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CONTRACTING ORGANIZATION: **University of Florida**

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Malignant peripheral nerve sheath tumors (MPNSTs) are very rare cancers that carry a poor prognosis. About half occur in the context of neurofibromatosis type 1 (NF1), an autosomal dominant syndrome that affects approximately 1/3000 individuals world-wide. NF1 patients develop multiple slow-growing benign Schwann cell tumors (neurofibromas) throughout life, anywhere on the peripheral nervous system, from spinal nerve roots to nerve endings in the skin. Neurofibromas affecting larger nerves are called plexiform, which are thought to be congenital in origin and have an 8-13% risk of transformation to MPNST, which may not be detected until an existing tumor becomes painful. Because of MPNST rarity, there is no evidence-based treatment protocol. Rather, each institution manages cases uniquely, choosing from treatment modalities, with variable results. In addition, as the World Health Organization 2016 classification indicates, "Clinically validated and reproducible grading systems for MPNST are generally lacking" (Reuss et al., 2016), so neuropathologists have less-than-optimal tools

15. SUBJECT TERMS
MPNST, sarcoma, NF1, histone modification, epigenetics, retrospective clinical outcomes research

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

Malignant peripheral nerve sheath tumors (MPNSTs) are very rare cancers with five-year survival rate <50%. About half occur in patients with neurofibromatosis type 1 (NF1), a progressive autosomal dominant tumor syndrome characterized by benign Schwann cell tumors (neurofibromas). Neurofibromas affecting larger nerves are called plexiform; these have an 8-13% risk of transformation to MPNST. There is no evidence-based treatment protocol for MPNST, so institutions manage patients with variable modalities. In addition, neuropathologists lack advanced tools for diagnosis and prognosis. The goal of this work is: (1) to gather retrospective chart review data on MPNST cases treated at the University of Florida (UF) College of Medicine (Aim 1), and (2) to immunostain MPNST sections for two antigens (Aim 2). The first Aim will provide data about UF therapeutic approach outcomes. The second Aim will test for relationships between H3K27me3 and HMGA2 immunostaining results with survival. This work involves retrospective chart review and analysis of existing specimens.

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

malignant peripheral nerve sheath tumor **(**MPNST), neurofibromatosis type 1 (NF1), epigenetics, H3K27me3, HMGA2, immunohistochemistry, retrospective chart review.

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.

Major Task 1. Analyze retrospective clinical data from UF MPNST cases (Aim 1).
Subtask 1. Search UF databases to identify MPNST cases (anticipate 50-80), gather clinical information (months. 1-6, completed).
Subtask 2. Perform statistical analysis on Subtask 1 data to identify clinical factors affecting patient survival (months 7-12, in progress).
Major Task 2. Collect and analyze data from MPNST tissue from the Aim 1 patients.
Subtask 1. Find FFPE blocks in Pathology Department for Aim 1 patients. (months 1-6, completed).
Subtask 2. Cut 4 slides from each FFPE block (months 2-8, completed).
Subtask 3. Stain 1 MPNST slide each with hematoxylin and eosin. (months 2-7, completed).
Subtask 4. Optimize & stain 1 MPNST slide each for H3K27me3 (months 2-7, completed).
Subtask 5. Optimize & stain 1 MPNST slide each for HMGA2 (months 4-8, completed).
Subtask 6: Score slides for staining result (months 5-10, completed).
Subtask 7: Statistical analysis of staining and clinical data (months 6-10, in progress).
Subtask 8: Interpretation of data, writing and submitting manuscript for publication. (months 5-12, to begin after statistical analysis).

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.

1. Major activities. We identified 70 potential MPNST patients in the retrospective study, from 1988-2021, out of an anticipated 80 in our original estimate. This included patients obtained through some archived records, as UF's electronic medical record doesn't always include records before 2000. 33 of these cases were in patients with neurofibromatosis type 1 (NF1), 33 were sporadic (non-NF1) MPNSTs, and in 4 cases it was unclear if the patient had NF1 or not (Table 1). We obtained fixed paraffin-embedded blocks from 51 cases, and in 13 cases we obtained 2 or 3 additional blocks from surgeries for tumor recurrence or metastasis, for a total of 81 blocks. Slides were cut from these blocks, followed by H&E staining, immunostaining for S100B, immunostaining for H3K27me3 epigenetic marker, and immunostaining for the HMGA2 transcription factor. Of the 51 cases for which we were able to evaluate slides, H&E staining revealed that the tumor was either a low-level MPNST or an ANNUBP (a neurofibroma in transition to becoming malignant), with no high-grade MPNST regions. We also stained a few additional neurofibroma (benign) slides for a comparison with the MPNSTs. Based on literature recommendation, slides were evaluated for presence/absence (or partial) of the S100B Schwann cell marker, H3K27me3 (loss if less than 5% of the tumor cells stained), and whether HMGA2 was overexpressed (yes if at least 50% of tumor cells had strong nuclear staining), by analyzing 4 high-power (40x) fields per slide. We have nearly finished scoring these, and converting the clinical data to scores, and will be ready for statistical analysis shortly, to evaluate relationships between survival, presence of NF1, tumor grade, gender, age, and the molecular markers, as described below.

Table 1. Breakdown of cases ascertained

70 potential cases	33 NF1	Tissue: 27	High-grade MPNST: 24
	33 sporadic	Tissue: 23	High-grade MPNST: 14
	4 unknown	Tissue: 1	High-grade MPNST: 1

2-4. Objectives, and outcomes to date. The first objective was to perform a comprehensive review and survival analysis of all MPNST cases seen at the University of Florida College of Medicine. A subset of 20 of cases was in the process of being studied as a medical student research project, in which the only cases analyzed were those treated in the UF Dept. of Orthopaedics, Division of Oncology (diagnosis to discharge). The student, Daniel Knewitz, finished his analysis and we published the paper in 2021 in *Sarcoma* (v.2021, article ID 9386823). The outcome showed that patients managed by UF Orthopaedics had a very high 5-year survival rate: 70%, with 60% 5-year survival for patients with metastases. We also observed that lack of S100B staining was associated with poorer survival. Our current study, with at least twice as many cases, will test whether this trend continues when considering all MPNST patients seen at UF, regardless of service. Cases after 2018 will not be included in the survival analysis, to have a 5-year survival view.

The second objective was to determine whether MPNST immunostaining of specific markers associate with tumor grade and/or survival. H3K27me3 is an epigenetic mark (H3 histone modification) of inactive/silenced chromatin. A few reports in the literature had suggested that MPNSTs tend to show loss of this marker (which is normally expressed in Schwann cells and benign Schwann cell tumors such as neurofibromas). At the time this grant was submitted, the field of neuropathology was considering whether to include H3K27me3 staining as recommended marker for MPNST, as there were none other than S100B and SOX10. Although H3K27me3 staining is now recommended, there are only a handful of studies providing data about sensitivity based on grade, but none with survival. Thus, our data will add substantially to the evaluation of the interpretation of H3K27me3 staining. The second marker to evaluate was HMGA2, a transcription factor involved in regulating gene expression, affecting chromatin condensation, DNA damage repair, and cell growth/differentiation. Two publications had suggested that overexpression of HMGA2 was a potential marker to differentiate benign Schwann cell tumors from MPNST, which would also make it an important diagnostic marker. Once the statistics are done, our data will shed light on this. The neuropathologists here are excited about the possibility of useful new markers for MPNST, because these tumors can have variable morphology (e.g. some can be rhabdoid/Triton-like, some can be epithelioid, some can mimic melanoma) and can be difficult to diagnose in the absence of NF1 co-morbidity. Additional markers will potentially increase the sensitivity detect transformation to malignancy within a benign neurofibroma, for patients with NF1.

The only goal not yet met is the statistical analysis of the data, and publication of those results. Our plan is to have the matrix of data to the statistician by the end of March. We hope that the analysis can be completed within 1-2 months, with subsequent plans for publications.

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.

A medical student was involved in Aim 1 (began similar work prior to this grant being funded, as part of a summer med student research opportunity). He learned about NF1, MPNSTs, orthopedic oncology, and clinical research. He was first author on the paper reporting survival data just from the UF Department of Orthopaedics (see below), so he also gained experience with writing, submitting, and revising scientific manuscripts. Also our neuropathologist Dr. Yachnis used this project's H&E slides (a rare, large collection of such tumors) to educate a younger neuropathologist, who had seen very few of these tumors. Fortunately, UF retains FFPE specimens, so we were able to study tumor samples back to 1988.

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.

Final Report, however, the statistical analysis has yet to be done. We intend to submit our results for publication within the next 6-12 months

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).
If there is nothing significant to report during this reporting period, state "Nothing to Report."

We do not yet know the impact specifically until we see the statistical outcomes. However, this will be a major addition to the literature regarding HMGA2 staining in MPNSTs, and contribute to evaluation of this marker, S100B, and H3K27me3 in diagnosing MPNSTs, as well as potential prognostic use for survival estimates.

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:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember

Nothing to Report, other than the delays described in the paragraph below.

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.

This research project suffered from multiple delays for a variety of reasons. Many are detailed in the previous progress report, but here's a summary. The first was due to communication problems with DOD (email black holes, with no phone numbers available), and extensive delay in DOD human subjects approval, despite this being approved as Expedited at UF very quickly. We could thus only initially work on non-human related work such as optimizing antibody staining during that time. Another major cause of delay was the COVID pandemic, which caused a lab shut down for several months, and restricted access thereafter for some time. After that, because the UF Pathology department was short-handed (like nearly every entity at that point), every step took unusually long: weeks to obtain requested FFPE blocks (in 4 batches), molecular pathology lab delays, appointments with the neuropathologist to read stained slides. I also had to dig through archived paper charts for early clinical data that had not been entered on the electronic medical record, but had limited access to that file room (5 visits spanning 6 weeks). The last delay has been from collating the data into spreadsheets for the statistician, which is nearly done, and my goal is to have this spreadsheet to the statistician within the next month. The statistical analysis should not take more than 1-2 months, and we will work on manuscript(s) after that. I have started a manuscript shell with the basics of the study.

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.

None.

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

None.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

None.

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).*

  Knewitz DK, Anderson CJ, Presley WT, Hordyski M, Scarborough MT, Wallace MR. Survival and *NF1* analysis in a cohort of orthopedics patients with malignant peripheral nerve sheath tumors. Published in Sarcoma, Volume 2021, Article ID 9386823, 6 pages. This grant was acknowledged.

  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).

None.

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.*

"Understanding the Basis for Neurofibromatosis Type 1 (NF1)." Invited talk, Moffitt Cancer Center, Tampa, FL, Feb. 2020.

"A 30+ year perspective on the NF1 gene and genetics: why "keep it simple, stupid" does not apply." 2020 NF Conference (virtual, live talk), June 15, 2020, 700 participants (international).

- **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.

None.

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

There were no new technologies developed.

- **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.

None.

- **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.*

None.

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".

Reporting for entire project period:
Margaret Wallace - no change except nearest person month worked is now 2.0
Hua Li - no change except nearest person month worked is now 2.0
Anthony Yachnis - no change
MaryBeth Horodyski - no change

Name: Daniel Knewitz
Project Role: medical student (until May 2022)
Research Identifier: ORCID ID 0000-0002-5123-9981
Nearest person month worked: 0.2 (in addition to collecting data from 20 subjects)
Contribution to project: contributed clinical data he collected on 20 Orthopaedics subjects (which he began prior to this project) and some of the additional subjects studied here.
Funding Support: none

Name: Elham Nasri, MD
Project Role: neuropathologist
Research Identifier: ORCID ID 0000-0001-5894-5865
Nearest person month worked: 0.06
Contribution to project: assisted Dr. Yachnis with pathology evaluation of H&E stained slides.
Funding Support: none

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.

Active support for the PI has changed since this grant was submitted in 2019, as follows: These R01s (on which the Dr. Wallace was co-investigator) ended: 1R01GM114290, 5R01DE019456, and 2R01AR055899. The NTAP grant has ended. The only new funding is a Clinical Research Award from the Children's Tumor Foundation: Study of NF1 in Families, 10.22 - 9.24, total award $146,348, 0.96 person months/year.

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.

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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.* **Not applicable.**

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.* **Not applicable.**

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.* NONE