AWARD NUMBER: W81XWH-19-1-0180

TITLE: Transcription, R-Loops, and RNA Splicing in Ewing Sarcoma

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REPORT DATE: October 2021

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

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1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2021	Annual	01Jul2019-30Jun2021

4. TITLE AND SUBTITLE

Transcription, R-Loops, and RNA Splicing in Ewing Sarcoma

5a. CONTRACT NUMBER W81XWH-19-1-0180

5b. GRANT NUMBER CA181177

5c. PROGRAM ELEMENT NUMBER

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)

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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 Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Ewing sarcoma (EwS) is an aggressive pediatric bone and soft tissue cancer driven primarily by the EWS-FLI1 fusion oncogene. EWS-FLI1 acts as a transcription factor and also interferes with normal regulation of transcription and transcription-associated RNA processing. We hypothesized that EWS-FLI1 driven hyper-activation of transcription causes a targetable dependence on RNA splicing in Ewing sarcoma. The specific aims of the project are to 1) determine the mechanistic relationship between EWS-FLI1-driven transcription, R-loops, and splicing vulnerabilities in EwS, and 2) establish splicing as a therapeutic target in EwS. We tested the effect of depletion of key splicing factors and found an increased sensitivity in EwS compared to control cells. This was partially rescued by depleting EWS-FLI1 or by overexpressing RNASEH1, which degrades R-loops. EwS cells were extremely sensitive to splicing inhibition, which showed synergy with multiple chemotherapeutic agents. Splicing inhibition also caused cell cycle arrest and induced apoptosis. This work provides novel insight into transcription regulation and its dysregulation by EWS-FLI1. In addition, our results point to RNA splicing as a potential new therapeutic target in Ewing sarcoma, which has the potential to benefit all Ewing sarcoma patients, especially those with chemo-resistant disease.

15. SUBJECT TERMS

Ewing sarcoma, splicing, transcription, R-loops

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	18	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION:

The purpose of this project is to evaluate splicing as a therapeutic target in Ewing sarcoma and provide insight into the mechanistic basis for splicing vulnerabilities in this disease. Ewing sarcoma (EwS) is an aggressive bone and soft tissue cancer that occurs in children, adolescents, and young adults[1]. It is most commonly driven by a chromosomal translocation resulting in the fusion protein EWS-FLI1. Chemotherapy has substantially improved overall survival, but the prognosis is still very poor for those with metastatic or recurrent disease, and toxicity is a major concern in pediatric patients. Consequently, effective, less toxic, targeted treatment strategies are much needed. Previous studies reported that EwS cells display changes in alternative splicing events, a phenomenon linked to the role of EWSR1 as an RNA-binding protein and proposed regulator of splicing[2-5]. Splicing occurs concurrently with transcription, and the regulation of the two processes is coordinated[6-9]. Our lab recently reported that EWS-FLI1 caused hyper-phosphorylation of RNA Polymerase II (RNAPII)[10]. Accordingly, EwS cells displayed high levels of transcription and R-loops and impaired transcription regulation in response to DNA damage. Notably, the set of genes that are alternatively spliced in EwS are highly expressed and have strong R-loop signal. In a genome-wide RNAi screen in the EwS cell line TC32, we found that RNA splicing was one of the top processes required for cell viability. We hypothesized that EWS-FLI1 driven hyper-activation of transcription causes a targetable dependence on RNA splicing in Ewing sarcoma. The specific aims of this project are to 1) determine the mechanistic relationship between EWS-FLI1-driven transcription, R-loops, and splicing vulnerabilities in EwS, and 2) establish splicing as a therapeutic target in EwS. During the first year of the project, we tested the effect of depletion of key splicing factors and found an increased sensitivity in EwS compared to control cells. This was partially rescued by depleting EWS-FLI1 or by overexpressing RNASEH1, which degrades R-loops. EwS cells were extremely sensitive to the splicing inhibitor E7107, which caused cell cycle arrest, induced apoptosis, and showed strong synergy with vincristine. During the second year of the project, we have made substantial progress towards completing the remaining experiments, including sequencing (RNA-seq, DRIP-seq, and GRO-seq), bioinformatics analysis, and protein interaction studies. We anticipate completing these experiments and submitting a manuscript for publication during year three of the project. This work provides insight into the dysregulation of transcription and splicing by EWS-FLI1 and suggests RNA splicing as a novel therapeutic target in Ewing sarcoma.

2. KEYWORDS:

Ewing sarcoma, splicing, transcription, R-loops

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. Major task 1: Confirm the dependence of Ewing sarcoma cells on the splicing machinery.
    a. Subtask 1: Cell viability with siRNA knockdown and cDNA overexpression of splicing factor genes in Ewing sarcoma and control cell lines. (target completion 10/19; 100% completed)
    b. Subtask 2: RT-qPCR of splice isoform expression with siRNA knockdown and cDNA overexpression of 3 splicing factor genes. (target completion 01/20; 50% completed)

c. Subtask 3: EU/flow cytometry transcription assay with siRNA knockdown and cDNA overexpression of 3 splicing factor genes. (target completion 01/20; 100% completed)
d. Subtask 4: Western blot for RNAPII-pSer2 and RNAPII-pSer5 with siRNA knockdown and cDNA overexpression of 3 splicing factor genes. (target completion 01/20; 100% completed)
e. Subtask 5: Dot blot for R-loops with siRNA knockdown and cDNA overexpression of 3 splicing factor genes. (target completion 01/20; 100% completed)

2. Major Task 2: Determine the causal relationship between transcription dysregulation, R- loops, and splicing in Ewing sarcoma.
   a. Subtask 1: RT-qPCR of splice isoform expression in the presence of DRB treatment, THZ1 treatment, and RNASEH1 overexpression. (target completion 07/20; 50% completed)
   b. Subtask 2: EU/flow cytometry in the presence of DRB treatment, THZ1 treatment, and RNASEH1 overexpression. (target completion 07/20; 50% completed)
   c. Subtask 3: Dot blot for R-loops in the presence of DRB treatment, THZ1 treatment, and RNASEH1 overexpression. (target completion 07/20; 50% completed)
   d. Subtask 4: Paired-end RNA-seq with and without RNASEH1 overexpression. (target completion 04/20; 0% completed)
   e. Subtask 5: GRO-seq in TC32 and MSC cells. (target completion 07/20; 50% completed)
   f. Subtask 6: DRIP-seq in TC32 and MSC cells. (target completion 07/20; 100% completed)
   g. Subtask 7: Bioinformatics analysis of RNA-seq, DRIP-seq, and GRO-seq data (target completion 10/20; 75% completed)
3. Major Task 3: Determine whether splicing inhibitors show EwS-specific toxicity, alone or in combination with standard therapeutic agents.
   a. Subtask 1: Cell viability dose response curves for E7107, E7107+vincristine, E7107+paclitaxel, and E7107+docetaxel in 9 cell lines. (target completion 01/20; 100% completed)
   b. Subtask 2: Apoptosis assay for E7107, E7107+vincristine, E7107+paclitaxel, and E7107+docetaxel in 9 cell lines. (target completion 01/20; 100% completed)
4. Major Task 4: Determine the biological effects of splicing inhibition in EwS cells.
   a. Subtask 1: RT-qPCR of splice isoform expression, with and without E7107, with and without RNASEH1 overexpression. (target completion 01/21; 0% completed)
   b. Subtask 2: EU/flow cytometry with and without E7107, with and without RNASEH1 overexpression. (target completion 01/21; 50% completed)
   c. Subtask 3: Dot blot for R-loops with and without E7107, with and without RNASEH1 overexpression. (target completion 01/21; 50% completed)
   d. Subtask 4: Flow cytometry cell cycle analysis with and without E7107, with and without RNASEH1 overexpression. (target completion 01/21; 50% completed)
   e. Subtask 5: DNA combing with and without E7107, with and without RNASEH1 overexpression, with and without HU treatment. (target completion 04/21; 0% completed)
   f. Subtask 6: DR-GFP assay for homologous recombination, with and without E7107, with and without RNASEH1 overexpression. (target completion 04/21; 0% completed)

What was accomplished under these goals?

Specific Aim 1: determine the mechanistic relationship between EWS-FLI1-driven transcription, R-loops, and splicing vulnerabilities in Ewing sarcoma.

The first major goal of the project was to confirm the dependence of Ewing sarcoma cells on the splicing machinery. During the first year of the project, cell viability experiments were performed to assess the effect of splicing factor depletion on Ewing sarcoma (EwS) versus control cell lines. Cell viability assays were performed in 384-well plates; siRNA was delivered by reverse transfection using RNAiMax and cDNA by forward transfection using Lipofectamine 3000. Cell growth was measured over three days by the IncuCyte® ZOOM live cell analysis system followed by an endpoint Cell Titer Glo assay in the same plates to quantify viability by ATP production. 9 cell lines were evaluated including 6 EwS (TC32, ES1, ES7, A673, EW8, CHLA10) and 3 control (MSC (mesenchymal stem cells) IMR90 (fibroblast), and U2OS (osteosarcoma)); 3 representative cell lines are shown in figure 1. EwS cells showed a heightened sensitivity to splicing factor knockdown

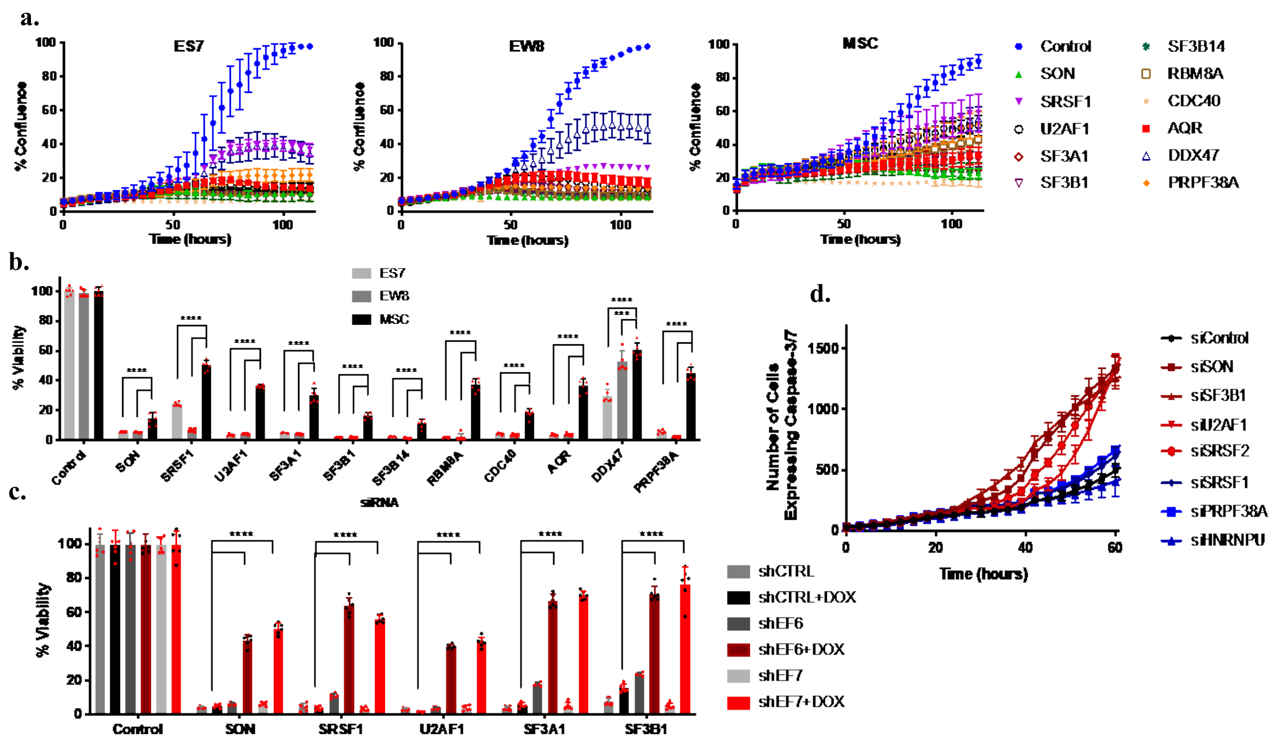

Figure 1. Growth (**a**) and Cell Titer-Glo viability (**b**) of EwS cells (ES7 and EW8) and MSC with siRNA knockdown of the indicated genes. **c**. Viability of TC32 cells with dox-inducible shRNA against EWS-FLI1 or control with knockdown of the indicated genes. **d**. Number of apoptotic TC32 cells (expressing caspase-3/7) over 60 hours following knockdown.

compared to control cells (Figure 1a, 1b). Further, shRNA knockdown of EWS-FLI1 in the EwS cell line TC32 conferred resistance to loss of key splicing factors (Figure 1c). Depletion of some but not all splicing factor genes induced apoptosis in TC32 cells (Figure 1d).

The second major goal of the project was to determine the causal relationship between transcription dysregulation, R- loops, and splicing in Ewing sarcoma. We found that overexpression of RNASEH1, an enzyme that degrades R-loops, conferred resistance to loss of key splicing factors (Figure 2a). However, we were unable to obtain high transfection efficiency using transient transfection of RNASEH1. Therefore, we established stable inducible cell lines expressing RNASEH1 to use for these experiments. Consequently, these experiments were not completed in the original projected timeframe, but we anticipate completing them within the next month.

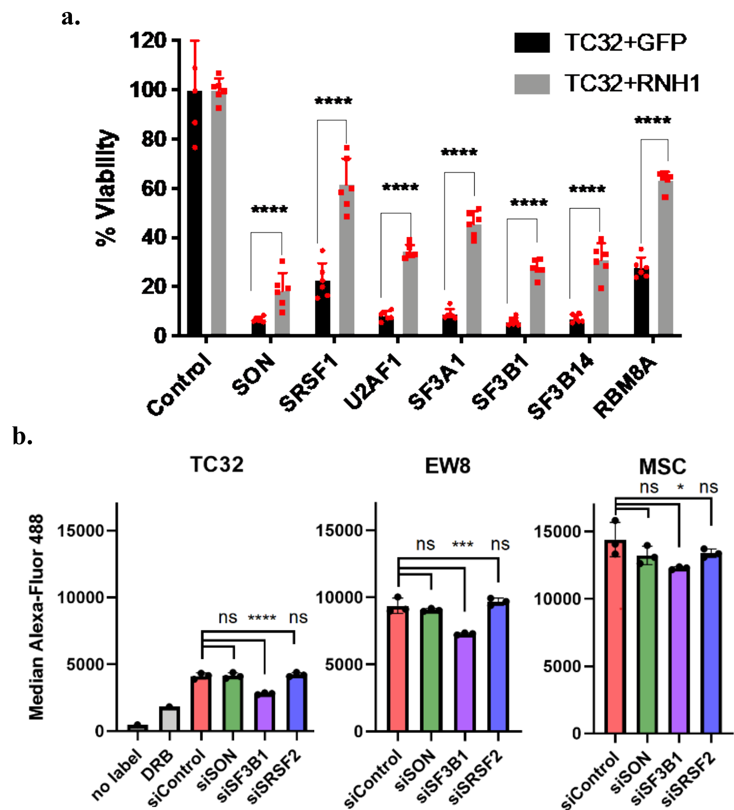

Figure 2. a. Viability of TC32 cells transfected with RNASEH1 or GFP with knockdown of the indicated genes. **b.** Quantification of ErU signal by flow cytometry with knockdown of the indicated genes.

To test effects on transcription, we used ethynyl ribouridine (ErU) incorporation/flow cytometry. Cells were treated with ErU to label nascent RNA, labeled by a click chemistry reaction, and analyzed by flow cytometry using the LSR Fortessa instrument. This revealed that knockdown of SON or SRSF2 had no significant effect on global transcription, although transcription decreased with knockdown of SF3B1 (Figure 2b).

During the second year of the project, we performed a number of large-scale sequencing experiments, each of which compared an EwS cell line (TC32) and control (MSC) with and without treatment with the splicing inhibitor E7107. First, we performed RNA-seq for gene expression analysis (by DEseq2) and splicing analysis. E7107 led to increased expression of 3562 genes (1456 in TC32 only, 821 in MSC only, and 1285 in both) and decreased expression of 3529 genes (1460 in TC32 only, 776 in MSC only, and 1293 in both), with top pathways including the spliceosome and RNAP2 regulation (Figure 3a). Differential transcript usage was identified in 4392 genes (1528 in TC32 only, 1186 in MSC only, and 1678 in both), with top pathways including DNA repair and

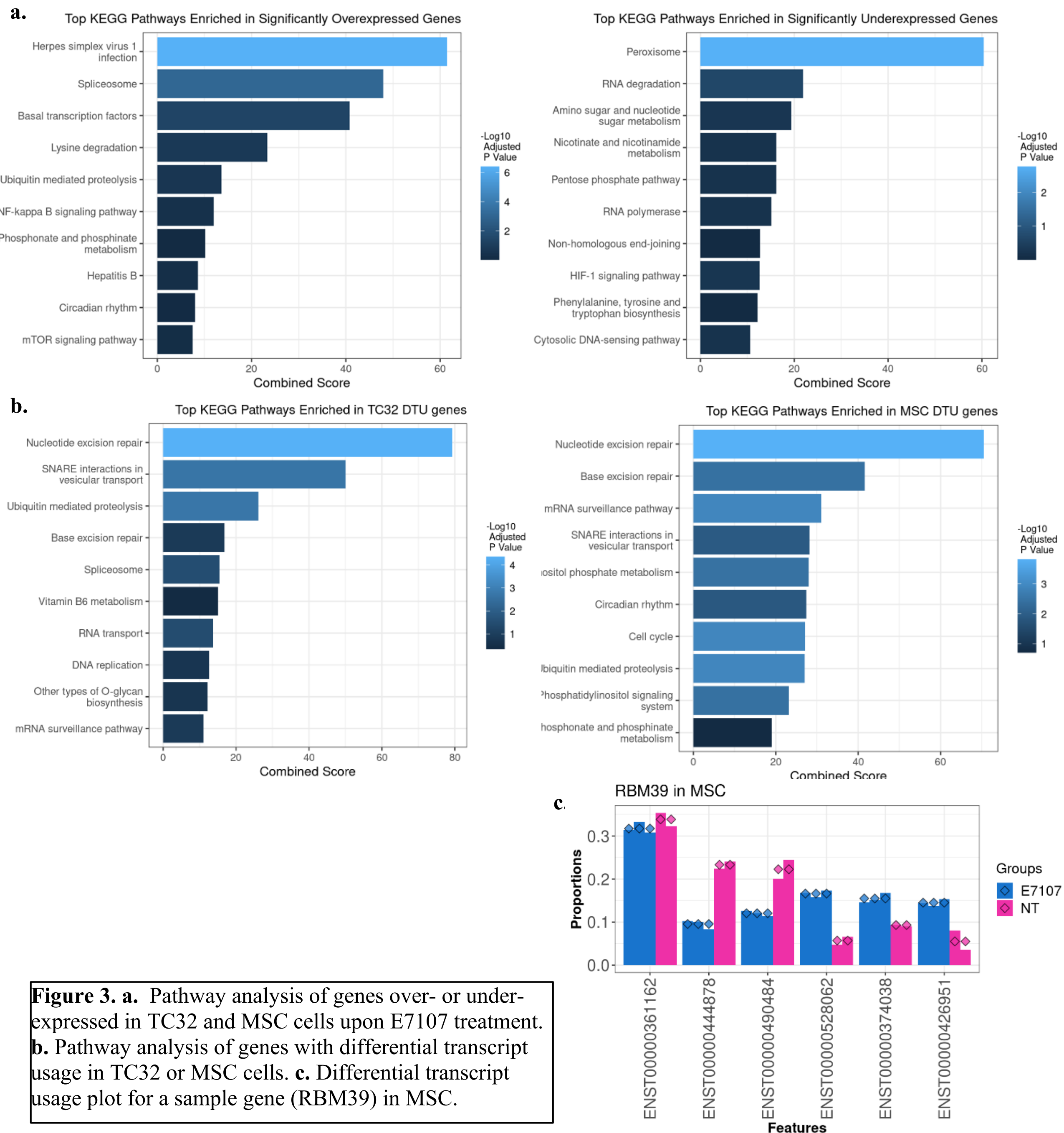

Figure 3. a. Pathway analysis of genes over- or under-expressed in TC32 and MSC cells upon E7107 treatment. **b.** Pathway analysis of genes with differential transcript usage in TC32 or MSC cells. **c.** Differential transcript usage plot for a sample gene (RBM39) in MSC.

RNA processing pathways (Figure 3b). Second, we planned to use Global Run-On (GRO-seq) to examine active transcription genome-wide. Briefly, nuclei are extracted and active transcripts labeled with BrU, then pulled down and sequenced. This is a technically challenging method that has not been previously performed in our lab and has required some troubleshooting. We performed the procedure twice; the first pulldown had low yield, and the second did not give DNA fragments of sufficient quality for deep sequencing. Currently, we have nuclei prepared for a third run and are working with experts within our institution to ensure the technique is completed successfully. Finally, we used DNA-RNA Immunoprecipitation (DRIP-seq) to examine R-loops genome-wide. Briefly, genomic DNA was extracted and fragmented using restriction enzyme digestion, then

pulled down with the S9.6 antibody (which recognizes DNA-RNA hybrids) and sequenced. We identified 11,187 total R-loops that increased upon E7107 treatment (4902 in TC32 only, 4287 in MSC only, and 1998 in both) and 23,912 that decreased (9108 in TC32 only, 6549 in MSC only, and 8255 in both) (Figure 4a). R-loops that increased upon E7107 treatment were enriched in promoter-proximal regions (Figure 4b). By far the highest pathway enrichment for R-loops that increased in both cell lines was in the spliceosome, with other RNA metabolism pathways also represented (Figure 4c).

Specific Aim 2. Establish splicing as a therapeutic target in Ewing sarcoma.

The third major goal of the project was to determine whether splicing inhibitors show EwS-specific toxicity, alone or in combination with standard therapeutic agents. In the first year of the project, we tested a panel of 9 splicing inhibitors (Table 1) and found the most promising results with the SF3B1 inhibitor E7107, which was effective at low nanomolar concentrations and showed a good therapeutic window between EwS and control cell lines (Figure 5). We initially proposed to test E7107 in combination with etoposide, cyclophosphamide, and olaparib. Etoposide and cyclophosphamide are two of the chemotherapeutics in the standard treatment of EwS, and olaparib is a PARP1 inhibitor previously described to show toxicity to EwS in cell culture[1]. However, as these combinations showed no more than an additive effect, we additionally tested combinations of E7107 with vincristine, paclitaxel, and docetaxel. Vincristine is another standard EwS chemotherapeutic agent, and paclitaxel and docetaxel have shown synergy with splicing inhibition in other models. These three drugs showed synergy with E7107 in EwS cells, with vincristine showing the best differentiation

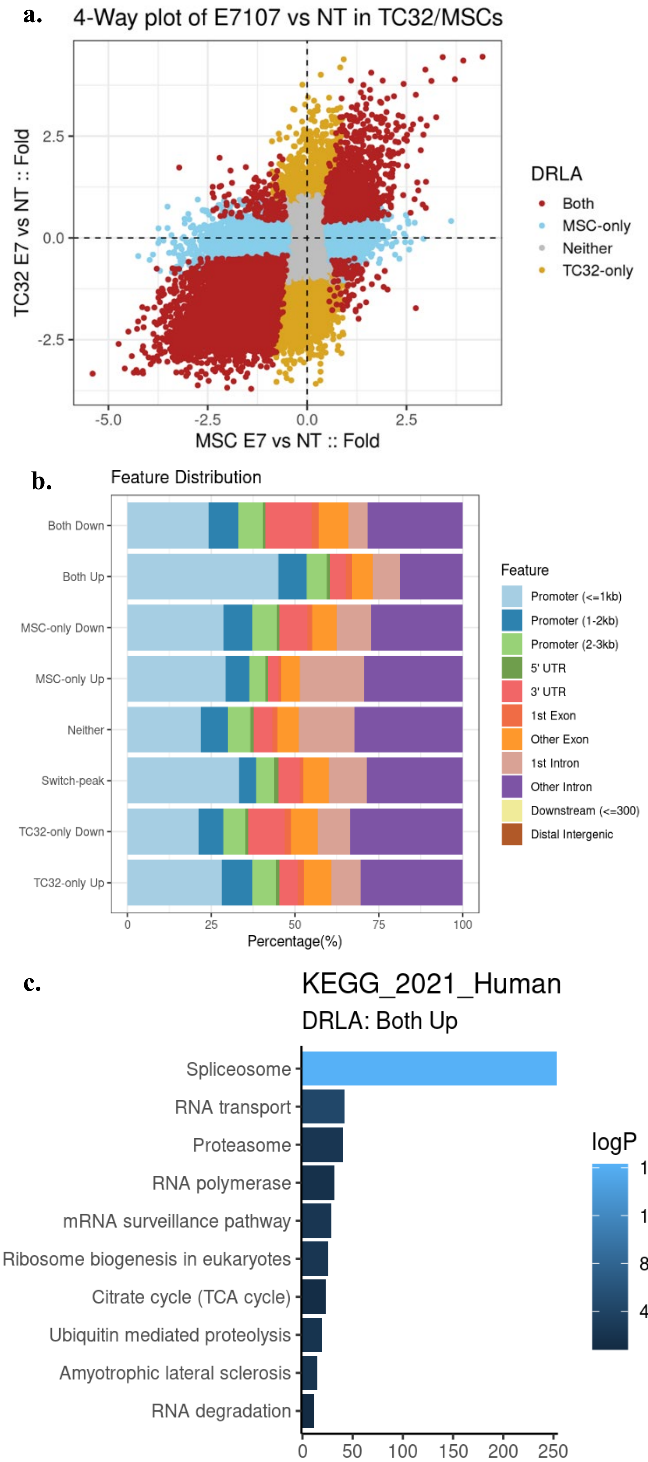

Figure 4. a. 4-way plot of R-loops showing differential abundance upon E7107 treatment in TC32, MSC, both, or neither. **b.** Genomic feature distribution of R-loops with increased or decreased signal upon E7107 treatment in MSC and/or TC32. **c.** Pathway enrichment for R-loops that showed increased signal upon E7107 treatment in both TC32 and MSC.

between EwS and control cells (Figure 6a). 4 representative cell lines are shown of the 9 that were tested.

The fourth major goal of the project was to determine the biological effects of splicing inhibition in EwS cells. ErU incorporation assays revealed no significant impact of splicing inhibition on global transcription. We assayed cell cycle progression using flow cytometry (cells labeled with EdU and Fx Cycle Violet stain) and found that E7107 caused cells to accumulate in G1 phase (Figure 6c). We assayed apoptosis using an IncuCyte imaging assay in which live cells and cells expressing Caspase-3/7 are labeled with fluorescent markers, and found that E7107, vincristine, and the combination induced apoptosis in Ewing sarcoma cells but not in MSC (Figure 6b).

Table 1. Splicing Inhibitors.

Drug	Target
E7107	SF3B1
GEX1A	SF3B1
Pladienolide B	SF3B1
SPHINX31	SRPK1
T-025	CLK1, CLK2, CLK3, CLK4
AZ191	DYRK1a, DYRK1b
MS023	PRMT type I
GSK3326595	PRMT5
borrelidin	FBP21 (WBP4)

During the second year of the project, we performed several experiments in order to better elucidate the protein-protein interactions between phospho-RNAPII, splicing factors and EWS-FLI1. Our initial model was that the hyperactivation of transcription simply produces such a large volume of transcripts that it overwhelms the RNA processing machinery, and this and the resulting R-loop accumulation leads to a sensitivity to any further perturbation of splicing. However, recent publications investigating interactions of phospho-RNAPII have indicated that phosphorylation

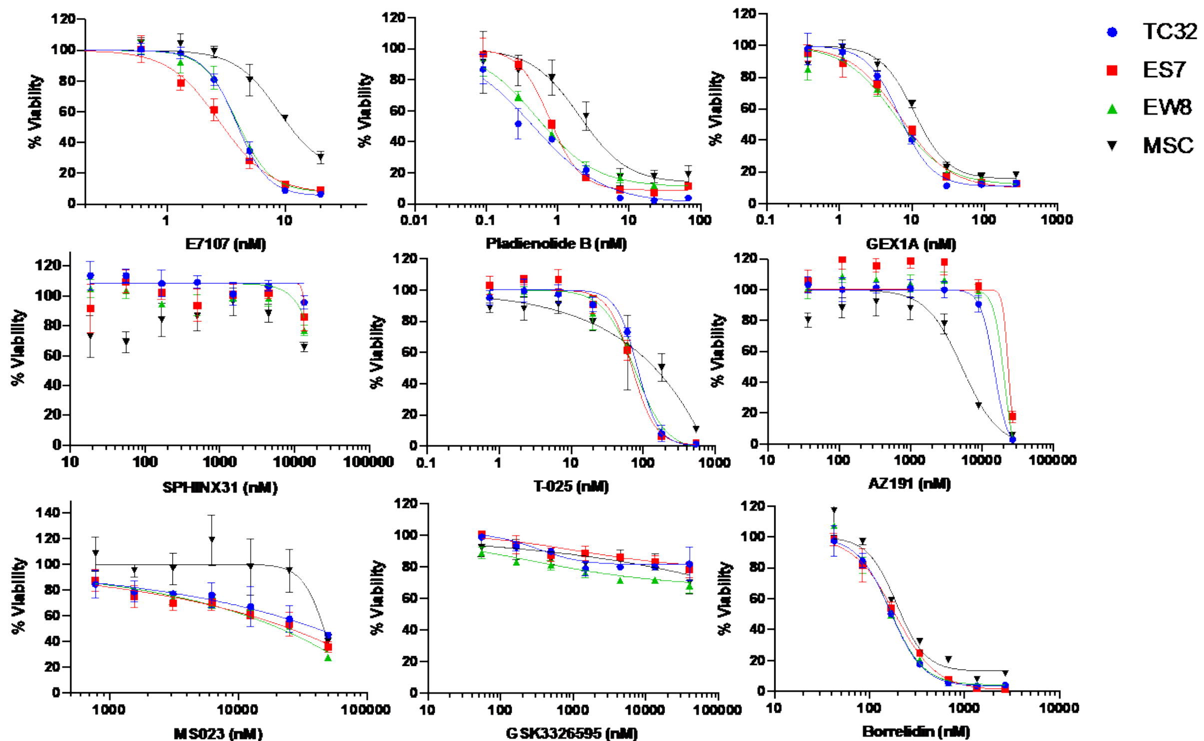

Figure 5. Cell viability of EwS or MSC cells following 3 days of treatment with splicing inhibitors (protein target indicated in parentheses).

removes RNAPII from phase separated condensates associated with Mediator and initiation and moves it to a new droplet including splicing factors and associated with elongation[11,12]. This process would likely be impacted by EWS-FLI1 and its disruption of RNAPII regulation. This raises the possibility that the splicing defect is in part due to sequestration of splicing factors with phosphorylated RNAPII as well as R-loop associated stress.

To test this, we first performed nuclear/chromatin co-immunoprecipitation in TC32 (EwS) and U2OS or MSC (control) cell lines. We were able to successfully pull down RNAP2 (total and phospho-Ser2), EWS-FLI1 and splicing factors (Figure 7a). During year three, we will repeat these experiments with RnaseA treatment to show if the interactions are RNA-dependent, and with E7107 treatment to see how these interactions are altered by splicing inhibition.

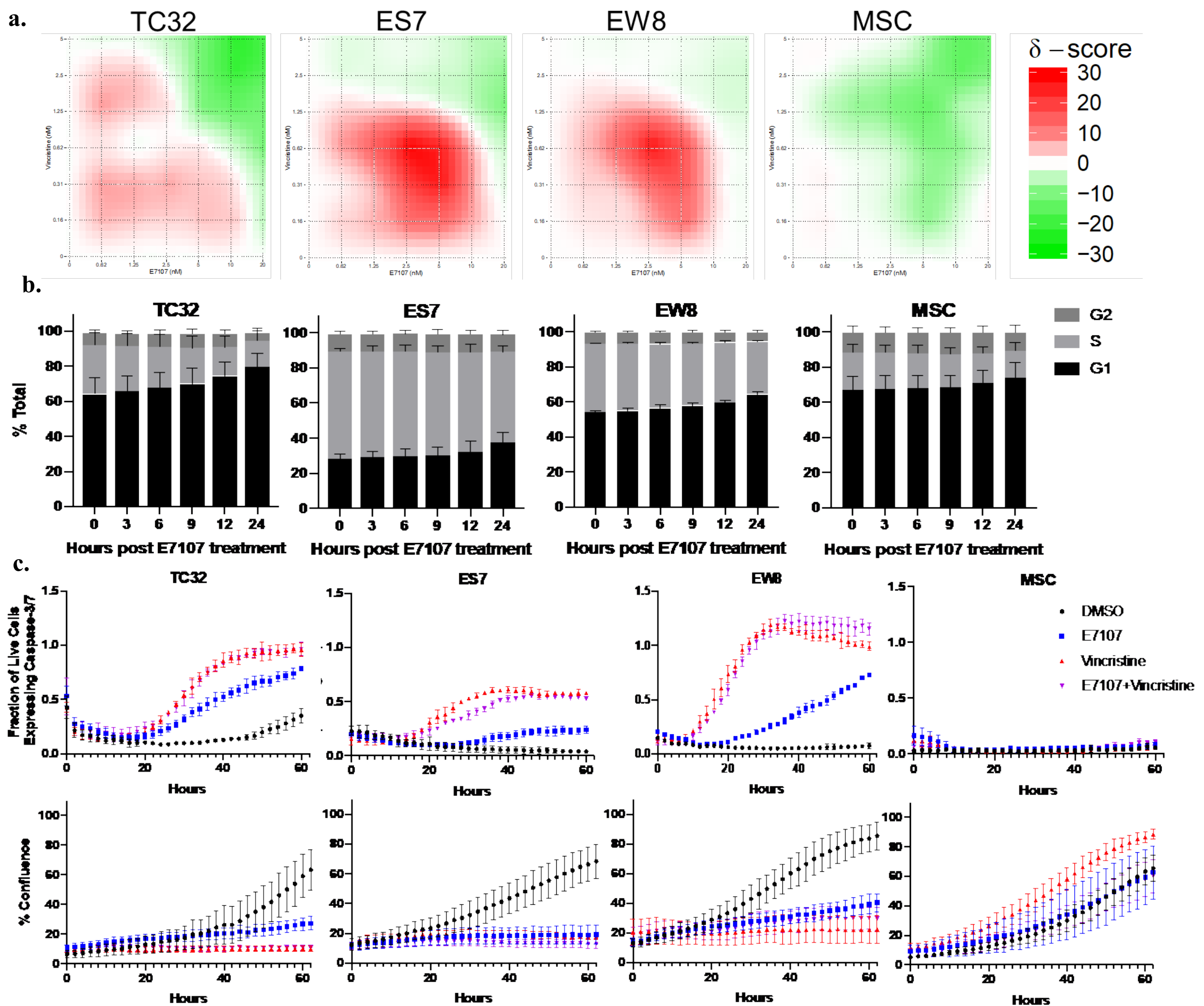

Figure 6. a. Heatmaps representing synergy between E7107 (x) and vincristine (y) analyzed using the Zero Interaction Potency model. Red indicates synergy and green indicates antagonism. **b.** Flow cytometry cell cycle analysis of cells treated with 2 nM E7107. **c.** Fraction of apoptotic cells (expressing caspase-3/7) over 60 hours of treatment (top) and corresponding growth curve (bottom).

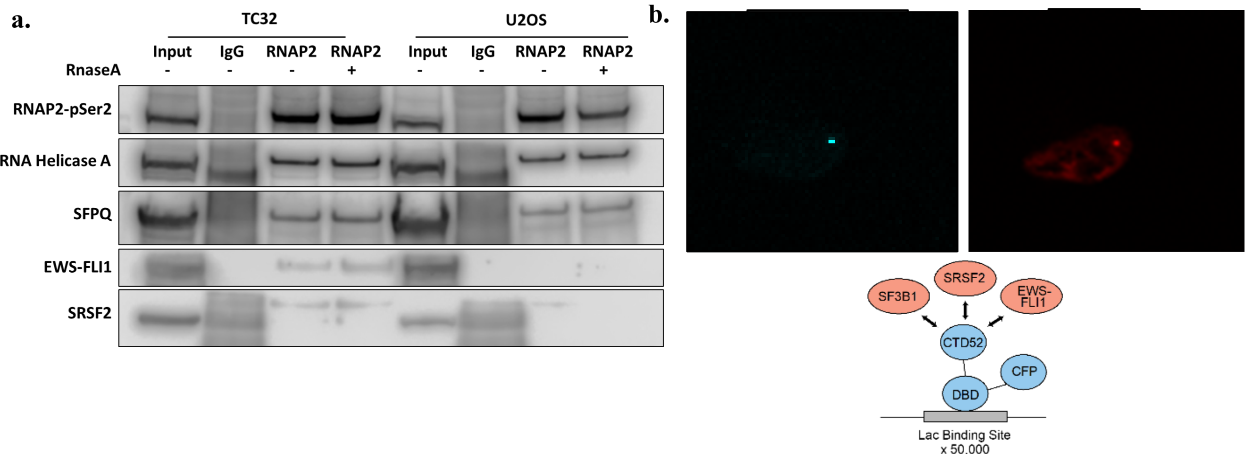

Figure 7. a. IP of RNAP2 in TC32 (EwS) and U2OS cells showing interaction with RHA, SFPQ, EWS-FLI1, and SRSF2. **b.** Schematic showing design of the lac binding assay and confocal images showing CTD52-CFP recruitment to the lac site and phosphorylation at Ser2.

We proposed to further characterize this with a reporter assay[12] in which one protein of interest is fused to Lac binding domain and CFP and transfected into U2OS cells carrying Lac operon repeats, and recruitment of interacting proteins is detected by IF. During the past year, we have successfully created a LacI construct with the CTD of RNAP2 and performed immunofluorescence staining to confirm that it is phosphorylated at Ser2 (Figure 7b), Ser5 and Ser7 (data not shown).. Next we plan to repeat these experiments in the presence and absence of EWS-FLI1 transfection to test the effect on these interactions. These experiments will provide further insight into the relationship between transcription dysregulation, R- loops, and splicing in Ewing sarcoma.

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

The main focus of this project is to train the PI as an independent investigator in the area of pediatric cancer research. Training activities during this period included regular one-on-one and group meetings with my dissertation mentor, Dr. Alexander Bishop, to discuss methods, analysis, interpretation, and reporting of data. In addition, I held two meetings with my Dissertation Supervising Committee (12/18/20 and 6/23/21) to report progress and discuss findings and future plans. I have also presented my work in a department presentation (5/24/21) to gain experience in presentation skills and obtain feedback from university faculty. Although COVID-19 travel restrictions prevented traveling to scientific meetings, I was able to present posters at the UT Health Cell Systems and Anatomy department retreat (5/20/21) to discuss my work with other investigators and get helpful feedback.

My training activities during the past year have also included participation in journal clubs and seminars. I have attended weekly Cancer Biology and Cell Biology/Genetics/Molecular Medicine journal clubs at UT Health San Antonio, in which students present and discuss recent original research articles with a substantial impact in biomedical research. I have also attended weekly seminars from a variety of seminar series including Cancer Development and Progression, Molecular Medicine, Pharmacology, and Precision of Science in Medicine, featuring both institutional faculty and top international scientists. This has helped me to keep up with current research in my own and related fields. Finally, I have attended a monthly Research Integrity workshop organized by the institutional training grant directors at UTHSA in which faculty and invited guests speak on topics such as

conflicts of interest, research compliance, record keeping, reproducibility, peer review, and ownership of data.

How were the results disseminated to communities of interest?

Nothing to report.

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

In year three of the project, we plan to complete the remaining experiments and submit a manuscript for publication. For GRO-seq, we have nuclei prepared and are working with experts within our institution to ensure the technique is completed successfully. Once this is completed, we will analyze this data and then complete the integrative analysis of RNA-seq, GRO-seq, and DRIP-seq data to compare differences in transcription and R-loops between EwS and control cells, with and without splicing inhibition. We will complete the experiments requiring RNASEH1 overexpression to deplete R-loops using the stable inducible cell lines that we have established. Finally, now that we have the co-immunoprecipitation and colocalization assays optimized we will complete the protein interaction experiments. The data we have obtained together with the experiments to be completed in year three of the project will then be submitted in a manuscript for publication.

4. IMPACT:

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

Ewing sarcoma is a rare but very aggressive tumor of the bone and soft tissue that is most frequent in children, adolescents, and young adults. It is treated with chemotherapy along with radiation or surgery. Although most patients respond well to this aggressive treatment regimen, there is no second-line treatment when it fails, and there are severe side effects in children that can continue to affect them long after treatment has ended. Consequently, effective, less toxic, targeted treatment strategies are much needed. Our lab performed a genome-wide screen to identify genes required for Ewing sarcoma cells to survive. We found that Ewing sarcoma cells are particularly sensitive to loss of genes involved in RNA splicing. RNA splicing is additional processing that occurs after a gene is copied from DNA to RNA (transcription) before it can be used to make proteins. We recently published that the fusion oncogene that drives Ewing sarcoma, EWS-FLI1, increases the overall level of transcription activity in a cell[10]. This results in problems in transcription, with some of the newly made RNA sticking to the DNA, creating a structure called an R-loop. Worse, the whole transcription process is normally turned off/down in response to cellular damage, but this does not happen in Ewing sarcoma. This project aims to understand the relationship between altered regulation of transcription and splicing in Ewing sarcoma. A number of splicing inhibitors have been developed, one of which is currently in clinical trials for hematologic malignancies. We tested splicing inhibitors in Ewing sarcoma cells to determine whether this could be used as a new treatment strategy, either alone or in combination with standard chemotherapeutics. We found that Ewing sarcoma cells were extremely sensitive to splicing inhibition, which synergized with several standard chemotherapeutic agents to kill the cells at very low doses. Our data indicated that this sensitivity is linked to the hyperphosphorylation of RNAPII that results from EWS-FLI1 expression and the resulting deregulated transcription and elevated R-loops. Our results are expected to impact research in basic science by improving our understanding of regulation and coordination of transcription and splicing.

In addition, our results point to RNA splicing as a potential new therapeutic target in Ewing sarcoma, which has the potential to benefit all Ewing sarcoma patients, especially those with chemo-resistant disease.

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:

Changes in approach and reasons for change

Nothing to report.

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

GRO-seq: This is a technically challenging method that has not been previously performed in our lab and has required some troubleshooting. We performed the procedure twice; the first pulldown had low yield, and the second did not give DNA fragments of sufficient quality for deep sequencing. Currently, we have nuclei prepared for a third run and are working with experts within our institution to ensure the technique is completed successfully.

Changes that had a significant impact on expenditures

Nothing to report.

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

Nothing to report.

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:

- **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.

  Poster presentations:
  Lawrence L, Gorthi A, Bishop A. Targeting the Dysregulation of Transcription and Splicing in Ewing Sarcoma. Cell Systems and Anatomy Departmental Retreat, UT Health San Antonio. San Antonio, TX. May 20, 2021.

- **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**

  - Cell lines expressing doxycycline-inducible RnaseH-M27 and catalytically dead mutant: TC32, EW8, MSC
  - Plasmid expressing RNAP2-CTD fused to lac-binding domain and CFP

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Liesl Lawrence
Project Role:	PI (Graduate Student)
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-8809-6278
Nearest person month worked:	12
Contribution to Project:	Ms. Lawrence performed laboratory experiments and data analysis.
Funding Support:	N/A

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?

H3 Biomedicine (Cambridge, MA) supplied E7107, a splicing inhibitor that has clinical relevance.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

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W81XWH-19-1-0180: Transcription, R-Loops, and RNA Splicing in Ewing Sarcoma

PI: Liesl Lawrence, University of Texas Health Science Center at San Antonio, TX. **Budget:** $220,350

Topic Area: Cancer in Children, Adolescents, and Young Adults

Mechanism: FY18 Peer Reviewed Cancer Research Program; Horizon Award

Research Area(s): 0209 (Transcription), 0805 (Targeted Therapies)
Award Status: 1 July 2019 – 30 June 2022

Study Goals:

Ewing sarcoma is an aggressive pediatric bone and soft tissue cancer driven primarily by the EWS-FLI1 fusion oncogene. EWS-FLI1 acts as a transcription factor and also interferes with normal regulation of transcription and transcription-associated RNA processing. The goal of this project is to evaluate the hypothesis that EWS-FLI1 driven hyper-activation of transcription causes a targetable dependence on RNA splicing in Ewing sarcoma.

Specific Aims:

1) Determine the mechanistic relationship between EWS-FLI1-driven transcription, R-loops, and splicing vulnerabilities in Ewing Sarcoma.
   a) Confirm the dependence of Ewing sarcoma cells on the splicing machinery.
   b) Elucidate the cause and effect relationships between transcription dysregulation, R-loops, and splicing in Ewing sarcoma cells.
2) Evaluate the potential of splicing as a therapeutic target in Ewing sarcoma.
   a) Determine whether splicing inhibitors show Ewing sarcoma-specific toxicity, alone or in combination with standard therapeutic agents.
   b) Determine the biological effects of splicing inhibition in Ewing sarcoma cells.

Key Accomplishments and Outcomes:

Publications:

Miller H, Gorthi A, Bassani N, Lawrence L, Iskra B, Bishop A. Reconstruction of Ewing Sarcoma Developmental Context from Mass-Scale Transcriptomics Reveals Characteristics of EWSR1-FLI1 Permissibility. Cancers. 2020 April 11; 12(4), 948.

Patents: none to date

Funding Obtained: none to date