AWARD NUMBER: W81XWH-18-1-0370

TITLE: Mapping the Routes to Tumor Cell Death in TSC

PRINCIPAL INVESTIGATOR: Dr. Brendan Manning, PhD

CONTRACTING ORGANIZATION: President and Fellows of Harvard College, Harvard T.H. Chan School of Public Health

REPORT DATE: AUGUST 2020

TYPE OF REPORT: Annual

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

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Mapping the routes to tumor cell death in TSC

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5b. GRANT NUMBER
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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Brendan Manning

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

President and Fellows of
Harvard College
Harvard T.H. Chan School of
Public Health
677 Huntington Ave Boston MA
02115-6028

8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Individuals with tuberous sclerosis complex (TSC) are at risk of developing a variety of tumors, primarily affecting the brain, kidneys, skin, lung, heart, and eyes. Rapamycin/sirolimus and its analogs, such as everolimus, are effective at shrinking the variety of tumors arising in TSC patients and appear to halt further tumor growth. However, these drugs do not eliminate tumors, and upon withdrawal of treatment, tumors regrow at a remarkably rapid rate. Thus, there is an unmet need for treatments that elicit a more complete and durable response that allows TSC patients to avoid continuous therapy with rapalogs. This current study uses cutting edge new techniques to study the molecular nature of tumor cell death and survival in TSC and represents a comprehensive and novel strategy to tackle this problem. The study will identify and test, in multiple preclinical tumor models, new therapeutic approaches to induce tumor cell death in TSC, thereby eliminating existing tumors. We will focus on an emerging and promising class of drugs, called BH3 mimetics, which are being developed and FDA approved for use in cancer therapy. We anticipate that a subset of these drugs, when used in combination with rapamycin or its analogs, will safely induce cell death specifically in the tumors of TSC patients, thereby eradicating these lesions. Through rigorous testing in multiple preclinical models, it is our hope that the completion of our study in the next three years will provide the preclinical evidence needed to advance these treatment strategies into the clinic to benefit TSC patients and offer an improved response to that currently achieved with rapamycin.

15. SUBJECT TERMS

Tuberous sclerosis complex, tumors, therapy, rapamycin, apoptosis, BCL2 family, BH3 mimetics, preclinical mouse models.

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

TABLE OF CONTENTS

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

The research under this proposal will determine the molecular effects of mTORC1 activation and inhibition in TSC on the key regulators and effectors of apoptosis to reveal novel approaches to induce tumor cell death. Rapamycin and its analogs (rapalogs) are effective at shrinking the variety of tumors arising in TSC patients and appear to halt further tumor growth. However, these drugs do not eliminate tumors, and upon withdrawal of treatment, tumors regrow at a remarkably rapid rate. Therefore, despite blocking the uncontrolled mTORC1 signaling that is a hallmark of TSC, tumor cell survival is unaffected by rapalogs, and these cells are poised to accelerate their growth when released from therapy. To understand and overcome this property of rapalogs in the TSC setting, we directly examine the status of pro- and anti-apoptotic proteins influencing mitochondrial membrane permeability and cytochrome C release, the initiating event in programmed cell death. We are measuring transcript and protein levels of these factors, the BCL2 family, in cell and tumor models of TSC, as well as available TSC patient samples. We are using functional assays to determine the effects of TSC1 and TSC2 mutations and mTOR inhibitors on mitochondrial apoptotic priming and apoptosis. Finally, we are using mouse tumor models to test the effectiveness of targeting these factors alone or in combination with mTOR inhibitors for durable anti-tumor responses that are sustained upon removal of treatment.

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

Tuberous sclerosis complex; Tumors, Therapy, Rapamycin, Apoptosis, BCL2 family, BH3 mimetics, Preclinical Mouse Models

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

What were the major goals of the project?

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

Specific Aim 1: Define the status of pro- and anti-apoptotic proteins of the BCL-2 family and apoptotic priming in TSC. *Mos 1-24 – 70% complete*
Task 1 Characterization of BLC-2 family members and BH3 profiling in TSC cell culture models. *Mos 1-12 – 100% complete*
Milestone *– ACURO approval complete*
Task 2 Characterization of BLC-2 family members and BH3 profiling in TSC mouse tumor models. *Mos 8-24 – 70% complete*
Task 3 Characterization of BCL-2 family member in human TSC and LAM tumor samples. *Mos 18-24 – 20% complete*

Specific Aim 2: Preclinical studies to enhance apoptotic priming in TSC cell and tumor models with BH3 mimetics developed for clinical use. *Mos 8-36 – 80% complete*
Task 1 Test the effects of inhibiting anti-apoptotic BCL-2 family members on the induction of apoptosis in TSC cell culture models, alone and in combination with mTOR inhibitors. *Mos 8-20 – 100% complete*
Task 2 Testing the effects of inhibiting anti-apoptotic BCL-2 family members on the induction of apoptosis in TSC mouse tumor models, alone and in combination with mTOR inhibitors. *Mos 20-36 – 70% complete*

Specific Aim 3: Determine the control mechanisms downstream of the TSC complex and mTORC1 altering apoptotic priming in TSC. *Mos 1-36 – 50% complete*
Task 1 Characterization of BLC-2 family members in multiple TSC cell culture models over time course of treatment with mTOR inhibitors. *Mos 1-12 – 100% complete*
Task 2 Protein stability and mRNA translation will be measured for those BCL-2 family proteins showing changes with TSC gene loss and/or mTOR inhibitors that are not accompanied by changes in mRNA levels. *Mos 13-24 – 50% complete*
Task 3 The precise molecular mechanisms regulating those BCL- 2 family members found to most strongly influence TSC-deficient cell survival and death decisions in TSC settings will be defined, with focus on potential post-translational modifications and transcription factors involved. *Mos 25-36 – 0% complete*

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 made steady progress toward the completion of the stated research aims in year 2 of this award. Representative data are presented in the five figures below, which cover specific experiments/tasks under Aim 1 (Figure 1), Aim 2 (Figures 2-4), and Aim 3 (Figure 5). The data accompanying each figure are summarized below.

2) Specific Objectives: Our main efforts focused on developing a further understanding of the primary changes in anti-apoptotic proteins upon loss of the TSC tumor supressors (Aim 1), testing the effects of available compounds that inhibit these proteins in TSC cell and tumor models, with or without rapamycin, with an emphasis on developing selective cytotoxic treatments for TSC (Aim 2), and defining the the molecular mechanisms by which mTORC1 activation and inhibition in TSC cells influence the levels of anti-apoptotic proteins.

3) Significant Results / Key Outcomes: *See document and five figures summarizing new data.*

4) Other achievements: Nothing to report.

(Please see full Accomplishments section beginning on next page.

What was accomplished under these goals?

1) Major Activities: We made steady progress toward the completion of the stated research aims in year 2 of this award. Representative data are presented in the five figures below, which cover specific experiments/tasks under Aim 1 (Figure 1), Aim 2 (Figures 2-4), and Aim 3 (Figure 5). The data accompanying each figure are summarized below.

2) Specific Objectives: Our main efforts focused on developing a further understanding of the primary changes in anti-apoptotic proteins upon loss of the TSC tumor supressors (Aim 1), testing the effects of available compounds that inhibit these proteins in TSC cell and tumor models, with or without rapamycin, with an emphasis on developing selective cytotoxic treatments for TSC (Aim 2), and defining the the molecular mechanisms by which mTORC1 activation and inhibition in TSC cells influence the levels of anti-apoptotic proteins.

3) Significant Results / Key Outcomes:

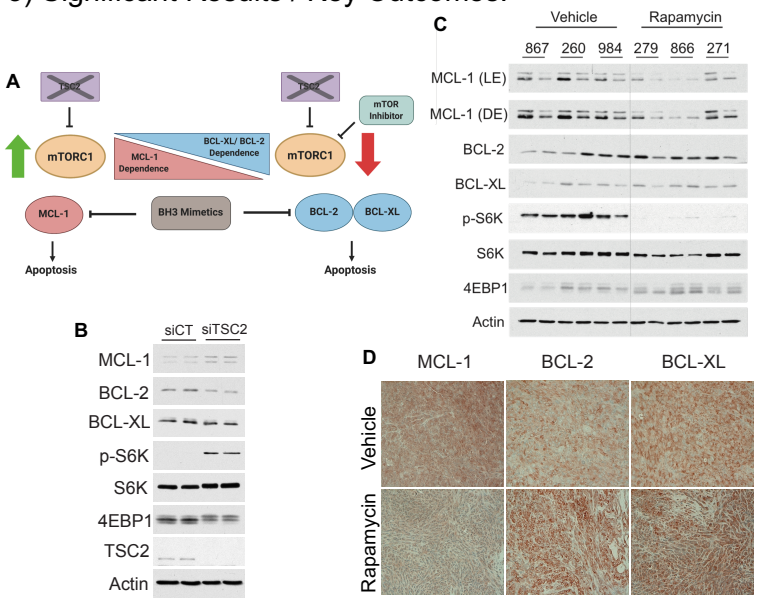

Figure 1: Data from Aim1, Task 1 and 2. (A) Working Model based on data to date:TSC loss and mTORC1 activation in TSC tumors leads to MCL-1 induction and dependence for survival, whereas mTORC1 inhibition leads to decreased MCL-1 and increased BCL-2/xL levels and dependence, which can be targeted with specific BH3 mimetics to induce apoptosis. (B) Transient siRNA-mediated knockdown of TSC2 leads MCL-1 upregulation and BCL-2 downregulation, as seen in other cellular settings with genetic ablation of TSC2. (C,D) BCL-2 family members in 105K xenograft tumors from mice treated with vehicle or rapamycin (1 mg/kg) for two days (C, immunoblots) or 20 days (D, immunohistochemistry). Similar to cell culture models, MCL-1 is decreased with rapamycin and BCL-2 and BCL-xL are increased or unchanged.

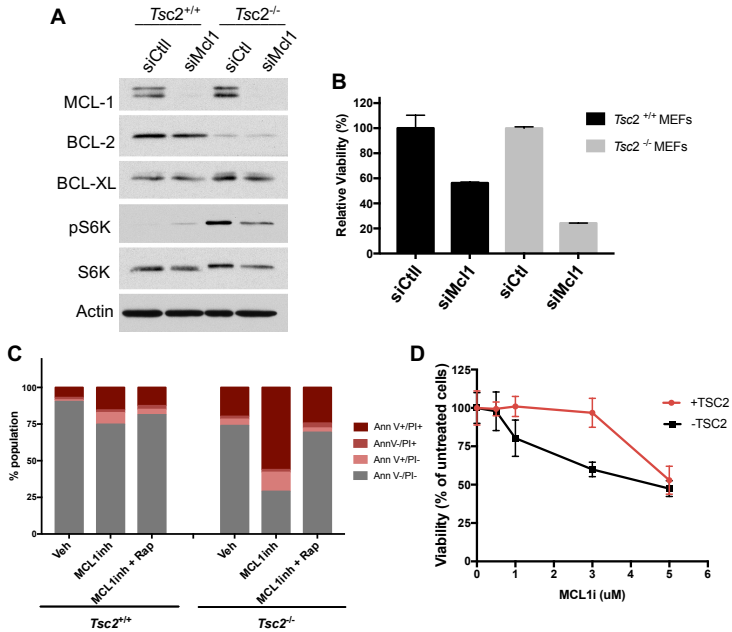

Figure 2: Data from Aim2, Task 1. (A, B) siRNA mediated knockdown of MCL-1 in *Tsc1*$^{+/+}$ and *Tsc2*$^{-/-}$ MEFs does not affect the other anti-apoptotic BCL-2 proteins but induces selective cell death in *Tsc2*$^{-/-}$ cells. (C) The MCL-1 inhibitor S63845 (2 uM, 24 h) induces selective cell death (FACs measurements of Annexin V/Propidium Iodide staining) in *Tsc2*$^{-/-}$ cells in a manner that is dependent on elevated mTORC1 signaling, as it is blocked by rapamycin (20 nM, 48 h). (D) Re-expression of TSC2 in *Tsc2-/-* 105K tumor cells causes dose-dependent resistance to S63845.

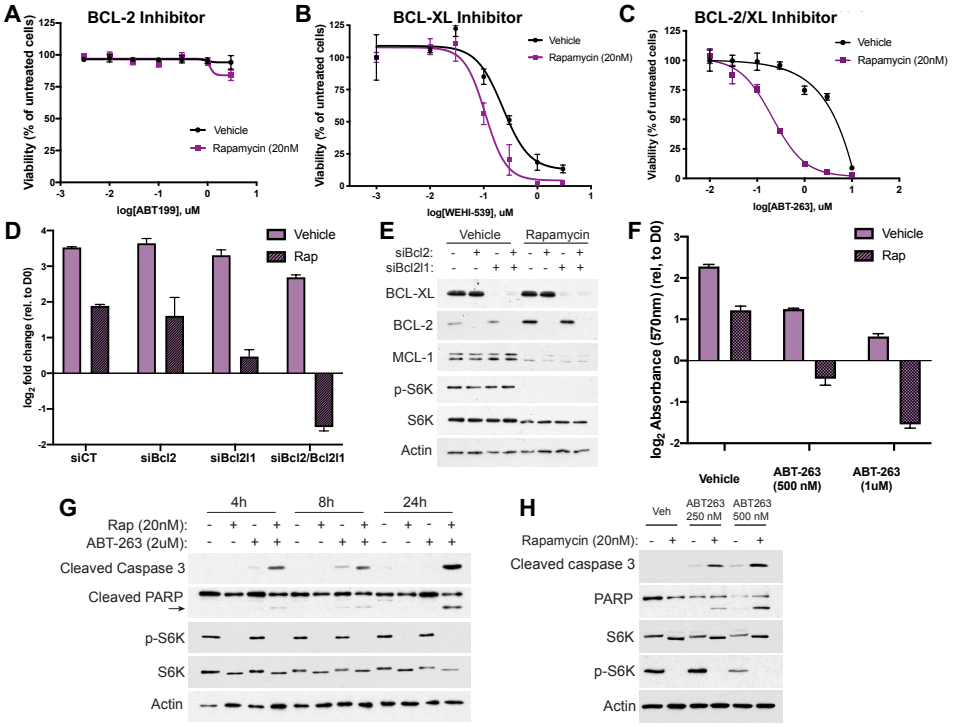

Figure 3: Data from Aim 2, Task 1. (A-C) Treatment with a dose range of BCL-2 inhibitor (ABT-199), Bcl-xL inhibitor (WEHI-539), or a dual inhibitor (ABT-263) in combination with rapamycin (20 nM) for 48 h reveals that only the dual inhibitor synergizes with rapamycin to decrease cell viability in 105K tumor cells. (D,E) SiRNA mediated knockdown of BCL-2, BCL-xL, and both BCL-2 and BCL-xL in 105K cells treated with vehicle or rapamycin (20 nM, 48 h), with log2-fold change relative to day 0 plotted (D) and effects of knockdowns and rapamycin on these proteins shown via immunoblot (E). These data further confirm the need to inhibit both BCL-2 and BCL-xL to see cell-killing synergy with rapamycin. (F) Dose-dependent synergy of ABT-263 with rapamycin, plotted as in D. (G, H) Effects on molecular markers of apoptosis (e.g., cleaved Caspase 3 and PARP) of a time course of treatment with ABT-263 and/or rapamycin in *Tsc2-/-* MEFs (G) or of different doses of ABT-263 in 105K tumor cells, assessed by immunoblot.

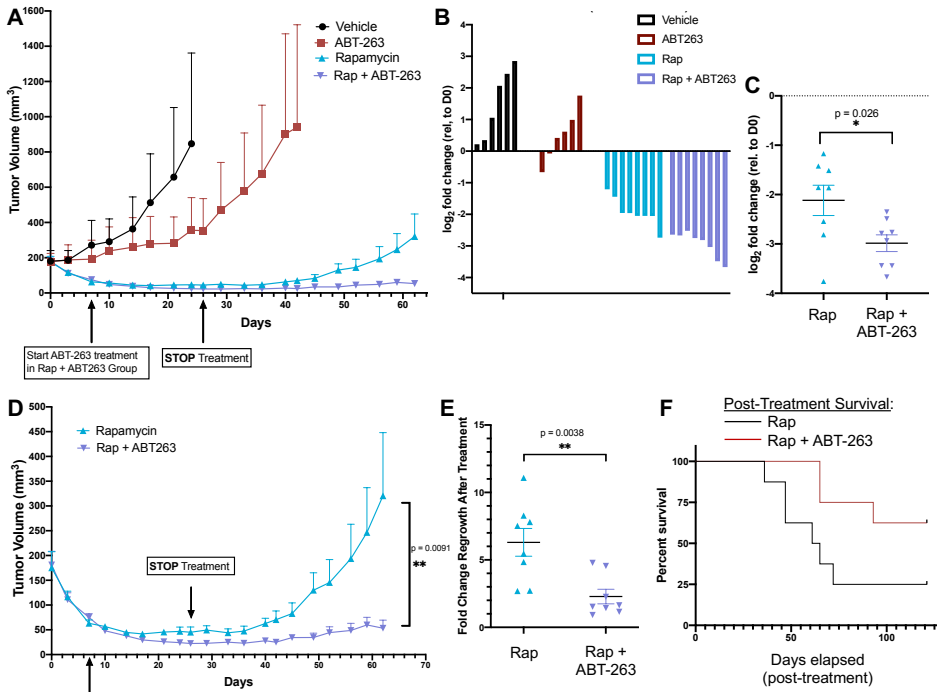

Figure 4: Data from Aim 2, Task 2. Preclinical use of a syngeneic mouse tumor model of TSC to compare rapamycin to ABT-263 with or without rapamycin cotreatment for effects on initial tumor progression (A-C) and tumor regrowth upon halting tumor growth (i.e., durability of the response; D-F). C57/BL6 mice bearing flank syngeneic *Tsc2*$^{-/-}$ 105K cell tumors of 200 mm^3 were treated for 26 days with vehicle (n=6), rapamycin (1 mg/kg via i.p. injection, MWF; n=6), ABT-263 (100 mg/kg via oral gavage, daily; n=8), or both rapamycin and ABT-263 (n=8). ABT-263 treatment started 6 days after rapamycin treatment initatied, and was therefore only administered for 20 days total. Treatment was halted for the rapamycin and rapamycin plus ABT-263 groups at day 26 and tumor volume was monitored over time to measure post-treatment tumor regrowth. (A) Tumor volume over time was measured. (B,C) Waterfall plot of log2-fold tumor change for each individual mouse at the end of the treatment phase (day 26; B) or mean change over this time in rapamycin versus rapamycin plus ABT-263 groups (C). (D, E) Data from (A) scaled to show differential tumor growth of rapamycin-treated mice, with or without ABT-263 (D) and fold change in tumor volume 36 days after treatment was halted. (F) Kaplin-Meier survival plot of these groups out to 120 days post-treatment, monitoring tumor growth to the humane endpoint of 1 cm^3 (Gehan-Breslow-Wilcoxon test: p = 0.043; Log-rank (Mantel-Cox) test: p = 0.0597). Conclusion: Adding the Bcl-2/Bcl-xL inhibitor ABT-263 to rapamycin greatly increases the durability of the anti-tumor response in this mouse model of TSC.

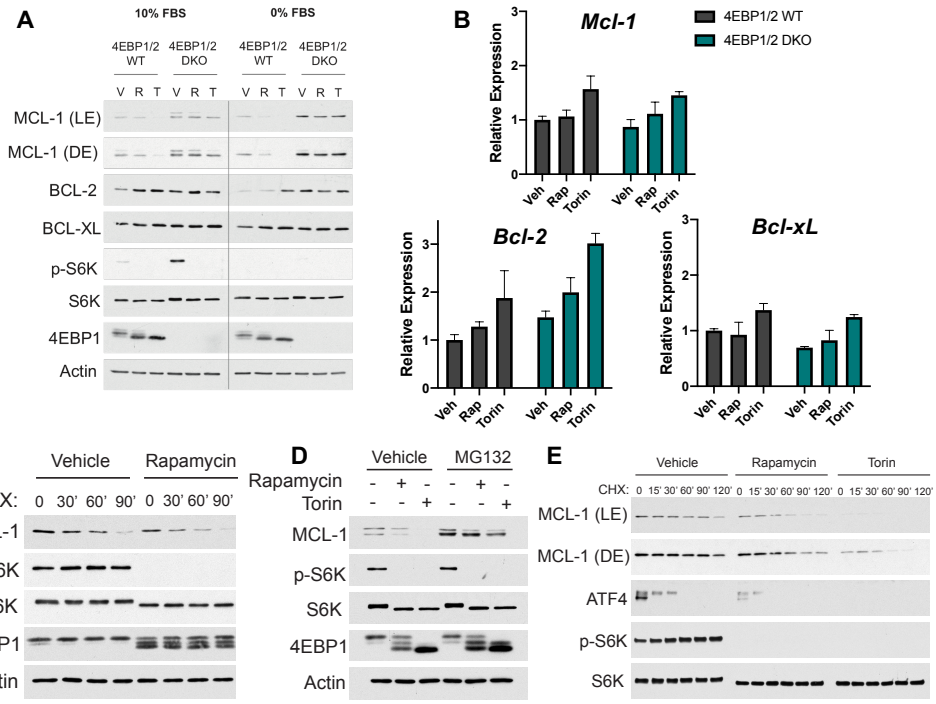

Figure 5: Data from Aim 3, Task 2. Studies to determine whether mTORC1 signaling increases MCL-1 protein levels via increased translation or reduced degradation. (A) Wild-type (WT) or 4EBP1/2 double knockout (DKO) MEFs were treated with vehicle (DMSO), rapamycin (20 nM), or Torin1 (250 nM) for 24 hours and anti-apoptotic BCL-2 family proteins were detected by immunoblot in the presence (10% FBS) or absence of serum. The ability of mTOR inhibitors to repress MCL-1 expression was greatly decreased in the DKO cells, demonstrating that 4E-BP-dependent suppression of translation initiation is required for this response. (B) There was no difference in transcript levels of these family members under the conditions in (A). (C) Effects of rapamycin on MCL-1 protein stability in *Tsc2*$^{-/-}$ MEFs. Cells were pretreated for 4 h with rapamycin (20 nM) prior to the indicated time course of translation inhibition with cycloheximide (100 uM). (D) As in (C), but cells were treated for 8 h with rapamycin (20 nM) or Torin1 (250 nM) in the presence or absence of the proteasome inhibitor MG132. (E) *Tsc2*$^{-/-}$ 105K tumor cells were treated as in (C) vehicle, rapamycin, or Torin1 and a time course of cycloheximide. (C-E) mTOR inhibitors decrease the half-life of MCL-1 in a manner that is blocked by proteasome inhibitors. Collectively, these data indicate that mTORC1 signaling increases MCL-1 protein levels via both increased translation and decreased proteasome-dependent degradation.

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.

Alexander Valvezan (postdoctoral fellow) and Molly McNamara (graduate student), have been working on specific elements of this project, which is the specific focus of Ms McNamara's doctoral research in the laboratory. Dr. Valvezan trained Ms McNamara in the techniques to generate and work with the TSC preclinical mouse models needed for this study and to deliver compounds by oral gavage, experiments that led to important new discoveries under Aim 2 regarding the greatly enhanced durability of combinatorial treatment with rapamycin plus the BH3 mimetic ABT-263, relative to rapamycin alone (data described in Figure 4 under accomplishments). Dr. Valvezan has recently been hired as an Assistant Professor at Rutgers University and will be starting in September of 2020. Ms. McNamara is continuing efforts to complete this project, the results of which we will publish in 2021.

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.

The PI, Brendan Manning, was scheduled to present the current results of this project at two international meetings this year, one on LAM research in Cincinnati, OH in April and one on TSC research in London, UK in November. Both of these meetings were postponed until 2021 due to Covid-19.

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.

We will complete the remaining tasks of this proposal, including examining BCL-2 family proteins in available human TSC and LAM specimens, BH3 profiling of tumors from mice treated with or without rapamycin or BH3 mimetics, and determining the mechanisms underlying mTORC1-dpendent changes in the levels and function of these proteins in TSC. Finally, we will assemble this data into a manuscript for publication in 2021, coinciding with the completion of the proposed aims.

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).

This project has potential to change the current treatment paradigm for tumors developing in patients with tuberous sclerosis complex and lymphangioleiomyomatosis, with improved and more durable treatment responses, perhaps over a shorter treatment time.

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.

As the TSC-mTORC1 pathway is frequently dysregulated in human cancers, the study has the real potential to alter treatment paradigms across multiple lineages of human cancer, where mTORC1 inhibitors alone have exhibited little to no anti-tumor efficacy.

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:*

Nothing to Report

that

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.

Due to limited access to our laboratory during the Covid-19-related shutdown of Harvard University, we have been delayed in our development of a cohort of *Tsc2*$^{+/-}$ mice for a subset of the described experiments. We have been able to re-initiate such breedings over the past month. For similar reasons, we were also delayed in our ability to perform BH3 profiling on tumors, which we will instead undertake this Fall, as part of our year 3 efforts.

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 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).*

  Nothing to Report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series.*

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

Nothing to Report.

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

Nothing to Report.

- **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".

Name:	Brendan D. Manning
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	eRA Commons ID: BMANNING1
Nearest person month worked:	1
Contribution to Project:	No change.
Funding Support:	See updated Current Support below.

Name:	Alexander J. Valvezan, PhD
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	eRA Commons ID: VALVEZAN
Nearest person month worked:	6
Contribution to Project:	Has developed the cell viability and apoptosis assays and trained Ms. McNamara for use of the TSC preclinical mouse models.
Funding Support:	DoD Award W81XWH-18-1-0659-TS170030 (PI: Manning)
Name:	Molly McNamara
Project Role:	Graduate Student
Researcher Identifier:	MOLLYMCNAMARA
Nearest person month worked:	12
Contribution to Project:	Has performed analyses of BCL2 family member expression levels by immunoblot and qRT-PCR and has carried out the BH3 profiling assays.
Funding Support:	NIH/NIEHS Training Grant 5T32ES016645-10 (PI: Wessling-Resnick, ended 06/30/2020)

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.

Since the last reporting period, the project sponsored by Zafgen has completed (as of 09/30/2019). Please see full, updated DoD Other Support info starting on the next page.

KEY PERSONNEL PREVIOUS/CURRENT/PENDING SUPPORT

MANNING, BRENDAN

PREVIOUS SUPPORT (last five years)

Tuberous Sclerosis Alliance 194641 (PIs: Manning & Valvezan) 12/01/2015 – 11/30/2018
Repurposing clinically approved inhibitors of purine synthesis for the treatment of TSC
$90,000 DC/YR, 0.6 calendar months
Tuberous Sclerosis Alliance
801 Roeder Road, Suite 750, Silver Spring, MD 20910-4487
Grants Officer: Kari Luther Rosbeck
This project screened nucleotide synthesis inhibitors for selective effects in TSC1/2-deficient cells, determined the underlying mechanism, and demonstrated anti-tumor efficacy in preclinical TSC tumor models.
Aim 1: Characterize the response of TSC1/2-deficient cells to available inhibitors of purine synthesis
Aim 2: Preclinical trials of IMPDH inhibitors in mouse models of TSC
Aim 3: Define the mechanism underlying the selective response of TSC cells to purine synthesis inhibitors
No overlap

Zafgen, No Award Number, (PIs: Manning & Mitchell) 09/01/2016 – 09/30/2019
Determining the mechanism of action of derivatives of the anti-obesity drug, fumagillin
$200,000 DC/YR, 0.6 calendar months
Zafgen, Inc.
3 Center Plaza, Suite 610, Boston, MA 02108
CFO: Patricia Allen
Under this grant, we are characterizing the effects of anti-obesity drugs on cellular and systemic metabolism, and nutrient signaling pathways.
No overlap.
***This award has switched from “Current” to “Past” since the last reporting period

CURRENT SUPPORT

NIH/NCI Outstanding Investigator Award: R35-CA197459 (PI: Manning) 07/01/2015 – 06/30/2022
Decoding and targeting the PI3K-mTOR signaling network in cancer
$543,503 DC/YR, 6 calendar months
National Cancer Institute
BG 9609 MSC 9760, 9609 Medical Center Drive, Bethesda, MD 20892-9760
Grants Management Specialist: Marianne Galczynski
There are no specific aims in this award, but research is focused on defining the upstream regulation and downstream functions of the PI3K-mTOR network.
No overlap

Department of Defense: TS170026 (PI: Manning) 09/01/2018 – 08/31/2021
Mapping the Routes to Tumor Cell Death in TSC
W81XWH-18-1-0370-TS170026
$150,000 DC/YR, 1.2 calendar months
U.S. Army Medical Research Acquisition Activity
820 Chandler Street, Fort Detrick, MD 21702-5014
Grants Specialist: Christopher Meinberg
Under this grant, we will examine how TSC gene loss and mTORC1 activation influences the cell intrinsic apoptosis machinery in TSC cell and tumor models, and the therapeutic implications.
Aim 1: Define the status of pro- and anti-apoptotic proteins of the BCL-2 family and apoptotic priming in TSC.

Aim 2: Preclinical studies to enhance apoptotic priming in TSC cell and tumor models with BH3 mimetics developed for clinical use.
Aim 3: Determine the control mechanisms downstream of the TSC complex and mTORC1 altering apoptotic priming in TSC.
No overlap

Department of Defense: TS170030 (CoPI: Manning and Perrimon) 09/01/2018 – 08/31/2021
An Evolutionary Approach to Vulnerability Mapping in Order to Identify Alternative and Synergistic Therapeutic Strategies for TSC and Related Diseases
W81XWH-18-1-0659-TS170030
$150,000 DC/YR for Dr. Manning, 0.6 calendar months
U.S. Army Medical Research Acquisition Activity
820 Chandler Steet, Fort Detrick, MD 21702-5014
Under this grant, we will perform synthetic lethality screens in *Drososphila* to identify therapeutically actionable targets conserved in mammalian systems, which will be studied in TSC cell and tumor models.
There were no "specific aims" submitted for this award, but We use state-of-the art functional genomics methods in the fruit fly, Drosophila, a proven model to study TSC, to identify drug targets that synergize with Rapalogs in the treatment of TSC. We are also characterizing a promising drug target that has already emerged from our screens for the treatment of TSC.
No overlap.

NIH/NCI P01 CA120964 (PI: Kwiatkowski; Project leader: Manning) 08/01/2018 – 07/31/2023
Molecular Pathogenesis of the Hamartoma Syndromes. **Project 1 (Manning and Perrimon): Identifying new therapeutic avenues to selectively target tumors with uncontrolled mTORC1 activation.**
$153,345 DC/YR, 1.2 calendar months
National Cancer Institute
BG 9609 MSC 9760, 9609 Medical Center Drive, Bethesda, MD 20892-9760
Grants Management Specialist: Rogers Gross
This project uses unbiased genomic, proteomic, and genetic approaches to reveal new components, connections, and targets within the TSC-Rheb signaling network. The co-project leaders are focused on identifying novel therapeutic strategies and biomarkers by merging high-throughput *Drosophila* studies with mechanistic biochemical and cell biological studies in mammalian systems.
No overlap.

PENDING SUPPORT

None.

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.*