AWARD NUMBER: W81XWH-22-1-0373

TITLE: Targeting Chromothripsis in Malignant Glioma

PRINCIPAL INVESTIGATOR: Deanna Tiek, PhD

CONTRACTING ORGANIZATION: Northwestern University, Evanston, IL

REPORT DATE: July 2023

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
JULY 2023	ANNUAL	01JUL2022 - 30JUN2023

4. TITLE AND SUBTITLE

Targeting Chromothripsis in Malignant Glioma

5a. CONTRACT NUMBER
W81XWH- 22-1-0373

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Deanna Tiek, PhD

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Northwestern University, Chicago, Illinois

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development 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 is Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT:

Clinical biomarker detection has paved the way in determining the patient population that will most benefit from a specific treatment. In this way, there will be fewer unnecessary side effects as a patient determined as a non-responder – via biomarker presence or absence – would not be selected to receive this treatment. A classic example is the expression of the HER2 receptor in breast cancers. If a patient is HER2 positive, they will receive the HER2-targeting drug, Herceptin. However, a non-HER2 positive patient would not receive Herceptin as they do not have the drug target expressed. Here, HER2 is a positive selection biomarker that dictates treatment therapeutic options to prevent over-treatment and assist in positive patient selection. While other cancers, like breast, have well-defined biomarkers that dictate drug options, the brain cancer glioblastoma (GBM) is lagging. GBM is the most common and deadly brain cancer in adults with an average survival post-diagnosis of ~14-16 months. This survival time greatly decreases once a patient becomes resistant to the current first line therapeutic option – temozolomide (TMZ). For these reasons, we are proposing to elucidate a biomarker of TMZ-resistant GBM, as well as better understand the disease, to determine the best second-line therapeutic option for these patients.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
U	U	U	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

This project was to determine the how chromothripsis – a new DNA mutational phenomenon – affects drug resistant glioblastoma treatment. Through its mechanism of action, the drug Selinexor was chosen as a target drug candidate as it prevents import/export of nuclear proteins through inhibiting the importin XPO1. The purpose is to find a second line therapy option as for glioblastoma (GBM) patients there is only a first line option. Patients will eventually become resistant to therapy and this is uniformly fatal.

2. KEYWORDS:

Brain cancer, glioblastoma, therapy resistant, drug resistant, chromothripsis, DNA damage

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. Analyze telomere sequences and correlation to chromothripsis. Month 1-3; completed in Month 3.
2. Identify localization of binding changes of shelterin. Month 4-5; completed in month 5.
3. Perform in vitro and in vivo studies of Selinexor +/- TMZ. Month 6-11; completed in vitro studies in Month 6, in vivo studies were limited as cell lines did not form tumors.
4. Identify the relationship between Selinexor sensitivity and telomere changes and chromothripsis in Selinexor clinical trial patient samples. Month 10-12; not completed; the patient samples ended up being fixed in a solution that did not allow for long read whole genome sequencing. Still trying to figure out what we can do with the limited patient samples available.

What was accomplished under these goals?

The major activities for this project were to characterize the chromothripsis phenotype and telomere changes in TMZ-resistant glioblastoma cell lines (TMZ-R GBM). I found that TMZ-R GBM was more sensitive to targeting of CT via the import/export protein inhibitor Selinexor/KPT. The original hypothesis was also strengthened that changes in G-rich regions via TMZ treatment affect protein docking to these regions which make them more dependent on import/export machinery. Combination of Selinexor and TMZ was the more synergistic treatment, and showed decreased growth in the deadly TMZ-R GBM model. Further and more detailed results are below.

Major Task 1. Analyze telomere sequences and correlation to chromothripsis:
Subtask 1 Complete 30X coverage whole genome sequencing and 2 Analyze whole genome sequencing for telomere changes - Figure 3A shows whole genome sequencing and analysis with Shatterseek to determine chromothripsis (CT) changes, where we see an increase in the TMZ-R lines, 42R and T98G. Subtask 2 is ongoing as a new bioinformatics method is needed.
Major Task 2 Identify localization of binding changes of shelterin:
Subtask 1 Create mutant telomere changes and determine the ability of shelterin to bind – Is ongoing as the sequences are still be determined as stated above.
Subtask 2 Identify localization of shelterin components with immunofluorescence - Figure 4A shows the changes in the shelterin protein TRF2 with and without KPT/TMZ treatment. Once Subtask 2 is complete, I will test the ability of TRF2 to bind them. In the meantime, I created a mutant TRF2 cell line in normal human astrocytes and show that when TRF2 is mutated (TRF2mut) these cells become more sensitive to KPT treatment (Figure 4B).
Major Task 3 Perform in vitro and in vivo studies of Selinexor +/- TMZ:
Subtask 1 Perform growth assays of TMZ-S and TMZ-R cell lines with Selinexor treatment +/- TMZ: Figure 2A-C shows the increased sensitivity of KPT +/- TMZ in TMZ-R lines. This data has now been extended in Figure 3D where when comparing other DNA damage drugs, we still see the greatest additive effect with TMZ. Furthermore, we see that KPT treatment decreases CT characteristics (Fig. 3B) and induces apoptosis in TMZ-R lines as well as pHis3 decreases with KPT suggesting a decrease in the G2/M cell cycle phase (Fig. 3C).
Subtask 2 – Submit documents for IACUC/ACURO approvals; IACUC has been approved for this project.
Subtask 3 Perform In vivo studies of Selinexor +/- TMZ from doses determined from Subtask 1. The in vivo work is still ongoing for this task.
Subtask 4 – At endpoint, excise the brains to analyze chromothripsis characteristics: Ongoing as it is dependent on the brains from the subtask 3 which is not complete.
Subtask 5 – Obtain HRPO approval to use and analyze de-identified patient samples in Major Task 4. Approved, and waiting for clinical samples as the patients are still on trial.
Major Task 4 Identify the relationship between Selinexor sensitivity and telomere changes and chromothripsis in Selinexor clinical trial patient samples:
Subtask 1 – Perform whole genome sequencing on flash frozen Selinexor treated patient samples. This task is ongoing as the patient samples are going to be fixed in paraffin preventing proper sequencing. We will have the embedded samples that can be used for IHC. For this reason, I have quantified other markers of CT. In Figure 1 I show that g-quadruplex staining is decreased in TMZ-R lines, nuclear envelope (NE) bridges and nuclear envelope rupturing during interphase (NERDI) is increased, and that this is in a G-rich binding motif dependent manner. Furthermore, looking two FET family members (EWSR1 and FUS) from the G-rich group, I see an increased stress response in the TMZ-R models vs the TMZ-S line (4C). Finally, the actual target of KPT, XPO1, shows a worse OS with increased expression in recurrent GBM (4D), suggesting an increased demand for nuclear import/export which can be targeted with Selinexor (KPT). This alternative characterization will allow me to hopefully determine CT features in the patient samples when these patients succumb to this disease post Selinexor treatment.
Subtask 2 – Correlate whole genome sequencing results to patient response in the Selinexor trial. Once the trial has finished I will correlate the CT characteristics with overall survival. As the study started 05/2021 and is not terminated yet as of 09/2023, we hope to see differences.

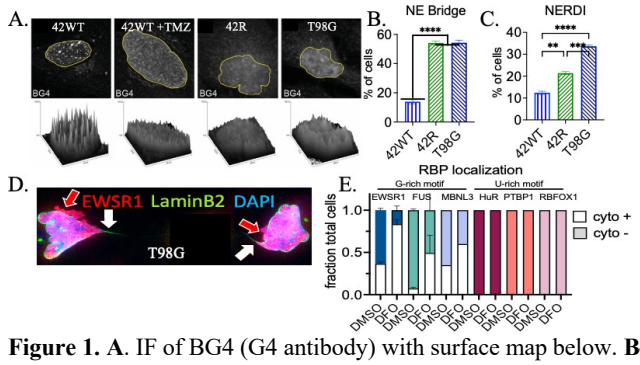

Figure 1. A. IF of BG4 (G4 antibody) with surface map below. **B-C.** IF quantification of 42WT, 42R, and T98G for NE Bridges (**B**) NERDI (**C**). **D**. IF of EWSR1, Lamin B2 (NE bridge marker) and DAPI showing nuclear protein leakage (EWSR1; red arrow) and NE bridge (LaminB2; white arrow). **E**. IF of RBP localization.

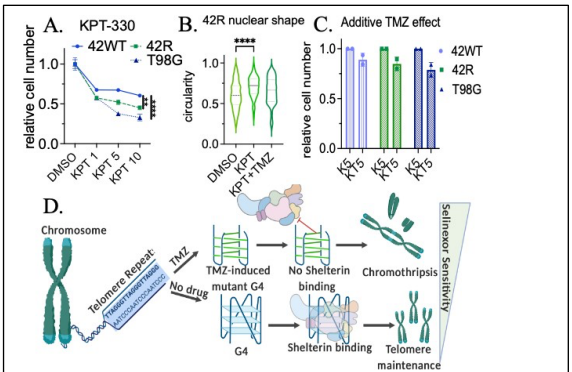

Figure 2. **A**. Crystal Violet (CV) staining for growth 4 days post KPT-330 µM concentrations.**B.** DAPI circularity with a perfect circle =1. **C.**CV for 5 µM KPT-330 (K) +/- 100 µM TMZ (KT). **D**. Cartoon of hypothesis.

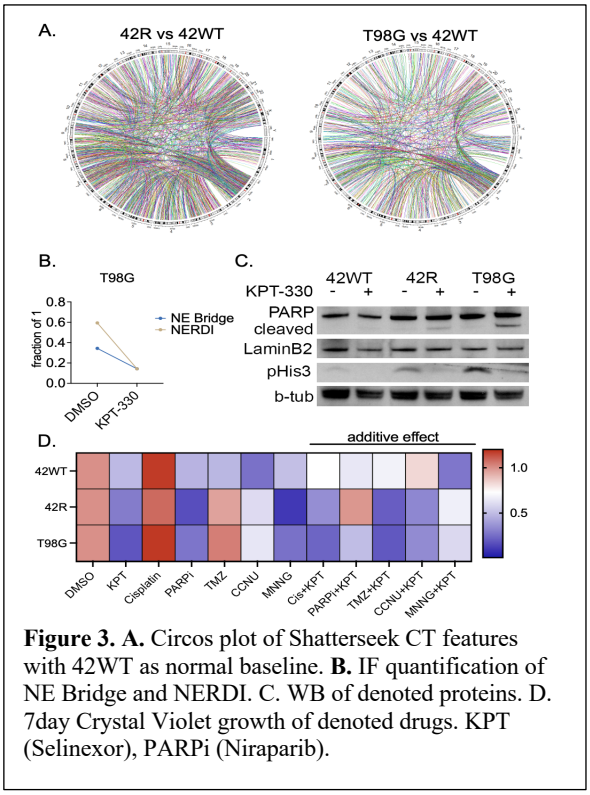

Figure 3. A. Circos plot of Shatterseek CT features with 42WT as normal baseline. **B.** IF quantification of NE Bridge and NERDI. C. WB of denoted proteins. D. 7day Crystal Violet growth of denoted drugs. KPT (Selinexor), PARPi (Niraparib).

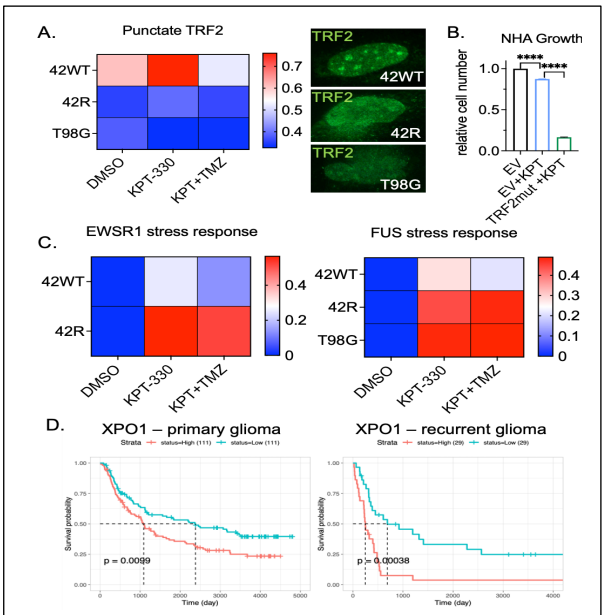

Figure 4. A. Left, quantification of TRF2 IF localization (Right) KPT+/-TMZ. **B.** Growth of normal human astrocytes (NHA) +/- EV or TRF2mutant protein +/- KPT. **C.** IF quantification of EWSR1 and FUS nucleolar localization KPT+/-TMZ. **D.** KMplot of CGGA data for XPO1 – the target of Selinexor.

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

This was not a training or development award, however I was able to learn a new technique in PacBio HiFi long read sequencing. This has allowed me to gain more collaborations through knowing this advanced technique.

How were the results disseminated to communities of interest?

I have presented these results in our departmental meetings at the Lurie Cancer Center as well as within my lab group to further explore these new forms of DNA damage – like chromothripsis – as well as the new techniques I've learned.

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

As I have an extension, I plan to best utilize the patient samples to determine if we can get any sort of sequencing or FISH data from this platform.

4. **IMPACT:**

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

This project has shown that TMZ treatment does affect the sensitivity of GBM cells to Selinexor. As most clinical trials are in the primary, newly diagnosed setting, this may be an issue when looking at the overall survival. This project suggests that either co-treatment with TMZ or after TMZ resistance may be a better time to intervene with Selinexor.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

The main change is how the patient samples will be utilized. Originally I had proposed sequencing to determine the Chromothripsis state, but these samples have been embedded in a material that is not conducive to our sequencing platform. I'm now determining how to best utilize these samples to answer our chromothripsis question, and plan to perform IHC to quantify the other markers of chromothripsis – shelterin complex changes, nuclear envelope rupture, and chromatin bridges.

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

I have had an issue with the fixation of the patient samples – as denoted above. I have requested an extension and now have a plan in place to perform a different analysis to determine chromothripsis markers and correlate this to overall patient survival with Selinexor treatment.

Changes that had a significant impact on expenditures

The major delay has been the patient samples. As most GBM clinical trials for recurrent disease end in 3-6 months, this trial was extended past a year. While this was great for patient outcomes, this has delayed our ability to gain access to patient samples. An extension has been asked for to be able to utilize the patient samples.

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

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to repot.

Significant changes in use of biohazards and/or select agents

Nothing to repot.

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

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

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

I have been solely working on this project with the help of core facilities here at Northwestern University.

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

Nothing to report.

What other organizations were involved as partners?

I have been working on this project at Northwestern.

8. SPECIAL REPORTING REQUIREMENTS
COLLABORATIVE AWARDS:
QUAD CHARTS:

9. APPENDICES: