**AWARD NUMBER: W81XWH-18-1-0496**

**TITLE:** The Role of ATRX/DAXX loss in NF1-associated Solid Malignancies

**PRINCIPAL INVESTIGATOR:** Fausto J. Rodriguez M.D.

**CONTRACTING ORGANIZATION:** Johns Hopkins University School of Medicine.

**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

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The Role of ATRX/DAXX loss in NF1-associated Solid Malignancies

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**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

Johns Hopkins University
3400 N. Charles St.
Baltimore, MD 21218

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**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)**

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**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

In this award period, we developed data that supported an important role for ATRX/DAXX loss and acquisition of ALT with an aggressive biology in NF1-associated gliomas, as well as a molecular subset of NF1-associated MPNSTs. This research effort is complementary to the research aim of the award but was completed using other funds available to the PI. In the laboratory, we demonstrate that ATRX knockdown results in ALT-like properties, including increased telomere lengths by PCR based methods and large telomeric foci using telomere specific FISH. ATRX knockdowns in MPNST-derived cell lines does not on its own result in an evident effect in cell growth. However, when the ATR inhibitor was administered, decreased growth in vitro was observed in a MPNST cell line that lacks TERT promoter alterations. These findings support a role of ATRX loss in a biologic subset of NF1-associated malignancies,and opens the opportunity for therapeutic targeting of these tumors using specific classes of drugs.

| 15. SUBJECT TERMS<br>NF1, ATRX, DAXX, Alternative lengthening of telomeres, telomeres, glioma, MPNST, pilocytic astrocytoma, diffuse glioma, neurofibroma | | | | | |
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**Standard Form 298 (Rev. 8-98)**
**Prescribed by ANSI Std. Z39.18**

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

Work from our laboratory has demonstrated that *ATRX* loss and the alternative lengthening of telomeres (ALT) occur frequently in astrocytomas developing in patients with NF1, predominantly adults, and may also develop in a subset of malignant peripheral nerve sheath tumors (MPNST). We have developed several murine and human models to study ATRX in the context of *NF1* loss, and are performing a comprehensive approach to delineate specific phenotypes and functional effects resulting from *ATRX* loss in the context of NF1 tumorigenesis, including effects on telomeres.

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

NF1, ATRX, DAXX, Alternative lengthening of telomeres, telomeres, glioma, MPNST, pilocytic astrocytoma, diffuse glioma, neurofibroma

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.*

| | |
|---|---|
| Subtask 1 Perform functional experiments using *Atrx* deficient/ $Nf1^{+/-}Trp53^{+/-}$ murine glioma lines. | 1-3 months (90%) |
| Subtask 2 Perform functional experiments using *ATRX* deficient human NF1- or *BRAF* mut gliomas lines and xenografts. | 3-12 (0%- studies not yet approved by HRPO) |
| Local IRB/IACUC Approval | 3 (100%) |
| Milestone Achieved: HRPO/ACURO Approval | 6 (50%, HRPO approval still pending) |
| **Major Task 2** | |
| Subtask 1 Establish the optimal oncogene sequence to transform human neural stem cells in the context of NF1 loss | 6-12 (0% studies not yet approved by HRPO) |
| Subtask 1: Develop MPNST xenografts with ATRX/DAXX loss | 15-21 |
| Subtask 2: Study phenotypic/telomere alterations resulting from *ATRX/DAXX* loss in plexiform neurofibroma and MPNSTs | 15-21 (25%) |

**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.*

**Major Activities and Objectives:**

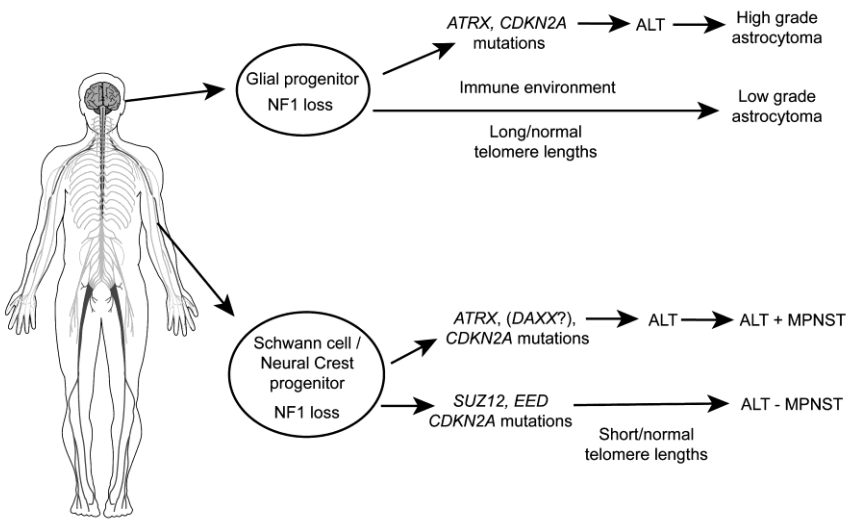
**1-**During this year of the award we finalized a manuscript describing the frequency of the alternative lengthening of telomeres in NF1-associated solid tumors. This substantial data formed the main substance of the preliminary data for the current award. The experiments were not technically done as part of the award, preceeded it, and were performed under other funding sources unrelated to the DOD. However, the manuscript generation and publication happened during the year of the award and support the translational significance of the overall project

2- Functional experiments using *Atrx* deficient/ $Nf1^{+/-}Trp53^{+/-}$ murine glioma lines. We continued working with these murine lines and performed functional analyses. These experiments are ongoing.

3- Study phenotypic/telomere alterations resulting from *ATRX/DAXX* loss in plexiform neurofibroma and MPNSTs. We performed these experiments using commercially available MPNST lines, given that HRPO has not yet provided approval to use cell lines developed inhouse.

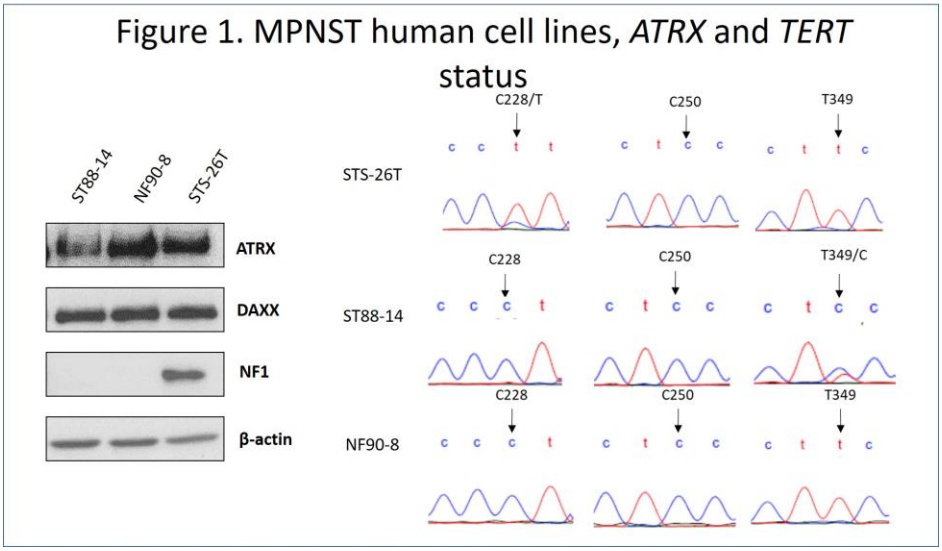
**Significant Results:**

1-Telomeres are altered in NF1-associated Solid Tumors.




The presence of Alternative lengthening of telomeres (ALT) and/or ATRX loss, as well as the role of other telomere abnormalities, had not been formally studied across the spectrum of NF1-associated solid tumors. Utilizing a telomere-specific FISH assay, we classified tumors as either ALT-positive or having long (without ALT), short, or normal telomere lengths. A total of 426 tumors from 256 NF1 patients were evaluated, as well as 99 MPNST tumor samples that were sporadic or of unknown NF1 status. We concluded that ALT occurs in a subset of NF1-associated solid tumors and is usually restricted to malignant subsets. In contrast, alterations in telomere lengths are more prevalent than ALT. These findings were published in *Acta Neuropathologica Communications*, and provided a new hypothesis of NF1 tumorigenesis (**Figure 4 in Appendix A**) and also formed part of the substance of a comprehensive review of the pathology of CNS manifestations of NF1, published in Acta Neuropathologica (**Appendix B**). The gathering of this data preceded the current DOD award, and was accomplished with unrelated funding sources. However, it was part of the preliminary data used for this DOD application and we thought it was relevant to document its successful completion in the form of publication here.

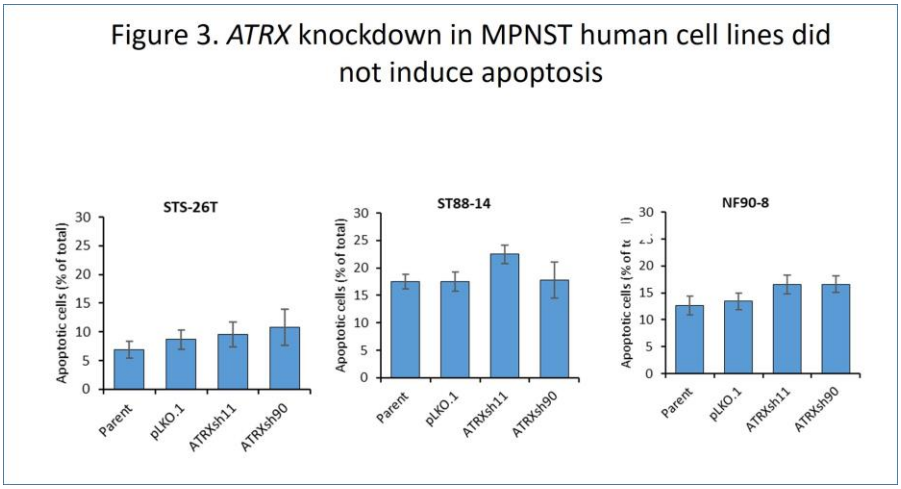
2- Functional experiments using *Atrx* deficient/ *Nf1*$^{+/-}$*Trp53*$^{+/-}$ murine glioma lines. These experiments formed part of the preliminary data that supported this award application. Since then




we performed basic experiments demonstrating a lack of significant growth differences in Atrx knockdown cell lines, and are currently performing functional inactivation of *Tert* using CRISPR technologis, to study more in depth the effect of Atrx and Nf1 loss in the context of *tert* inactivation, a more realistic scenario for human disease.

3-Study phenotypic/telomere alterations resulting from *ATRX/DAXX* loss in MPNSTs

Given the lack of HRPO approval, we used commercially available cell lines to study the effect of *ATRX* loss in MPNST (ST88-14, NF90-8, STS-26T). We screened these lines for ATRX, DAXX,

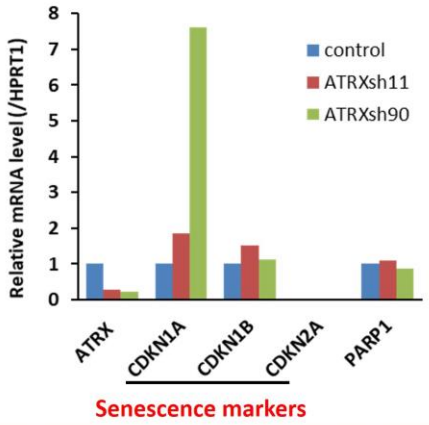



NF1 and *TERT* promoter alterations. These cell lines all expressed ATRX and DAXX, but STS-26T had a TERT promoter mutation and ST88-14 had a known SNP in the *TERT* promoter, while NF90-8 had no alterations (**Figure 1**).

Next we performed ATRX knockdowns using siRNA. Although knockdown was efficiently performed in all cell lines, no significant effects in cell proliferation was noted, suggesting that *ATRX* loss is not necessary for some basic tumor related properties, once tumor is initiated (**Figure 2**). Next we calculated the percent of apoptotic cells after *ATRX* knockdowns and found no significant difference in apoptosis when measuring the percent of cleaved caspase-3 positive cells (**Figure 3).**

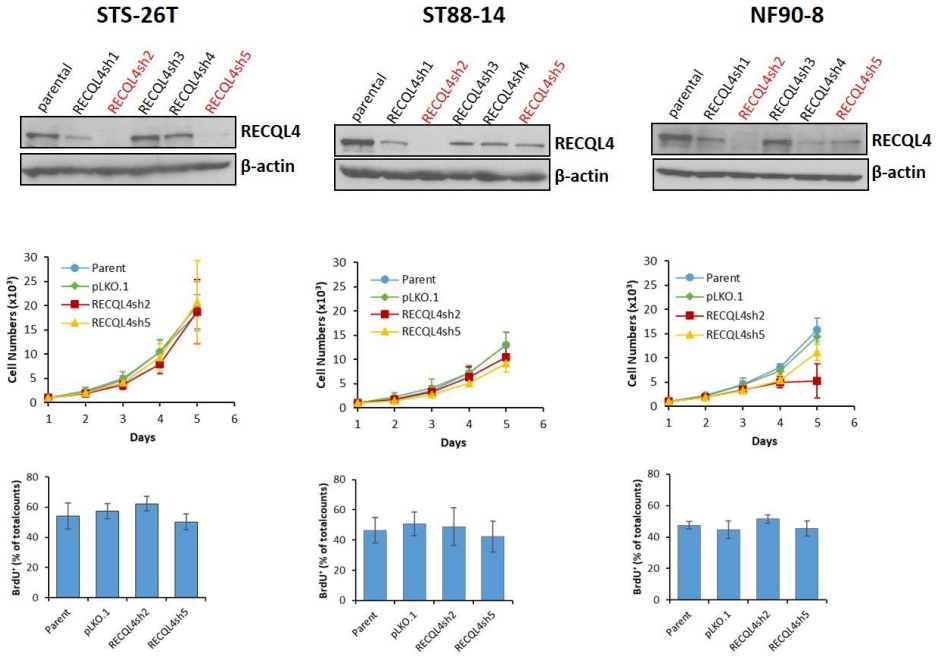
Next, we studied the effect of ATRX loss in a non-neoplastic context, using a commercially available human Schwann cell line. *ATRX* knockdown in these cell line resulted in the induction of senescence markers, particularly CDKN1A and CDKN1B (**Figure 4**).

Figure 4. *ATRX* knockdown in Schwann cells induced cell senescence



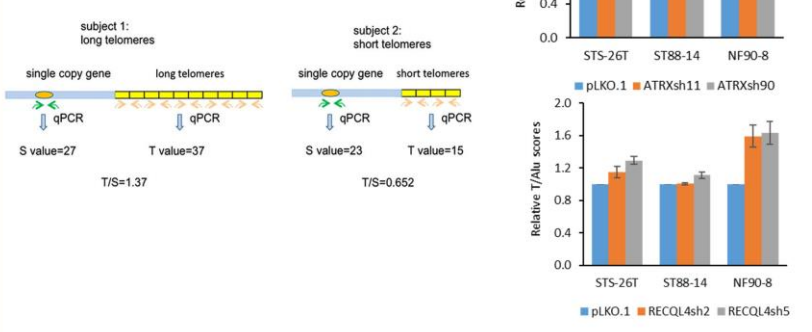

4-Study the effect of *RECQL4* loss in MPNST cell lines. Our published work has demonstrated that rare NF1-associated gliomas and MPNSTs with ALT lack *ATRX* and *DAXX* mutations, but have sequence variants in *RECQL4*, using next generation sequencing (**Appendix A** and Palsgrove DN, et al. Subependymal giant cell astrocytoma-like astrocytoma: a neoplasm with a distinct phenotype and frequent neurofibromatosis type-1-association. Mod Pathol. 2018 Dec;31(12):1787-1800). *RECQL4* encodes an ATP-dependent DNA helicase that is part of a group of the RecQ helicase protein family, which also includes WRN and BLM, and plays critical roles in genome maintenance and stability. Germline mutations in *RECQL4* are associated with Rothmund-Thomson syndrome and related disorders. These patients

## Figure 5. *RECQL4* knockdown in MPNST cell lines




develop poikiloderma, juvenile cataracts, and a predisposition to develop osteosarcoma. It must be noted that osteosarcoma is one of the human cancers with the highest frequency of alternative lenghtening of telomeres, and indeed cancer is the leading cause of death in

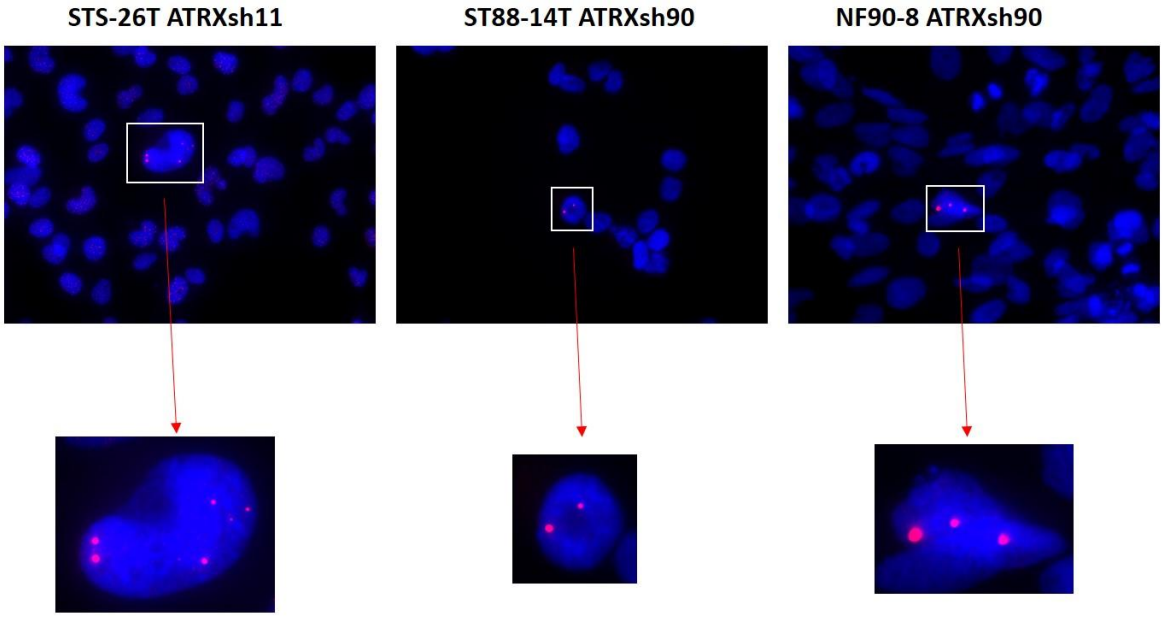
**Figure 6. Telomere length measurement by qPCR (relative telomere length score)**




patients with germline *RECQL4* mutations. Many of the pathogenic mutations that have been described involve the helicase and the PARP1 interaction domains, and usually spare the N-terminus. The interaction with PARP1 appears to play a role in RECQL4

localization to the nucleolus in response to oxidative stress. Interestingly, experiments have shown that RECQL4 associates with activity on telomeric D-loops and telomeric substrates containing thymine

Figure 7. *ATRX* knockdown in MPNST cells induced rare ALT-like foci



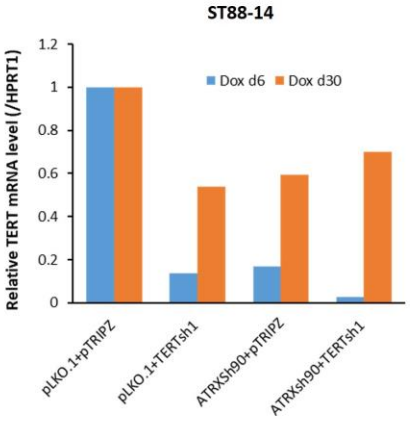

glycol, suggesting a role in telomere maintenance. Therefore we studied the effect of *RECQL4* loss in parallel with the experiments focusing on *ATRX* inactivation. Interestingly, knowckdown of *RECQL4* in these cell lines resulted in decreased growth of the cell line with intact *TERT* (NF90-8) but not with the other two cell lines with *TERT* alterations (ST88-14, NF, STS-26T) (**Figure 5**). *ATRX* and *RECQL4* knockdowns resulted in increased telomere lengths, particularly in NF90-8 using a qPCR quantitative assay (**Figure 6**) and in rare ALT-like foci using telomere specific FISH (**Figure 7**).

Given that the ALT phenotype and related properties requires in addition to ATRX loss to have low telomerase activity, we created an inducible doxycycline responsive cell culture model of TERT knockdown)(**Figure 8**). We have ongoing experiments using this model that will be completed in the second year of the award.

Given the vulnerability of ALT positive/ATRX deficient cells to specific chemotherapeutic agents, particularly ATR inhibitors, we studied *in vitro* the effect of the ATR inhibitor VE-821 in MPNST cell

lines with either *RECQL4* or *ATRX* knockdown. While all three cell lines demonstrated decreased growth after *RECQL4* knockdown and VE-821 treatment, only NF90-8 (lacking *TERT* alterations as mentioned above) demonstrated a reproducible decrease in growth after ATRX

Figure 8. *TERT* knockdown in MPNST cell lines




knockdown and VE-821 treatment (**Figure 9**). However, neither ATRX nor RECQL4 knockdowns affected cell sensitivity to carboplatin, another chemotherapeutic agent that works independently of ATR **(Figure 10**).

Collectively, our findings support a role for ATRX loss and the alternative lengthening of telomeres in a biologic subset of NF1-associated malignancies, and opens the opportunity for therapeutic targeting of these aggressive tumors using specific classes of drugs. In the subsequent two years of the award our aim is to extend these experiments to more relevant cell lines and models and in vitro and in vivo, and test the feasibility of ATR inhibitors as as therapeutic strategy in preclinical in vivo models.

# Figure 9. *RECQL4* knockdown in MPNST cells increased sensitivity to ATR inhibitor

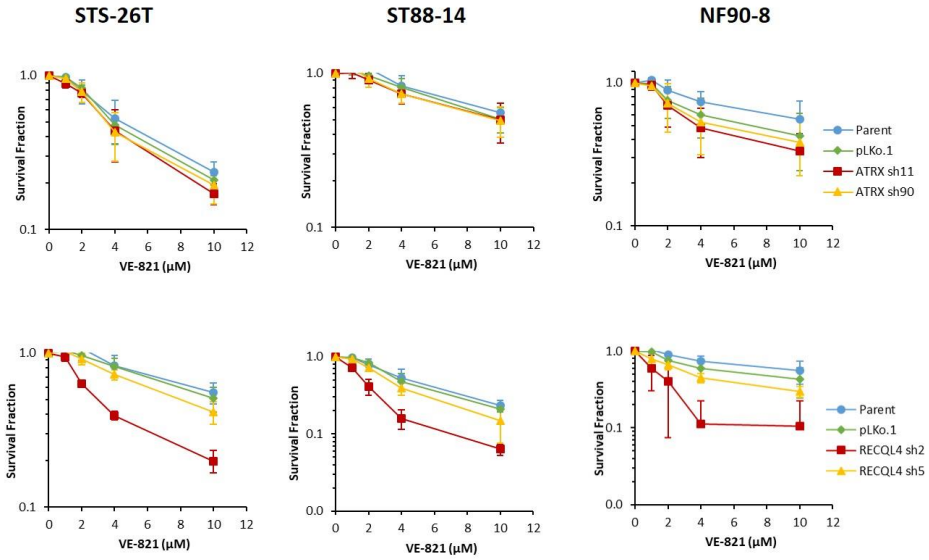
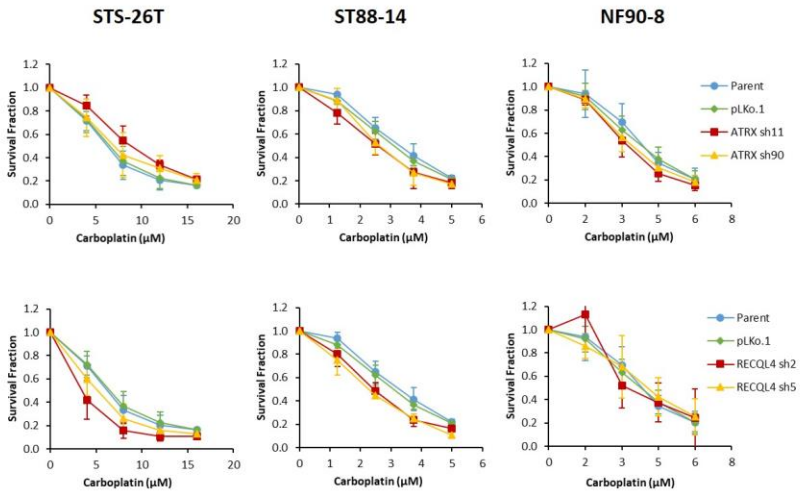


Figure 10. *ATRX* knockdown and *RECQL4* knockdown in MPNST cells did not affect sensitivity to carboplatin




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

Dr. Christopher Heaphy who is a junior faculty of the department and co-investigator in the project was able to attend the yearly meeting of the American Association of Neuropathologists (AANP) in Atlanta June of 2019 to present the paper titled "Telomere alterations in neurofibromatosis type 1-associated solid tumors". This presentation received the **Moore Award** for the best clinicopathologic paper presented at the meeting.

Data gathered for this project formed the basis for a travel grant to Dr. Fausto J. Rodriguez as part of an ongoing collaborative effort in the study of NF1 gliomas with the *Brain Research Institute, Niigata University, Japan*. Dr. Rodriguez visited the brain research institute as a component of this grant in 8/2019 and was the main presenter at a brain tumor symposium, presenting the lecture titled "*Role of ATRX and alternative lengthening of telomeres (ALT) in NF1-associated solid malignancies*".
Details of the grant:

| | |
|---|---|
| 4/1/2019-3/31/2020 | Role of autophagy in NF1-associated gliomas |
| | Brain Research Institute, Niigata University, Japan |
| | $3000 |
| | Role: PI |

Dr. Fausto J. Rodriguez was invited to attend a selected conference **NF1-Low Grade Glioma/Anaplastic Piloid Astrocytoma Conference** hosted by Dr. Roger Packer at Children's National Hospital, in Bethesda MD 2/2019. Dr. Rodriguez presentation titled "*Johns Hopkins Hopkins NF1-low grade glioma, Adult Experience*" was based on his unique experience as the leading pathologist reviewing the pathology specimens of patients seen at the *Johns Hopkins Comprehensive Neurofibromatosis Center*. The purpose of this conference was to brainstorm and discuss recent scientific progress in the field of gliomas developing in NF1 patients. These fruitful discussion will help guide future directions and impact clinical trial design and approaches. The findings will also be published as as consensus paper that will be shortly submitted. Data gathered as part of this DOD award/project was a key component of Dr. Rodriguez participation at this conference.

**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.*

| |
|---|
| Nothing to report |

**What do you plan to do during the next reporting period to accomplish the goals?**
*If this is the final report, state "Nothing to Report."*

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

| |
|---|
| The most important next steps is to extend our initial functional experiments to more relevant models of NF1 and ATRX loss, particularly patient derived glioma and MPNST cells lines in the context of murine xenografts. Now that all the HRPO and AUCU approvals are in place, we are proceeding to develop in vitro models of ATRX/NF1 loss in the context of TERT inactivation (a more accurate combination reflective of human disease). Once we select the best clones, we can start studying the effects of specific drugs in vitro (ATR inhibitors) and create the appropriate xenografts to study in vivo. |

***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.*

Nothing to report.

**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:*

The main problems we have encountered is a significant delay from ACURO and HRPO to approve the standard approaches we propose to study glioma and MPNST cell lines in vitro and in vivo (xenografts). We have been told that there are staff shortages, which explains why our emails/submissions have been entirely ignored or addressed weeks to months after. As of this date we have final ACURO approval (just recently), but the HRPO is still pending after weeks of deliberation.

**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.*

In the past year we have worked with cells in vitro that do not require ACURO or HRPO approval, i.e. mice derived glioma lines and human derived MPNST lines from commercial sources. We have refined our delivery techniques to perform knockdowns of ATRX and TERT intctivation (required for the ALT phenotype) using these models, and will extend these experiments in the second year of the grant to more realistic models of human disease once the approval of HRPO is final.

**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.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

*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**

Nothing to report.

**Significant changes in use or care of vertebrate animals**

Nothing to report.

**Significant changes in use of biohazards and/or select agents**

| |
|---|
| Nothing to report. |

**6. PRODUCTS:** *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).*

**1-Rodriguez FJ**, Graham MK, Brosnan-Cashman JA, Barber JR, Davis C, M., Vizcaino MA, Palsgrove DM, Giannini C, Pekmezci M, Dahiya S, Gokden M, Noë M, Wood LD, Pratilas CA, Morris C, Belzberg A, Blakeley J. Heaphy CM. Telomere alterations in neurofibromatosis type 1-associated solid tumors. *Acta Neuropathol Comm* 2019;7(1):139.

2- Costa FD, Dias TM, Lombardo KA, Raghunathan A, Giannini C, Kenyon L, Saad AG, Gokden M, Burger PC, Montgomery EA, **Rodriguez FJ**. Intracranial Cellular Schwannomas: A Clinicopathologic Study of 20 Cases. *Histopathol* (advanced online publication).

3-Ahlawat S, Blakeley J, **Rodriguez FJ**, Fayad LM. Imaging Biomarkers for the Characterization of Malignant Peripheral Nerve Sheath Tumors in Neurofibromatosis type 1. *Neurology* (advanced online publication)

4-Martínez H, Nagurney M, Wang ZX**,** Eberhart CG, Heaphy CM, Curtis MT, **Rodriguez FJ**. *ATRX* Mutations in Pineal Parenchymal Tumors of Intermediate Differentiation**.** *J Neuropathol Exp Neurol* (advanced online publication)

5- Nix JS, Blakeley J, **Rodriguez FJ**. An Update on the Central Nervous System Manifestations of Neurofibromatosis 1. *Acta Neuropathol* (advanced online publication).

**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).*

**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.*

1- **Rodriguez FJ**, Graham MK, Brosnan-Cashman JA, Barber JR, Davis C, M., Vizcaino MA, Palsgrove DM, Giannini C, Pekmezci M, Dahiya S, Gokden M, Noë M, Wood LD, Pratilas CA, Morris C, Belzberg A, Blakeley J. **Heaphy CM**. Telomere Alterations in NF1-associated Solid Tumors are Associated with Clinical Outcome. *J Neuropathol Exp Neurol* 2019; 78 *p554* (oral presentation at the American Association of Neuropathologists Annual Meeting, received the Moore award for best clinicopathologic paper)

2- **Rodriguez FJ**, Graham MK, Brosnan-Cashman JA, Barber JR, Davis C, M., Vizcaino MA, Palsgrove DM, Giannini C, Pekmezci M, Dahiya S, Gokden M, Noë M, Wood LD, Pratilas CA, Morris C, Belzberg A, Blakeley J. **Heaphy CM**. Telomere alterations in neurofibromatosis type 1-associated solid tumors (accepted for poster presentation to the 2019 NF conference, *Children's Tumor Foundation*)

- **Website(s) or other Internet site(s)**

*List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

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.*

| |
|---|
| Nothing to report. |

- **Other Products**
  *Identify any other reportable outcomes that were developed under this project. Reportable 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 support is provided from other than this award.)* |

Dr. Ming Yuan (no change)
Dr. Christopher Heaphy participated in the past year in the project as outlined in the application. However, Dr. Heaphy will be moving to another institution and his close colleague and mentor, Dr. Alan Meeker will be taking over his responsibilities in the grant.

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
*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.*

Nothing to report.

**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.*

**Appendix A: Rodriguez FJ**, Graham MK, Brosnan-Cashman JA, Barber JR, Davis C, M., Vizcaino MA, Palsgrove DM, Giannini C, Pekmezci M, Dahiya S, Gokden M, Noë M, Wood LD, Pratilas CA, Morris C, Belzberg A, Blakeley J. Heaphy CM. Telomere alterations in neurofibromatosis type 1-associated solid tumors. *Acta Neuropathol Comm* 2019;7(1):139.

**Appendix B**: Nix JS, Blakeley J, **Rodriguez FJ**. An Update on the Central Nervous System Manifestations of Neurofibromatosis 1. *Acta Neuropathol* (advanced online publication).