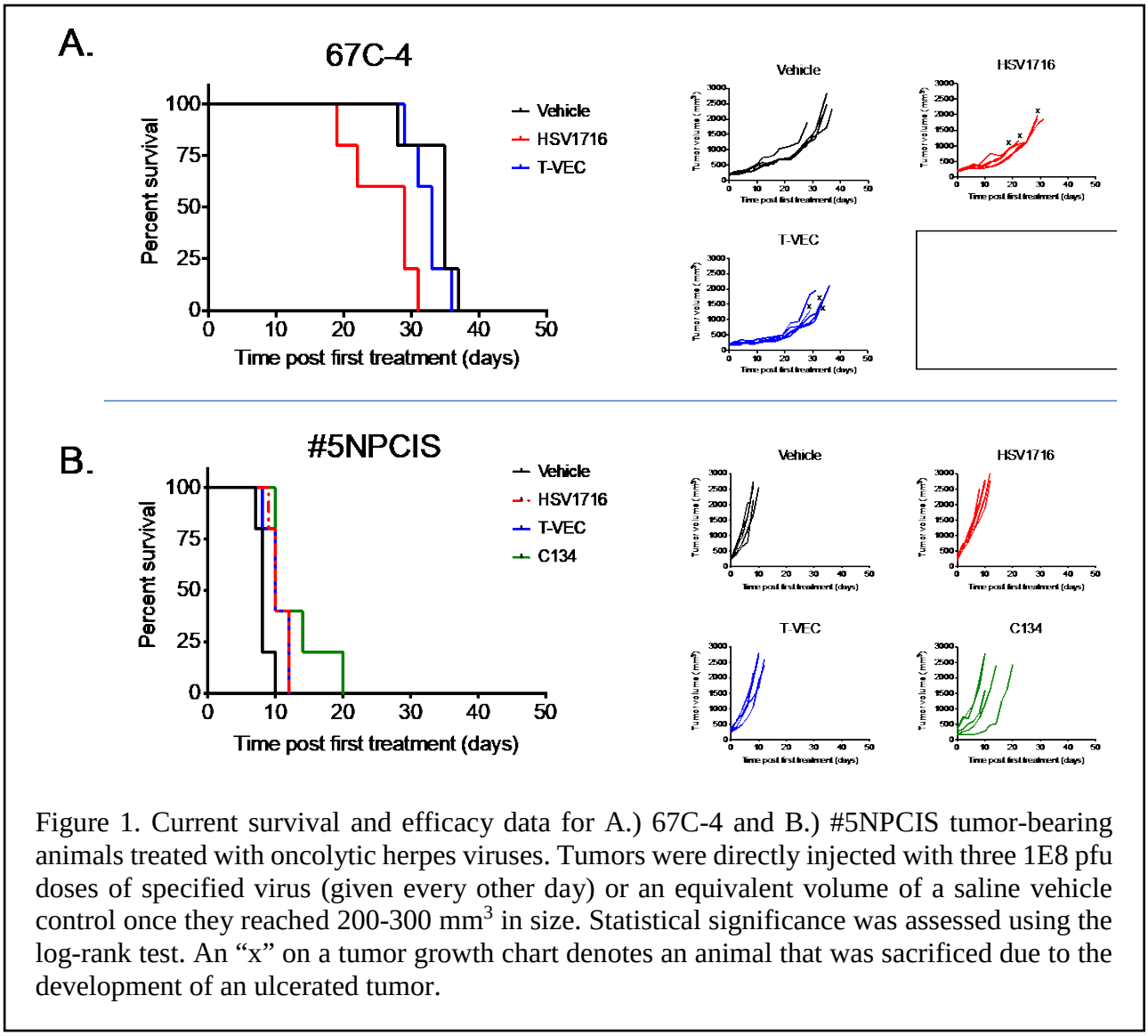


Figure 1. Current survival and efficacy data for A.) 67C-4 and B.) #5NPCIS tumor-bearing animals treated with oncolytic herpes viruses. Tumors were directly injected with three 1E8 pfu doses of specified virus (given every other day) or an equivalent volume of a saline vehicle control once they reached 200-300 mm^3 in size. Statistical significance was assessed using the log-rank test. An "x" on a tumor growth chart denotes an animal that was sacrificed due to the development of an ulcerated tumor.

Task 1.2: Measure the permissivity of mouse MPNSTs to each oncolytic herpes virus. As part of our comparison process, we wanted to determine if there were any differences in replication rates (permissivitiy) among the oHSVs following infection of mouse MPNST cell lines and tumors. For the in vitro experiments, we infected 67C-4 and #5NPCIS cells with HSV1716, T-VEC or C134 at a multiplicity of infection (MOI) of 0.5 plaque-forming units of virus per cancer cell. We then collected the infected cells at the listed timepoints and quantified the amount of virus produced with plaque assays (Figure 2A). For these in vitro studies, we noted that T-VEC appears to have the greatest permissivity, as it alone showed a small, but steady increase in the amount of infectious virus that could be recovered over time in both 67C-4 and #5NPCIS. HSV1716 and C134 on the other hand showed the opposite trend. We also conducted this study using a lower MOI of 0.05 pfu and observed similar trends (not shown).

To see if these observations held true in our mouse models, we implanted 67C-4 or #5NPCIS tumors on the flanks of immunocompetent mice and treated them with a single intratumoral 1E8 pfu dose of virus. These animals were then sacrificed 3, 24, 48 or 72 hours later and their tumors were collected and processed to allow for quantification of virus by plaque assay (Figure 2B). In 67C-4 tumors, oHSV titers dropped steadily over the course of 72 hours regardless of which virus had been administrated. We also noted decreasing virus titers in the #5NPCIS tumors, although not to quite the same extent as that witnessed in 67C-4. Interestingly, we found that T-VEC had greater persistence in #5NPCIS compared to HSV1716 and C134, although this did not seem to have an impact on antitumor efficacy or animal survival (refer to Figure 1B).

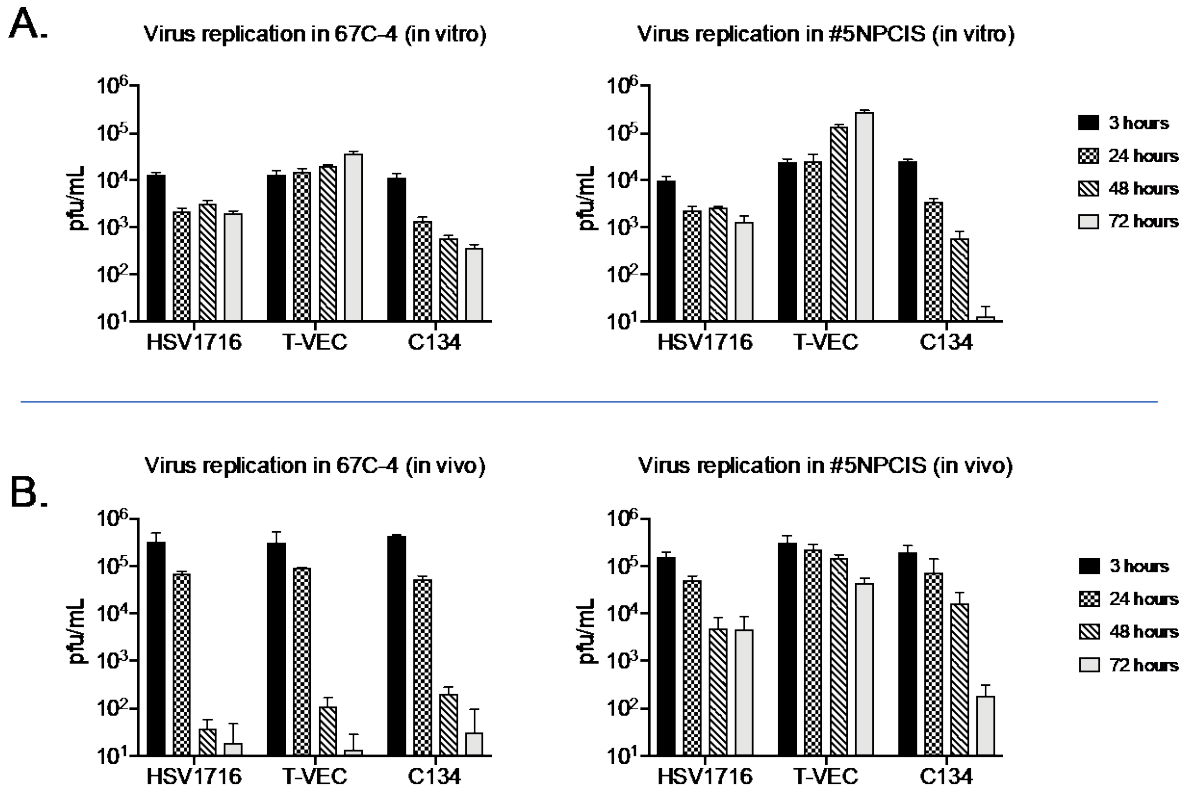
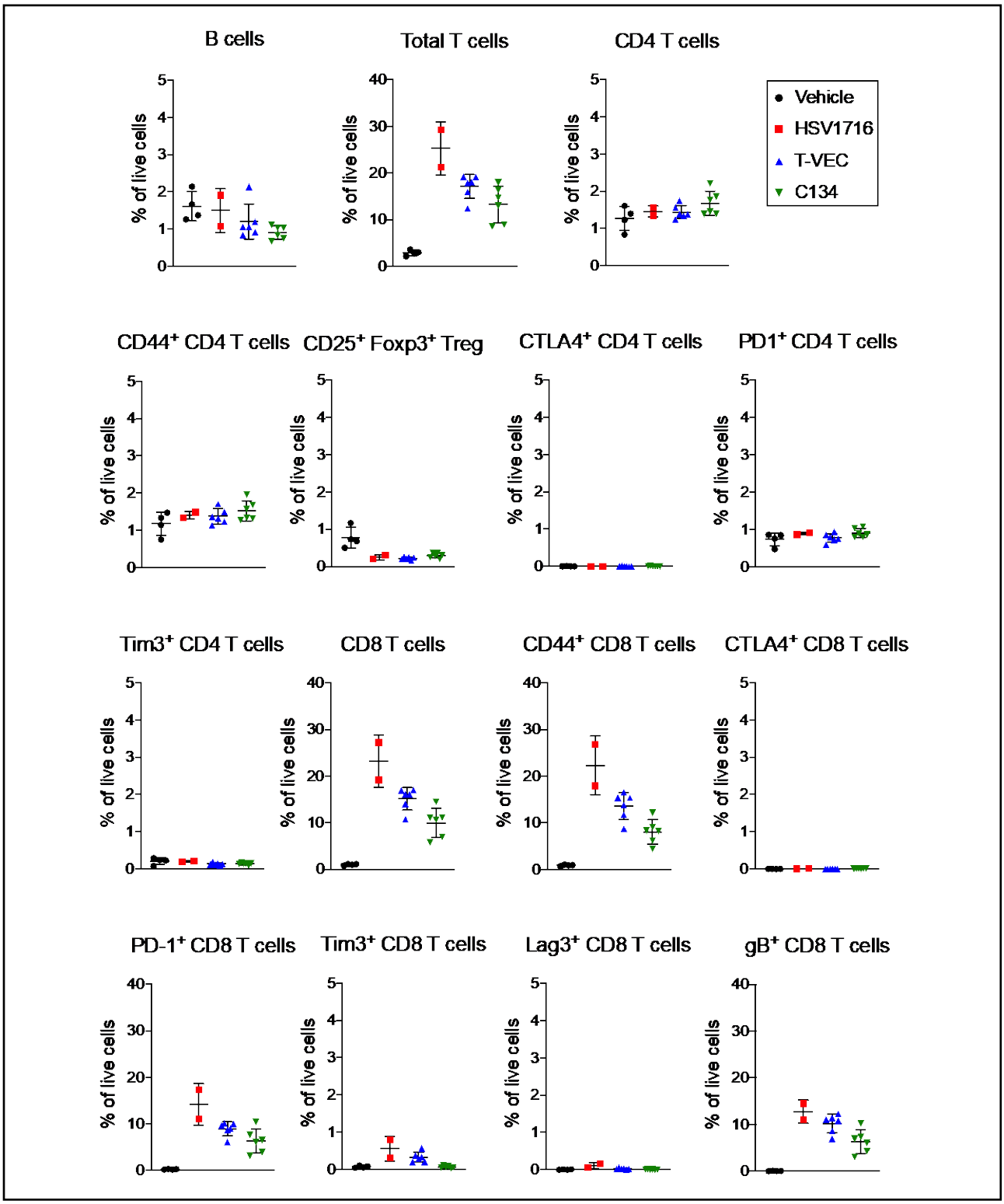


Figure 2. Average virus replication in 67C-4 and #5NPCIS A.) cell lines and B.) tumors. Each sample was run in quadruplicate. Error bars represent standard deviation.

Task 1.3: Compare viruses for their modulation of the immune cells within the tumor microenvironment. We know from previous studies that oncolytic herpes virus treatment promotes the influx of various immune effector cells to the tumor microenvironment. The purpose of task 1.3 was to determine if HSV1716, T-VEC or C134 treatment resulted in differential modulation of this response, which we would assess by using flow cytometry techniques to examine single-cell suspensions of processed tumors at various timepoints post infection. These experiments are currently underway as this report is being prepared and more data will be forthcoming over the next several

weeks. The first set of these experiments, examining immune cell infiltrates in the #5NPCIS model at a 72 hour timepoint, is shown in Figure 3 as an example. We will continue to acquire data for the #5NPCIS model and then move onto completing the 67C-4 arm of these studies once we address the issues described in task 1.1.



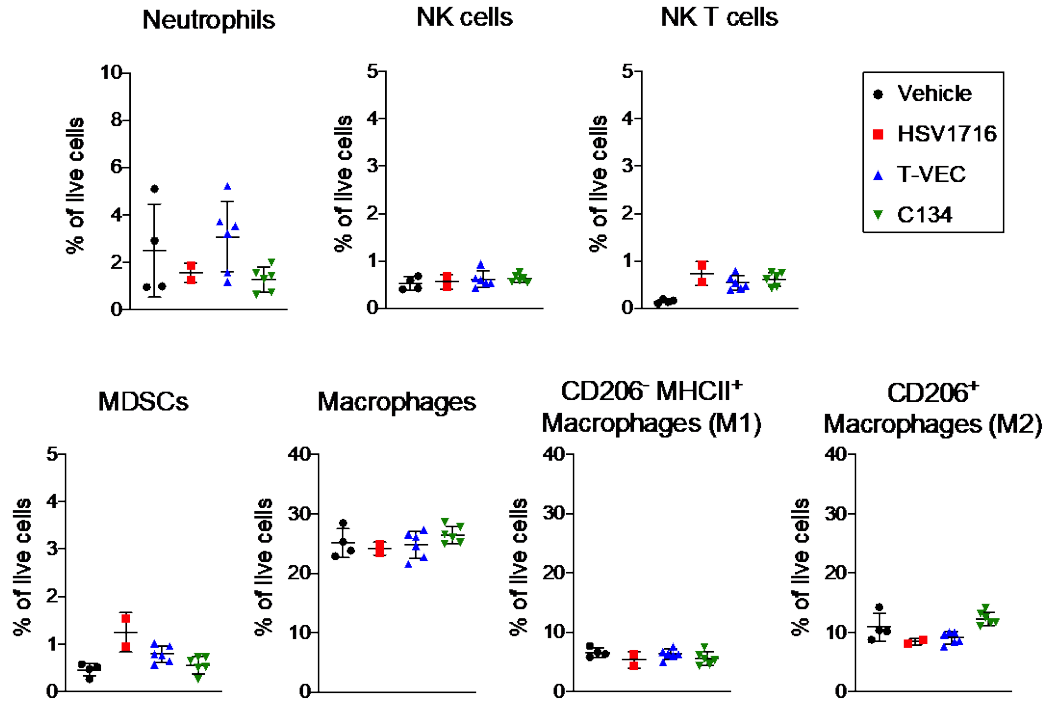


Figure 3. Immune cell infiltrate data for #5NPCIS tumors following three intratumoral injections of specified virus (1E8 pfu per dose). Tumors were harvested at 72 hours after the final injection for flow cytometry analysis. Similar experiments for 24 hours and 168 hours timepoints are expected to commence shortly.

Task 1.4: Compare viruses for their induction of immunostimulatory cytokines and chemokines. Immunostimulatory and immunosuppressive cytokines in the tumor microenvironment influence T cell recruitment and function. The purpose of this task was to compare cytokine/chemokine expression profiles of infected MPNSTs and determine if there were any differences linked to the choice of oHSV. We accomplished this using the commercially available Proteome Profiler Mouse XL Cytokine Array (R&D Systems, Minneapolis, MN), which allowed us to detect and make qualitative assessments of the expression of 111 different mouse cytokines and chemokines in tumors treated three times with 1E8 pfu doses HVS1716, T-VEC, C134 or a vehicle control. We have completed these experiments in the #5NPCIS model thus far, and plan to run the 67C-4 portion of these experiments alongside our repeat of the efficacy studies outlined in task 1.1. Data acquired from treated #5NPCIS tumors are shown in Figure 4 (limited to analytes showing clear differences across treatment groups).

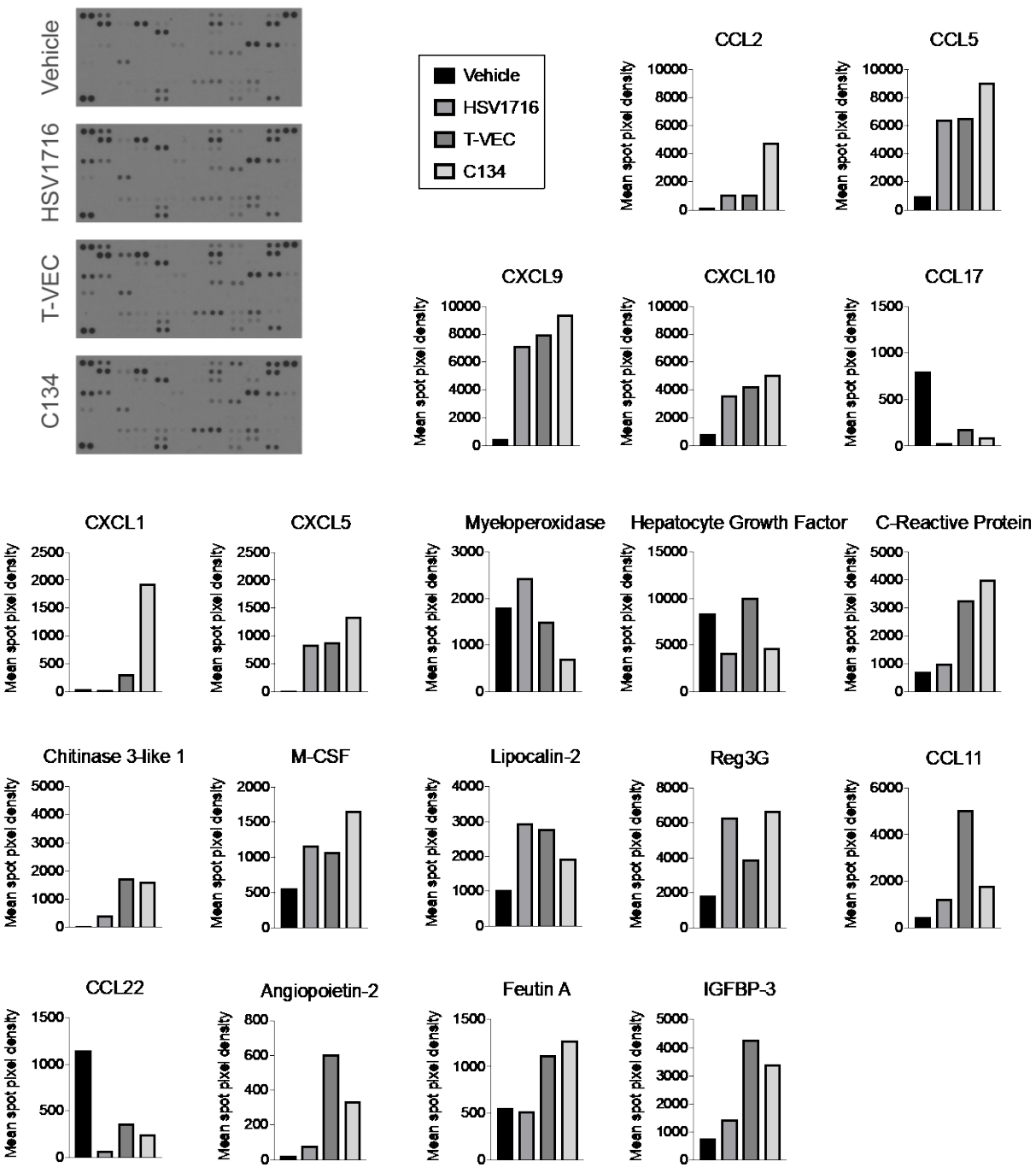


Figure 4. Proteome profile array data for #5NPCIS tumors treated with Vehicle, HSV1716, T-VEC, or C134. Tumors were treated three times with 1E8 pfu doses of respective virus or equivalent volume of vehicle control and harvested for analysis 24 hours after the final treatment. Densitometric quantification of listed cytokines/chemokines was performed with ImageJ software (National Institutes of Health, USA).

Task 1.5: Determine the dependence of virotherapy efficacy on T cell subsets. In this experiment, we planned to systemically deplete $CD4^+$ and/or $CD8^+$ T cell populations in MPNST-bearing mice to gauge their relative contribution to the therapeutic response following oHSV infection. However, based on available data obtained from task 1.1, it appears that these viruses promote little if any antitumor efficacy as single agents, which calls into question the practical relevance of conducting the experiments in this task. Although we intend to repeat experiments in task 1.1 to increase our sample sizes, it is likely that we will arrive at the same conclusion. If that is indeed the case, we will use the mice allocated for this task in other studies related to this grant.

Task 2.1: Measure the effect of TGFβ inhibition on the antitumor efficacy of herpes virotherapy in mouse models of MPNST. Nothing to report.

Task 2.2: Determine the effect of TGFβ inhibition on the influx of T cell subsets and their activation following virotherapy. Nothing to report.

Task 2.3: Determine the effect of TGFβ inhibition on intratumoral expression of T cell chemokines. Nothing to report.

Task 2.4: Test the role of T cell effectors in the enhancement of virotherapy due to TGFβ inhibition. Nothing to report.

Task 2.5: Confirm the dependence of A8301 activity on TGFβR2 signaling in T cells. Nothing to report.

Task 2.6: Determine if TGFβ inhibition is redundant to or complementary with PD-1 inhibition. Nothing to report.

Task 3.1: Determine the effect of Ruxolitinib on TGFβ and IL-10 expression in mouse MPNST tumors. In these experiments, we plan to use a combination of quantitative real-time PCR (qPCR) and Western blotting to examine what effect(s) Ruxolitinib treatment has on the expression of the immunosuppressive cytokines TGFβ and IL-10. The unexpected departure of research personnel impacted our ability to complete these studies within this reporting period, thus the data we are able to show are limited to a single timepoint of the 67C-4 model (Figure 5). We implanted C57Bl/6 mice with 67C-4 cells, and once the tumors reached 25-150 mm^3 in size, we treated them for three consecutive days with 60 mg/kg with Ruxolitinib or an equivalent volume of a saline vehicle control. The tumors were harvested 24 hours after the last treatment for qPCR analysis. Ruxolitinib treatment not only significantly reduced expression of mouse TGFβ and IL-10, but also reduced expression of RIG-I, a pattern recognition receptor that typically acts to restrict herpes virus infection. We are currently attempting to expand these studies by examining gene expression at other timepoints in both the 67C-4 and #5NPCIS models. We also intend to supplement these data with Western blots to determine the effects of Ruxolitinib treatment on TGFβ signaling activity and the expression of IL-10 protein.

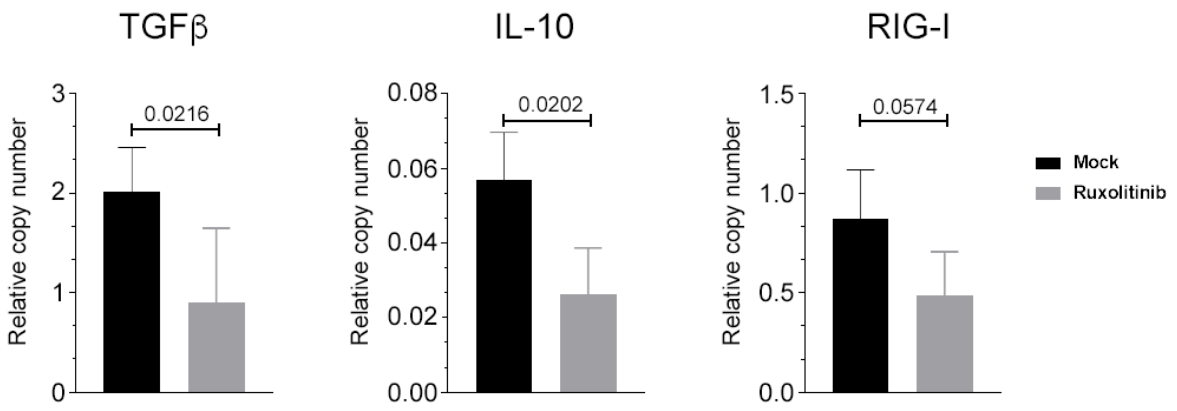


Figure 5. Ruxolitinib treatment reduces TGFβ and IL-10 gene expression in 67C-4 tumors. Mice were treated with 60 mg/kg Ruxolitinib or vehicle control and sacrificed 24 hours later for qPCR analysis (n = 4 per group). Gene expression data are presented as relative copy numbers. Statistical significance was determined using unpaired t-tests. Error bars represent standard deviation.

Task 3.2: Determine if inhibition of TGFβ and/or IL-10 accounts for the effects of Ruxolitinib on antitumor immunity. Nothing to report.

Task 3.3: Test the effects of Ruxolitinib on intratumoral virus production and persistence. In this task, we plan to measure the effect of Ruxolitinib treatment on oHSV replication in treated MPNST tumors. We will treat the animals similarly to those described in task 3.1 and examine both early and late timepoints. Preliminary data for the 67C-4 model is shown in Figure 6, where we used TaqMan qPCR to quantify virus from tumors harvested on days 1, 3 and 5 following treatment with C134 and Ruxolitinib or a vehicle control.

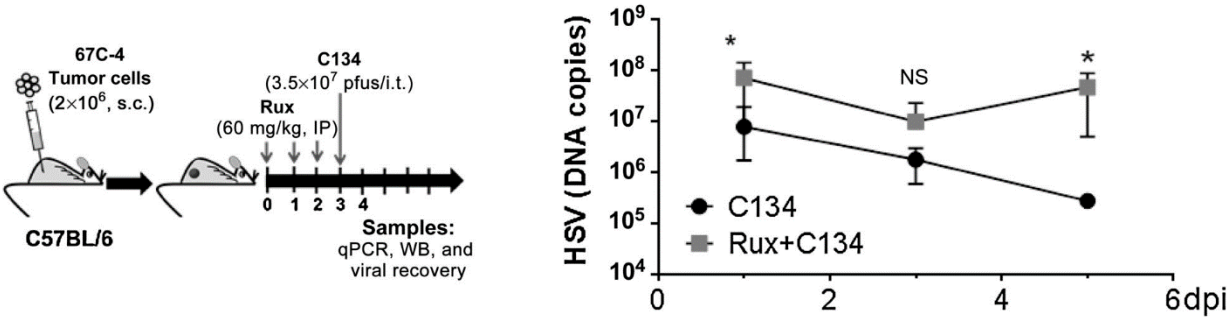


Figure 6. Ruxolitinib treatment increases the amount of C134 virus that can be recovered from 67C-4 tumors. Mice were treated three times with 60 mg/kg Ruxolitinib or vehicle control and sacrificed 1, 3 or 5 days later for qPCR analysis (n = 5 per group). Gene expression data are presented as relative copy numbers. Statistical significance was determined using two-way ANOVA. Error bars represent standard error of means. dpi = days post infection.

Task 3.4: Measure the effect of Ruxolitinib on the generation of anti-viral $CD8^+$ T cells. Nothing to report.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The project is helping provide the foundation for the graduate studies of a new student in Dr. Cripe's lab, Siddhi Nath Paudel. It also helped further the post-doctoral training of Mohammed Ghonime in Dr. Cassady's lab.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

A research paper and review article were published, the latter of which will be available under open access. See "Journal publications" below for more information.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We will continue to use the proposed Statement of Work as our guide and hold regular meetings to update one another on our progress and seek feedback/assistance when necessary. We expect our productivity to increase as we bring on new personnel and bring them up to speed.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

The oncolytic herpes virus, C134, was recently licensed to Mustang Bio (Worcester, MA).
Specifically, prior to this licensure was C134 the intellectual property of the PI, Co-I, or the institution? "
RESPONSE: C134 was originally the IP of UAB who coordinated with NCH to commercialize the invention after Dr. Cassady's arrival there.
"Was this drug (MB-108) created using any DoD funds?"
RESPONSE: No, DOD funds were not involved in production or licensure of C134 with Mustang Bio.
Additionally, how will this impact use of C134 for any further studies related to this award?
RESPONSE: No negative impact only positive. The NCH and Mustang agreement does not preclude our continued research using C134 or use of the virus. It does provide, however, a commercially viable translational path for us to introduce this virus into patients in the future.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

As mentioned in task 1.1, we encountered issues getting the 67C-4 MPNST cell line to establish tumors in a timely and reliable fashion. This impacted the number of mice we had available for treatment groups and is why the majority of data we have thus far is in the #5NPCIS model. To address this issue, we recently cryopreserved sections of an already established 67C-4 tumor for implantation into future mice. We will passage and propagate these tumors in mice as opposed to generating them from cell culture. This is a standard technique that we have had success with in the past and we expect that it will allow us to complete the 67C-4 arms of each task.

In the survival studies we were able to conduct, we observed very little antitumor efficacy in the mice treated with HSV1716, T-VEC or C134 as single agents. While this was not wholly unexpected, it does argue against proceeding with task 1.5, where we proposed to look at the dependence of efficacy on T cells. We will make the final determination regarding this task after completing repeats of both the 67C-4 and #5NPCIS survival studies. If we determine that completing this task will not yield meaningful data, we will re-allocate the mice to other studies in Aims 2 and 3.

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The unexpected departure of Mohammed Ghonime continues to impact our ability to complete tasks associated with Aim 3. We are currently trying to hire new personnel to assist with this research.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

This project does not involve human subjects.

Significant changes in use or care of vertebrate animals

There are no changes to report regarding our use of vertebrate animals.

Significant changes in use of biohazards and/or select agents

There are no changes to report regarding our use of biohazards and/or select agents.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

- Ghonime, MG, and Cassady, KA (2018). Combination Therapy Using Ruxolitinib and Oncolytic HSV Renders Resistant MPNSTs Susceptible to Virotherapy. Cancer Immunol Res 6: 1499-1510.

- Hutzen, BJ, Ghonime, MG, Lee, JM, Mardis, ER, Wang, R, Lee, DA, Cairo, MS, Roberts, RD, Cripe, TP and Cassady, KA (2019). Immunotherapeutic Challenges for Pediatric Tumors. Molecular Therapy Oncolytics (in press)

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Research related to this grant was integral to the licensing of C134 virus.

outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding*

Name: Timothy Cripe, MD, PhD
Project Role: Principal investigator
Research Identifier:
Nearest person month worked: 2.0
Contribution to Project: Dr. Cripe helped conceive and plan the experiments and oversees the research related to Aims 1 and 2.
Funding Support: NIH, St. Baldrick's Foundation, Hyundai Foundation, Vyriad, Inc., institutional support

Name: Chun-Yu Chen, PhD
Project Role: Research scientist
Research Identifier:
Nearest person month worked: 3.0
Contribution to Project: Dr. Chen runs the flow cytometry experiments related to this grant.
Funding Support: NIH, DOD, St. Baldrick's Foundation, institutional support

Name: Kevin Cassady, MD
Project Role: Co-principal investigator
Research Identifier:
Nearest person month worked: 2.0
Contribution to Project: Dr. Cassady helped conceive and plan the experiments and oversees the research related to Aim 3.
Funding Support: NIH, Alex's Lemonade Stand Foundation, CancerFree Kids Foundation, institutional support

Name: Pin-Yi Wang, PhD
Project Role: Research scientist
Research Identifier:
Nearest person month worked: 1.0
Contribution to Project: Dr. Wang assists with virus production for studies in Aims 1 and 2.
Funding Support: NIH, DOD, institutional support

Name: Brian Hutzen, PhD
Project Role: Research scientist
Research Identifier:
Nearest person month worked: 4.0
Contribution to Project: Dr. Hutzen assists with the animal studies (efficacy, immune infiltrates, cytokine/chemokine expression) related to Aims 1 and 2.
Funding Support: DOD, CancerFree Kids Foundation, institutional support

Name: Mohammed Ghonime, PhD
Project Role: Post-doctoral research associate
Research Identifier:
Nearest person month worked: 6.0
Contribution to Project: Dr. Ghonime assisted with studies related to Aim 3.
Funding Support: CancerFree Kids Foundation, institutional support

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Cripe, Timothy
Completed:
N/A (Streby) 08/08/2016- 08/08/2019 0.72 calendar
United Therapeutics Corporation
Increasing ADCC in the tumor microenvironment with HSV1716 enhances ch14.18 anti-tumor efficacy

Changes to Active:
W81XWH1910371 (Cripe) 8/01/2019- 7/31/2021 2.40 calendar
Department of Defense $601,012
BiTE Gene Therapy to Augment Oncolytic Virotherapy

R21CA223104 (Cripe/Wang) 01/16/2019 – 12/23/2020 0.6 calendar
NIH/NCI $130,500
Improving Lytic Potency of Herpes Virus Cancer Therapeutics

R21CA237505 (Kudryashov) 4/12/2019- 3/31/2021 0.12 calendar
The Ohio State University/NIH $47,850
An innovative modular strategy for highly specific elimination of human osteosarcomas

U54CA232561 (Cripe/Mardis) 09/12/2019-08/31/2024 1.8 calendar
NIH $7,544,791
The Pediatric Ohio-New York Cancer (Peds-ONC) Immunotherapy Center

Cassady, Kevin
Completed:
N/A (Cassady) 10/01/16 - 12/31/18 1.8 calendar
Hyundai Foundation $250,000
Improving oncolytic virotherapy by suppressing Interferon Stimulated Gene over-expression in MPNST tumors

N/A (Cassady) 09/01/16 - 08/31/18 1.8 calendar
Alex's Lemonade Stand $250,000
Improving Immune-mediated oncolytic viral therapy: Engineering Tumor Vaccine Elements into the Virus

Changes to Active:
N/A (Markert/Cassady) 07/01/18 – 06/30/21 1.8 calendar
University of Alabama Birmingham/NIH/NCI $574,460
Oncolytic Immunotherapy using Chimeric HSV C134: A Phase I Trial and Establishment of Response Indicators in Recurrent Glioma Patients

U54CA232561 (Cripe/Mardis) 09/12/2019-08/31/2024 1.8 calendar
NIH $7,544,791
The Pediatric Ohio-New York Cancer (Peds-ONC) Immunotherapy Center

R21CA223104 (Cripe/Wang) 01/16/2019 – 12/23/2020 0.6 calendar
NIH/NCI $130,500
Improving Lytic Potency of Herpes Virus Cancer Therapeutics

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.