AWARD NUMBER: W81XWH-18-1-0384

TITLE: Mechanistic and therapeutic implications of spliceosomal gene mutations in ER+ breast cancer

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CONTRACTING ORGANIZATION: Sloan Kettering Institute for Cancer Research

REPORT DATE: Sept 2020

TYPE OF REPORT: Annual Technical

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2020	Annual	15 Aug 2019 – 14 Aug 2020

4. TITLE AND SUBTITLE

Mechanistic and therapeutic implications of spliceosomal gene mutations in ER+ breast cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0384

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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1275 York Avenue
New York, NY 10065

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

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

Recent evidence has revealed that altered mRNA splicing is a mechanism by which tumors can derive constitutive, tumor-promoting signals. Recurrent, somatic mutations in the core RNA splicing factor SF3B1 have been found in several malignancies. Through analyses of metastatic breast cancer patients at our center, we have noted hotspot mutations in SF3B1 in up to 6% of cases and these are strongly associated with the ER+/HER2- subtype and inferior outcomes in patients. We therefore conducted studies to understand the potential implications of SF3B1 mutations on breast cancer pathogenesis and have so far found that expression of the most common mutant, K700E, leads to characteristic alterations in RNA splicing, promotes the invasiveness and lethality of PIK3CA mutant breast cancer, and increases the sensitivity of PIK3CA mutant breast cancer cells to AKT inhibition. Based on these observations, we are further studying the consequences of SF3B1 mutation on breast cancer progression and sensitivity to spliceosomal targeted therapy.

15. SUBJECT TERMS

Antisense oligonucleotides, breast cancer, estrogen receptor, E7107, E7820, PIK3CA, RBM39, RNA splicing, SF3B1.

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	17	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.*

Recurrent, hotspot mutations in the RNA splicing factor SF3B1 occur in estrogen receptor positive (ER+) metastatic breast cancer (MBC). Here we aimed to (1) determine the mechanism by which the SF3B1 K700E mutation promotes the pathogenesis of breast cancer, (2) examine the effect of SF3B1 K700E mutation on mutant PI3K-initiated mammary tumorigenesis and develop novel SF3B1 mutant-selective splicing reporters, and (3) determine the therapeutic benefit of modulating RNA splicing in genetically defined breast cancers with and without spliceosomal gene mutations.

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

Antisense oligonucleotides, breast cancer, estrogen receptor, E7107, E7820, PIK3CA, RBM39, RNA splicing, SF3B1

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: Determine if SF3B1 K700E breast cancer cells depend on ER and/or ER signaling by testing the response of SF3B1 K700E models to inhibitors of ER or of its key targets (e.g. cyclin D1).

Milestone(s): Define the effects of SF3B1 K700E mutations on ER dependence and signaling as well as gene expression and splicing. Also, identify the global effects of SF3B1 K700E on splicing and gene expression in the context of breast cancer as well as those splicing events critical for estrogen-independent growth.
Timeline: Months 1-12. 100% complete.

Major Task 2: Examine the effect of *Sf3b1*K700E mutation on mammary tumorigenesis *in vivo*.

Milestone(s): Determine the effects of mutant Sf3b1 as well as mutant Pik3ca, alone and together, on splicing and gene expression in mammary breast epithelial cells as well the tumor promoting potential for mutant SF3B1 *in vivo.*
Timeline: Months 1-36. 70% complete.

Major Task 3: Determine the therapeutic benefit of modulating RNA splicing in breast cancers with spliceosomal gene mutations.

Milestone(s): Determine if breast cancer cells with SF3B1 mutations and/or MYC overexpression are preferentially sensitive to H3B-8800 or E7070 and E7820 as well as the mechanistic basis for their sensitivity.
Timeline: Months 1-36. 50% 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.

Major Task 1

Subtask 1. Generate additional tetracycline inducible models of SF3B1 K700E (or WT) in MCF7 or CAMA-1 ER+ models. (OAW and SC)

We have generated tetracycline inducible models of SF3B1 WT or K700E in MCF7 and CAMA-1 breast cancer cell lines. Western blots showed expression of WT or K700E at similar abundance induced by doxycycline treatment in both MCF7 (Fig 1A) and CAMA-1 (Fig 1D) cell lines.

Subtask 2. Knock the *SF3B1* K700E allele into MCF7 using CRISPR-Cas9 (SC)

We have now generated the following MCF7 cell lines with and without expression of SF3B1 K700E mutation as follows (Fig. 1E):

-MCF10A cells with heterozygous knockin of SF3B1K700E allele.
-MCF10A cells with heterozygous knockin of PIK3CA H1047R allele.
-MCF10A cells with dual heterozygous knockin of SF3B1K700E and PIK3CA H1047R alleles.

Subtask 3. Characterize the dependence of the mutant versus WT expressing models on hormones, ER level, and levels of key ER target genes as well as sensitivity to combination endocrine regimens. (SC)

We found no significant difference in the expression of ER and its key target genes in MCF7 and CAMA-1 cells expressing mutant SF3B1 by western blot (Fig 1A and 1D) and qRT-PCR (Fig 1B and 1E). While the mutant SF3B1 cells showed slightly slower proliferation in media with 10% FBS, these cells were yet dependent on estrogen as they did not grow in media supplemented with 10% charcoal stripped serum (css) in which hormones were depleted (Fig 1C and 1F). Similarly, these models were equally sensitive to ER antagonism with fulvestrant inhibiting growth at equivalent IC50s and GR50s (data not shown).

Subtask 4. Perform and analyze mRNA sequencing (RNA-seq) of T47-D, MCF-7, and CAMA-1 cells with inducible expressing vector, SF3B1 WT, and SF3B1 K700E (equal expression to endogenous WT) to characterize gene expression as well as RNA splicing events. (OAW)

We have performed RNA-seq analysis on MCF10A (WT or SF3B1 K700E knock-in), MCF7 and T47D doxycycline-inducible cells. The expression of mutant SF3B1 caused extensive usage of alternative 3' splice sites (Fig 2C) that are conserved in all three cell lines. For differential gene expression analysis, the major variance was dominated by cell lines (Fig 2D). Only a small fraction of genes were commonly regulated in all three cell lines (Fig 2F). Interestingly, this list includes genes that are alternatively spliced, such as MAP3K7 and PPP2R5A (Fig 2E and Fig 2F).

Subtask 5. Design antisense oligonucleotides (AONs) to mimic splicing events by SF3B1 K700E in SF3B1 WT ER-dependent cell lines to identify the functional contribution of individual splicing events. (OAW)

We tried to design AONs to target the 3' alternative splice sites of *MAP3K7* and *PPP2R5A*. However, due to the low GC content in these regions, it was hard to get a Morpholino oligo with Tm to work at 37ºC culture condition.

Major Task 2

Subtask 1 Assess *Mmtv*-cre *Sf3b1*$^{K700E/WT}$, *Mmtv*-cre *R26R-LSL-Pik3ca*^{H1047R,} *Mmtv*-cre *Sf3b1*$^{K700E/WT}$ *R26R-LSL-Pik3ca*^{H1047R,} along with WT controls for tumor development. (OAW and SC)
We have generated four group of mice: *Mmtv*-cre, *Mmtv*-cre *Sf3b1*$^{K700E/WT}$, *Mmtv*-cre *R26R-LSL-Pik3ca*^{H1047R} and *Mmtv*-cre *Sf3b1*$^{K700E/WT}$ *R26R-LSL-Pik3ca*^{H1047R} double mutant (Fig 3A). While extensive growth of mammary ducts was found in Pik3ca single mutant and Sf3b1K700E Pik3ca double mutant mice, the Sf3b1 K700E single mutant mice showed reduced mammary ductal branching (Fig 3B). Hastened death was observed in MMTV-Cre Sf3b1K700E/WT R26-Pik3caH1047R double mutant mice (Fig 3C), where ER+/PR+, high Ki67 mammary gland adenocarcinoma developed (Fig 3D).

Subtask 3. We have now performed and analyzed RNA-seq of purified luminal (DAPI^{-} CD24^{low}) and basal (DAPI^{-} CD24^{high}) epithelial cells from the mouse models above. This has revealed the same global change in aberrant, intron-proximal 3' splice site usage in SF3B1 mouse epithelial cells and breast tumors as was seen in SF3B1 mutant breast cancer cell lines (Fig. 3E). This prominently includes mis-splicing of Map3k7 and Ppp2r5a, as seen in human breast cancer cells with mutant SF3B1 (Fig. 3F).

Major Task 3

Subtask 1. Test the effects of 2 different inhibitors of SF3b complex in a panel of ER dependent cell lines isogenic for SF3B1K700E or WT. (OAW and SC)
We have tested the effect of SF3B1 inhibitor E7107 on in vitro proliferation in MCF10A and T47D cells expressing mutant SF3B1 (Fig 4A and 4B), and no significant difference was observed when compared to WT control cells. E7107 is very potent and toxic to all these cells under in vitro culture condition with IC50 less than 0.3nM. Based upon the findings in Major Task 1, we identified AKT inhibition as potential pharmacologic strategy for targeting dual SF3B1 and PIK3CA mutant cancers and have found models with both alterations to be hypersensitive to AKT inhibitors.

Subtask 2: Test the effects of 2 different inhibitors of SF3b complex in a panel of genetically annotated ER/PR+ and TNBC cell lines defined by MYC expression status (OAW and SC)

Subtask 4: Evaluate the sensitivity of human and murine breast cancer cells with and without an SF3B1 mutation to pharmacologic or genetic loss of the accessory splicing factor RBM39 (OAW and SC)
We have tested the effect of E7070, a sulfonamide inducing RBM39 degradation, on MCF10A WT or SF3B1 K700E knock-in cells (Fig 4C). SF3B1 mutant cells were more sensitive to E7070 treatment in proliferation experiment with lower IC50.

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.

Dr. Bo Liu (shared postdoctoral fellow of the Abdel-Wahab and Chandarlapaty labs) presented his work at the 2019 RNA Processing in Cancer meeting at Yale University and the 2019 AACR Annual Meeting in Atlanta. He also presented his work as an oral presentation at a large Departmental seminar series in 2020.

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.

For Major Task 1, We will generate additional RNA-seq data in CAMA-1 cells. Finally, we will generate and test anti-sense oligonucleotides for additional mutant SF3B1-induced aberrant splicing events.

For Major Task 2, we will attempt to serially passage tumors that arise in primary mice for downstream analyses of drug sensitivity in orthotopic xenografts.

For Major Task 3, we will test the effects of 2 different inhibitors of SF3b complex in genetically annotated patient derived xenografts (PDX). In addition, we will perform RNA-seq in select cells from above treated with SF3b inhibitor, RBM39 degrader, and vehicle.

In addition to the above specific plans, we would like to publish our initial findings. We have generated a manuscript that was reviewed in the *Journal of Clinical Investigation (JCI)* and is now being revised for the same journal. We hope to resubmit the manuscript in Sept. 2020.

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).

One of the main findings from our work thus far is that cells which contains mutations in genes that regulate RNA splicing can cause breast cancer cells to be come more invasive and lethal. Part of this effect appears to be through a common growth pathway known as the PI3K/AKT pathway and so these tumors may be treated with AKT inhibitors. We have also identified drugs which alter splicing by inhibiting arginine methylation of splicing proteins (by enzymes known as PRMTs) may also target splicing factor mutant cells. Inhibitors of PRMT5 are currently in phase I clinical trials in solid tumors and our data suggest that mutations in RNA splicing factors (such as SF3B1) may serve as a biomarker for responsiveness to these therapies.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Although we did have a ~4 month laboratory shutdown at our institution with cessation of animal breeding due to COVID-19, we were able to use that time to generate a manuscript of our initial data which has now been successfully reviewed. In addition, we did not lose any strains of mice.

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.

Nothing to report.

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.

As noted above, we did have a 4 month period of time where no experiments were performed at our institution due to COVID-19. Therefore, minimal funds were spent during this time.

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

None.

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).

Bo Liu, Zhaoqi Liu, Michelle Ki, Caroline Erickson, Jorge Reis-Filho, Raul Rabadan, Omar Abdel-Wahab (co-corresponding), Sarat Chandarlapaty (co-corresponding). Mutant SF3B1 promotes AKT and NF-□B driven mammary tumorigenesis. In revision, *Journal of Clinical Investigation*.

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.*

2019 RNA Processing in Cancer Presentation entitled "Role of SF3B1 Mutations in Breast Cancer", Yale University, New Haven, CT.

2019 AACR Annual Meeting Presentation entitled "Hotspot mutations in the core spliceosomal protein SF3B1 promote breast tumorigenesis", Atlanta, GA.

- **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.

We have generated a novel technique to identify aberrant 3' splice site usage from RNA-seq data. In addition, we have performed Morpholino based anti-sense oligonucleotides to modulate RNA splicing in breast cancer cells.

- **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.*

  We have generated substantial deep RNA-seq data on isogenic breast cancer cell lines with and without mutations in SF3B1. These include MCF10, MCF7, T47D cells and mouse breast epithelial cells. These data will be deposited in GEO at the time of publication of the manuscript describing our splicing and gene expression analyses of these data.

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".

No change for Dr. Omar Abdel-Wahab, Dr. Sarat Chandarlapaty, and Dr. Bo Liu.

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.

See attached.

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)

Name:	*Omar Abdel-Wahab*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	*.91*
Contribution to Project:	*Overseeing all experiments in conjunction with Dr. Chandarlapaty.*
Funding Support:	*US National Institutes of Health (NIH)-NHLBI grant R01 HL128239 & NIH-NCI grant R01 CA201247-03*
Name:	*Sarat Chandarlapaty*
Project Role:	*co-PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-4532-8053*
Nearest person month worked:	*1.2*
Contribution to Project:	*Overseeing all experiments in conjunction with Dr. Abdel-Wahab.*
Funding Support:	*US National Institutes of Health (NIH)-NCI grants – R01CA244812, R01CA234361, and R01204999 and Breast Cancer Research Foundation (BCRF)*
Name:	*Bo Liu*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	*8.4*
Contribution to Project:	*Bo generated all of the data presented here.*
Funding Support:	*US National Institutes of Health (NIH)-NHLBI grant R01 HL128239 & NIH-NCI grant R01 CA201247-03*
Name:	*Hana Cho*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	*None*
Nearest person month worked:	*1.2*
Contribution to Project:	*Assist Bo Liu in generating data presented here.*
Funding Support:	*US National Institutes of Health (NIH)-NHLBI grant R01 HL128239 & NIH-NCI grant R01 CA201247-03*

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.*

Figure legends

Figure 1. A and D) Western blots of FLAG-SF3B1, ER, key ER target genes and phosphorylated AKT in MCF7 (A) and CAMA-1 (D) cells transduced with doxycycline-induced WT or mutant SF3B1 expressing vectors. **B and E**) qRT-PCR analysis of ESR1 and key ER target genes in MCF7 (B) and CAMA-1 (E) cells transduced with doxycycline-induced WT or mutant SF3B1 expressing vectors. **C and F**) Proliferation curve of cells above in media supplemented with either 10% FBS or 10% charcoal-stripped serum (CSS). **G)** Sanger sequencing traces showing creation of *SF3B1*K700E/WT mutation in the endogenous locus of *SF3B1* in MCF10A cells via CRISPR. **H)** Western blot for signaling intermediates and protein products of mutant SFB1 mis-spliced genes in MCF10A cells with knockin of *SF3B1*K700E, *PIK3CA*H1047R, or both mutations together.

Figure 2. A) Sanger sequencing of nucleotides around codon K700 of SF3B1 in MCF7 cells transfected with CAS9, SF3B1 sgRNA and K700E repair template ssDNA. **B**) Sanger sequencing of nucleotides around codon K700 of SF3B1 in MCF10A cells with heterozygous K700E knock-in. **C**) Heatmap clustering for 3' alternative splice sites from RNA-seq analysis in MCF10A, MCF7 and T47D cells. **D**) Alternative splicing of MAP3K7 and PPP2R5A in MCF10A, MCF7 and T47D cells. **E**) Heatmap of differential gene expression from RNA-seq analysis in MCF10A, MCF7 and T47D cells. **F**) Venn diagrams showing common genes that are upregulated or downregulated in MCF10A, MCF7 and T47D cells expressing mutant SF3B1.

Figure 3. A) Generation of MMTV-promoter driven conditional knock-in Sf3b1K700E/WT and Sf3b1K700E/WT Rosa26-Pik3caH1047R double mutant mice. **B**) Effect of Sf3b1K700E, Pik3caH1047R and double mutant on mammary gland duct development. **C**) Survival curve for mice in each group. **D**) H&E staining and immunohistochemical staining for Ki67, estrogen receptor α (ER) , progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) of mammary gland tumors from MMTV-Cre Pik3caH1047R and double mutant mice. **E)** FACS plots used for purification of mammary breast epithelial cells from *Mmtv*-cre control, *Mmtv*-cre *Sf3b1*$^{K700E/WT}$, *Mmtv*-cre R26-LSL*Pik3ca*^{H1047R}, and *Mmtv*-cre *Sf3b1*$^{K700E/WT}$ *Pik3ca*^{H1047R} 4-month old mice. **F)** Venn diagrams of genes significantly differentially expressed ($p<0.05$; DESeq2) and spliced ($q<0.05$; t-test) across SF3B1 mutant versus wild-type human breast cancers from TCGA (23) (left), isogenic human breast cell lines (right), and overlapping across human tumors, human cell lines, and mouse mammary epithelial cells mutant for Sf3b1 (middle).

Figure 4. A) Dose-response curve for E7107 in T47D cells with doxycycline-induced expression of WT or mutant SF3B1. **B**) Dose-response curvce for E7107 in WT or SF3B1 K700E knock-in MCF10A cells. **C**) Dose-response curvce for E7070 in WT or SF3B1 K700E knock-in MCF10A cells.

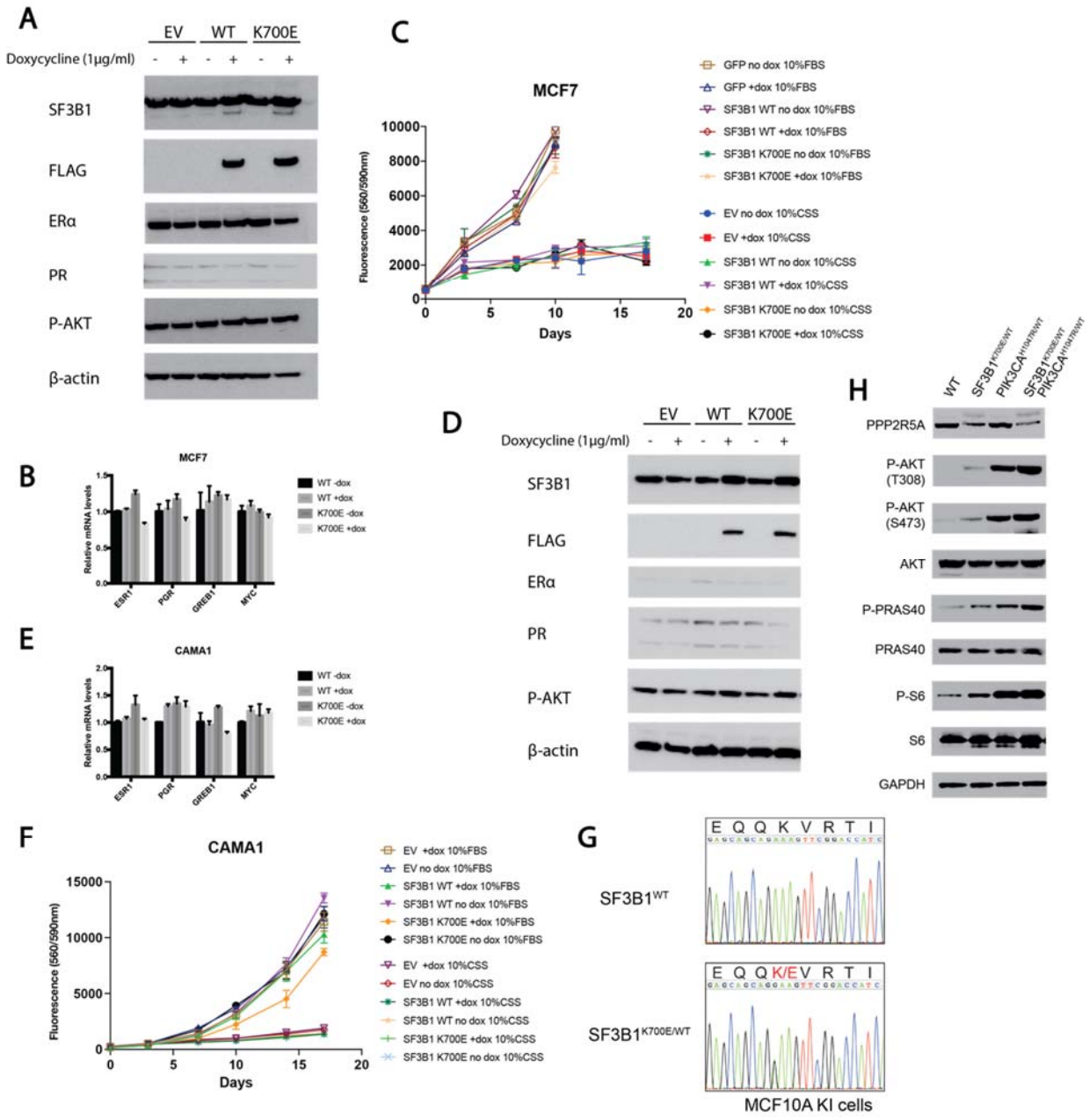

A
EV
WT
K700E
Doxycycline (1μg/ml)
SF3B1
FLAG
ERα
PR
P-AKT
β-actin
B
MCF7
Relative mRNA levels
WT -dox
WT +dox
K700E -dox
K700E +dox
ESR1
PGR
GREB1
MYC
C
MCF7
Fluorescence (560/590nm)
Days
GFP no dox 10%FBS
GFP +dox 10%FBS
SF3B1 WT no dox 10%FBS
SF3B1 WT +dox 10%FBS
SF3B1 K700E no dox 10%FBS
SF3B1 K700E +dox 10%FBS
EV no dox 10%CSS
EV +dox 10%CSS
SF3B1 WT no dox 10%CSS
SF3B1 WT +dox 10%CSS
SF3B1 K700E no dox 10%CSS
SF3B1 K700E +dox 10%CSS
D
EV
WT
K700E
Doxycycline (1μg/ml)
SF3B1
FLAG
ERα
PR
P-AKT
β-actin
E
CAMA1
Relative mRNA levels
WT -dox
WT +dox
K700E -dox
K700E +dox
ESR1
PGR
GREB1
MYC
F
CAMA1
Fluorescence (560/590nm)
Days
EV +dox 10%FBS
EV no dox 10%FBS
SF3B1 WT +dox 10%FBS
SF3B1 WT no dox 10%FBS
SF3B1 K700E +dox 10%FBS
SF3B1 K700E no dox 10%FBS
EV +dox 10%CSS
EV no dox 10%CSS
SF3B1 WT +dox 10%CSS
SF3B1 WT no dox 10%CSS
SF3B1 K700E +dox 10%CSS
SF3B1 K700E no dox 10%CSS
G
E Q Q K V R T I
SF3B1WT
E Q Q K/E V R T I
SF3B1K700E/WT
MCF10A KI cells
H
WT
SF3B1K700E/WT
PIK3CAH1047R/WT
SF3B1K700E/WT PIK3CAH1047R/WT
PPP2R5A
P-AKT (T308)
P-AKT (S473)
AKT
P-PRAS40
PRAS40
P-S6
S6
GAPDH

Figure 1.

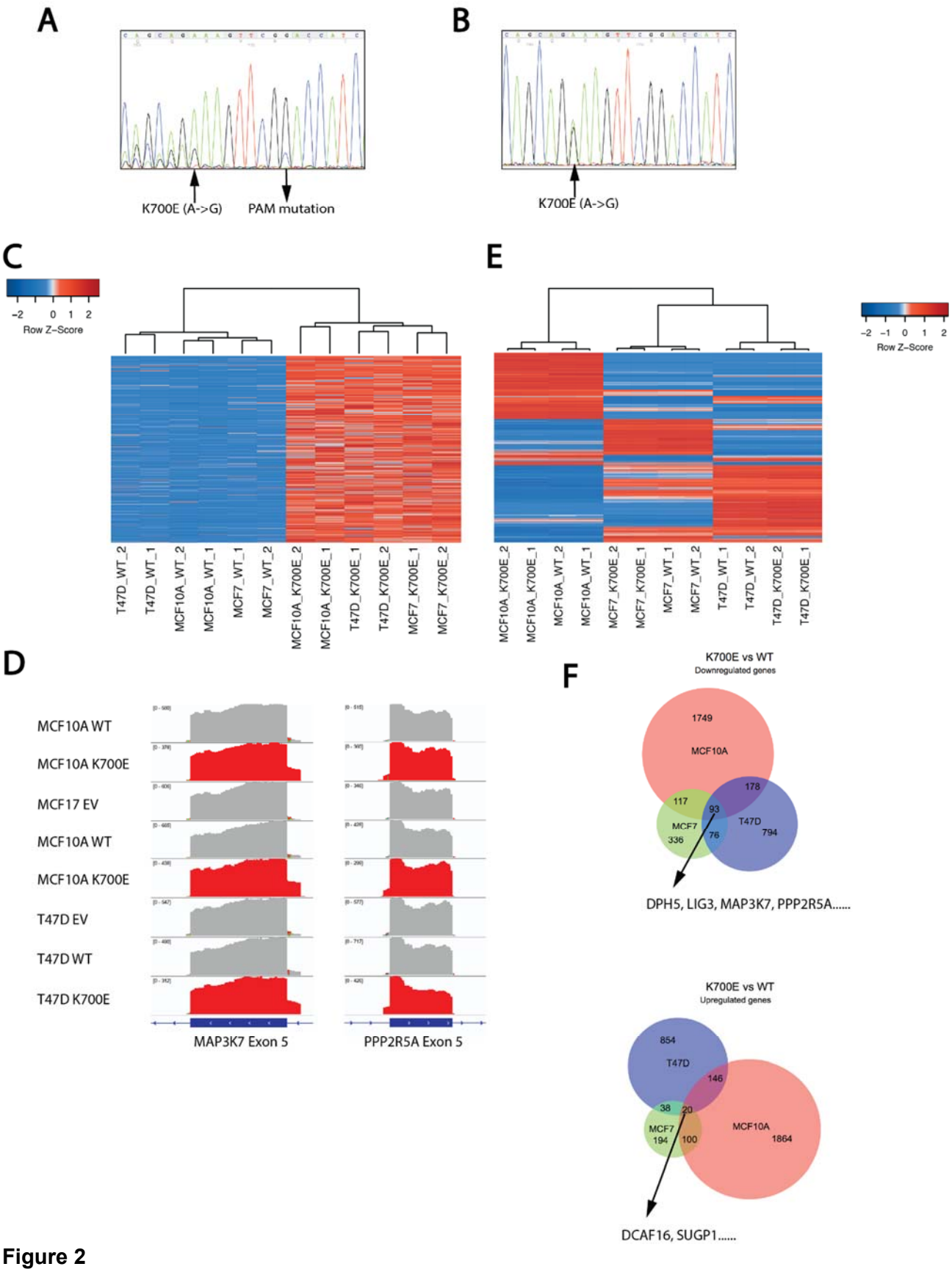
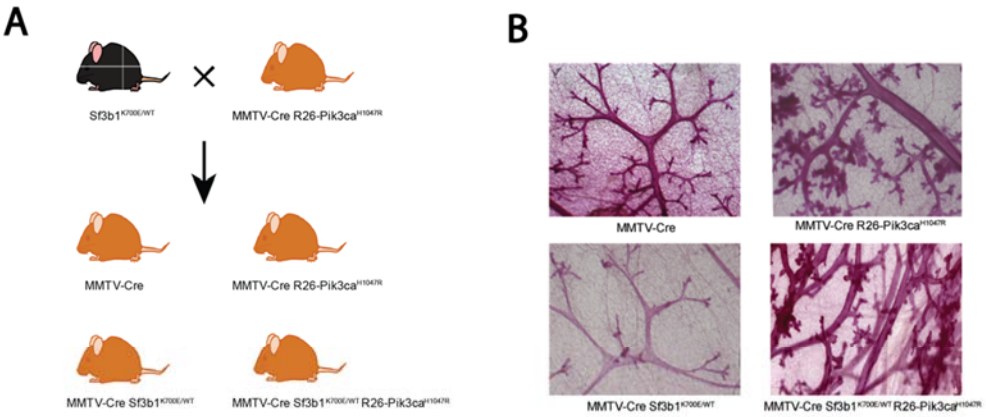
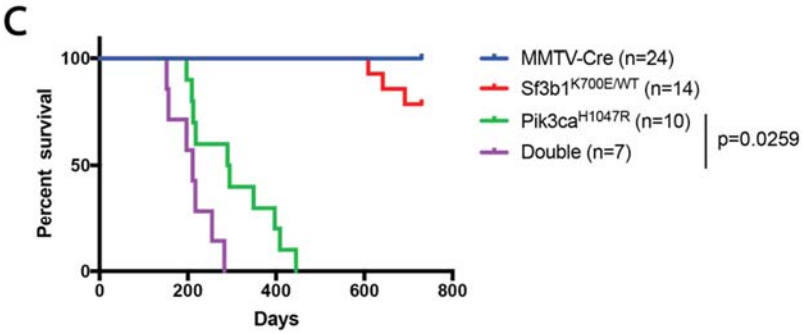
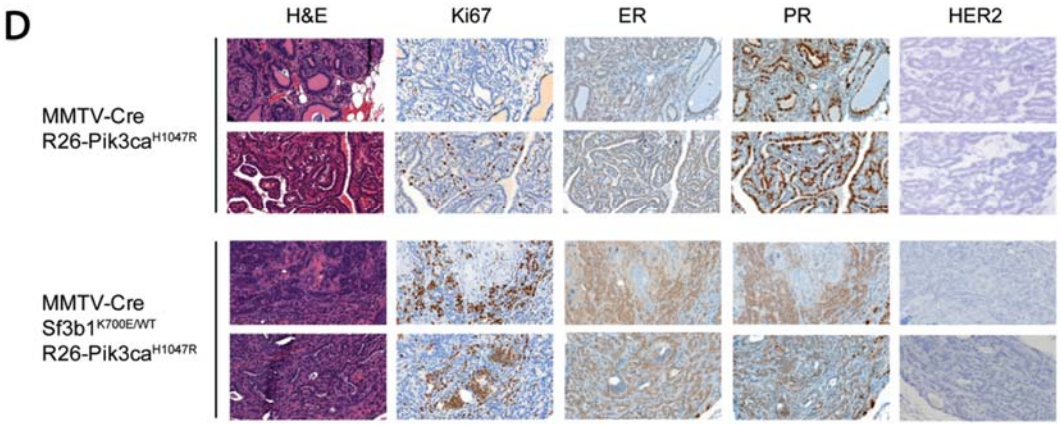
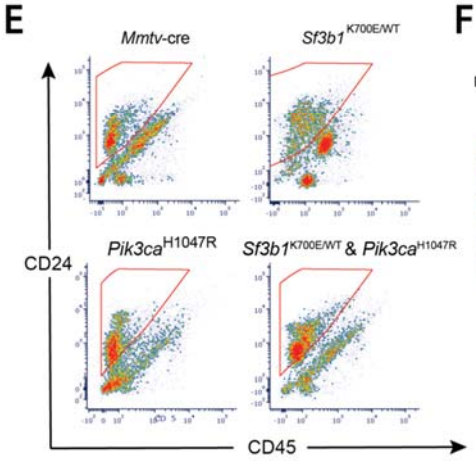

A
K700E (A->G)
PAM mutation
B
K700E (A->G)
C
−2 0 1 2
Row Z−Score
T47D_WT_2
T47D_WT_1
MCF10A_WT_2
MCF10A_WT_1
MCF7_WT_1
MCF7_WT_2
MCF10A_K700E_2
MCF10A_K700E_1
T47D_K700E_1
T47D_K700E_2
MCF7_K700E_1
MCF7_K700E_2
E
−2 −1 0 1 2
Row Z−Score
MCF10A_K700E_2
MCF10A_K700E_1
MCF10A_WT_2
MCF10A_WT_1
MCF7_K700E_2
MCF7_K700E_1
MCF7_WT_1
MCF7_WT_2
T47D_WT_1
T47D_WT_2
T47D_K700E_2
T47D_K700E_1
D
MCF10A WT
MCF10A K700E
MCF17 EV
MCF10A WT
MCF10A K700E
T47D EV
T47D WT
T47D K700E
MAP3K7 Exon 5
PPP2R5A Exon 5
F
K700E vs WT
Downregulated genes
1749
MCF10A
178
117
93
MCF7
336
76
T47D
794
DPH5, LIG3, MAP3K7, PPP2R5A.....
K700E vs WT
Upregulated genes
854
T47D
146
38
20
MCF7
194
100
MCF10A
1864
DCAF16, SUGP1.....

Figure 2


A
Sf3b1K700E/WT
MMTV-Cre R26-Pik3caH1047R
MMTV-Cre
MMTV-Cre R26-Pik3caH1047R
MMTV-Cre Sf3b1K700E/WT
MMTV-Cre Sf3b1K700E/WT R26-Pik3caH1047R
B
MMTV-Cre
MMTV-Cre R26-Pik3caH1047R
MMTV-Cre Sf3b1K700E/WT
MMTV-Cre Sf3b1K700E/WT R26-Pik3caH1047R


C
Percent survival
100
50
0
0
200
400
600
800
Days
MMTV-Cre (n=24)
Sf3b1K700E/WT (n=14)
Pik3caH1047R (n=10)
Double (n=7)
p=0.0259


D
H&E
Ki67
ER
PR
HER2
MMTV-Cre
R26-Pik3caH1047R
MMTV-Cre
Sf3b1K700E/WT
R26-Pik3caH1047R


E
Mmtv-cre
Sf3b1 K700E/WT
Pik3caH1047R
Sf3b1K700E/WT & Pik3caH1047R
CD24
CD45

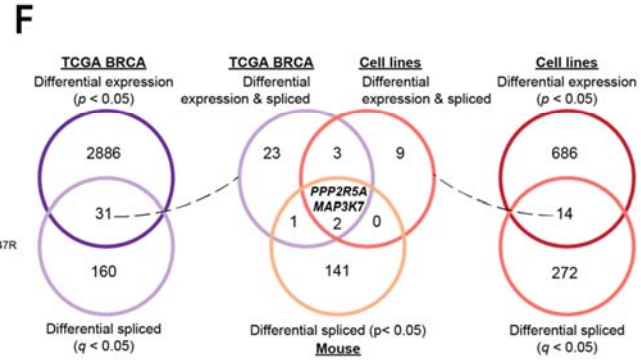

F
TCGA BRCA
Differential expression
(p < 0.05)
2886
31
160
Differential spliced
(q < 0.05)
TCGA BRCA
Differential
expression & spliced
23
3
9
PPP2R5A
MAP3K7
1
2
0
141
Differential spliced (p< 0.05)
Mouse
Cell lines
Differential
expression & spliced
Cell lines
Differential expression
(p < 0.05)
686
14
272
Differential spliced
(q < 0.05)

Figure 3

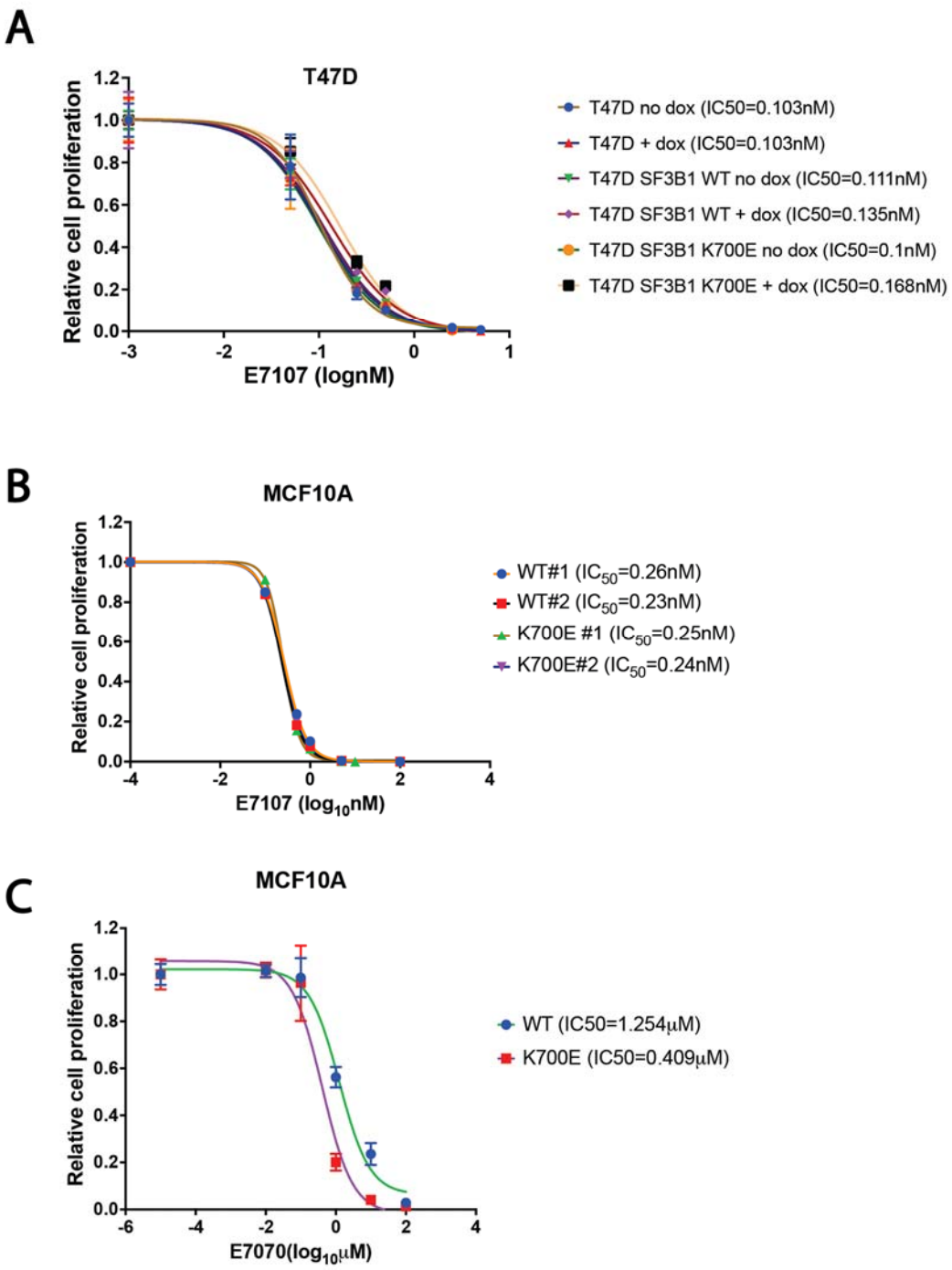

A
T47D
Relative cell proliferation
E7107 (lognM)
T47D no dox (IC50=0.103nM)
T47D + dox (IC50=0.103nM)
T47D SF3B1 WT no dox (IC50=0.111nM)
T47D SF3B1 WT + dox (IC50=0.135nM)
T47D SF3B1 K700E no dox (IC50=0.1nM)
T47D SF3B1 K700E + dox (IC50=0.168nM)
B
MCF10A
Relative cell proliferation
E7107 (log10nM)
WT#1 (IC50=0.26nM)
WT#2 (IC50=0.23nM)
K700E #1 (IC50=0.25nM)
K700E#2 (IC50=0.24nM)
C
MCF10A
Relative cell proliferation
E7070(log10μM)
WT (IC50=1.254μM)
K700E (IC50=0.409μM)

Figure 4

OMAR I. ABDEL-WAHAB, M.D.

NEW ACTIVE SUPPORT

1R43CA246950-01 (PI: Akerman) 9/9/2019 - 8/31/2024 0.24 calendar
NIH $ 36,192
SpliceIO: A machine learning approach to discover novel cancer specific antigens through splicing analysis using RNA seq data
Goals: Here, we propose to leverage and extend this resource to develop SpliceIO, a novel discovery platform that integrates the SpliceCore knowledge base with machine learning (ML) to identify novel TAEs resulting from aberrant splicing. This strategic development will lead Envisagenics to disrupt IO with an unprecedent modality for RNAseq analysis, creating partnership opportunities for co-develop of IO products with pharma.
Specific Aims: Aim 1. Develop SpliceIO to identify and predict viable alternatively spliced ectodomains. Aim 2. Identify and predict antigenicity of AS-derived TAE from public pediatric AML data to assess utility of SpliceIO in-silico. Aim 3. Leverage SpliceIO to discover viable AS-derived ectodomains associated with AML relapse and validate targets in-vitro.
Agency Contract: PI Name: Martin Akerman, PhD Email: makerman@envisagenics.com Tel: (631)697-9471
Overlap: None

1 R01 CA242020-01A1 (PI: Abdel-Wahab/Aifantis) 6/1/2020 - 5/31/2025 1.80 calendar
NCI $ 428,033
Targeting an RNA-binding Protein Network in Acute Myeloid Leukemia
Goals:Recent studies have identified that proteins that bind RNA to regulate RNA translation, stability or splicing, playkey roles in human cancer. We recently identified a group of such RNA binding proteins (RBPs) that are required for the survival of acute myeloid leukemia (AML), a deadly form of cancer in great need of improved therapy. In this proposal we aim to understand the role of one such protein called RBM39, which is a putative, novel, and exciting new AML drug target.
Specific Aims: Aim 1: Determine the biological role of RBM39 in normal and malignant hematopoiesis. Aim 2: Identify the mechanistic basis for cell-type and disease-specific roles for RBM39 in normal and malignant hematopoietic cells.
Agency Contract: Funmi Elesinmogun, Grants Management Specialist, elesinmf@mail.nih.gov, 240-276-6313
Overlap: None

1 R01 CA251138-01 (PI: Abdel-Wahab) 7/1/2020 - 6/30/2025 1.80 calendar
NCI $ 515,431
Interrogating the minor spliceosome to understand and treat leukemia
Goals: Leukemias are cancers in which the bone marrow produces too many abnormal blood cells at the expense of normal blood cells. Here we will determine how a commonly occurring change in a gene called ZRSR2, which encodes a protein that participates in the process of RNA splicing, gives rise to leukemia. We will determine how ZRSR2 mutations drive leukemia and find new ways to treat leukemias with ZRSR2 mutations.
Agency Contract: N/A
Overlap: None

GC260033 (PI: Abdel-Wahab) 7/7/2020 – 6/6/2021 0.24 calendar
Edward P. Evans Foundation $ 181,818
Elucidating Critical Targets, Transcripts, and Collaborating Events in Spliceosomal-Mutant MDS
Goal: The project goal is to understand which abnormally spliced products are functionally important for the development of myelodysplastic syndrome phenotypes.
Agency Contract: Michael Lewis, Ph.D. President,mdl@epefoundation.org 978 494-6009
Overlap: None

CONTINUTING ACTIVE SUPPORT

6581-20 (PI: Liu) 7/1/2019 - 6/30/2022 0.24 calendar
Leukemia and Lymphoma Society $ 60,000
Development of therapeutic strategy for the treatment of MDS
Goals & Specific Aims: AIM 1: Elucidate the mechanisms by which mutant p53 alters pre-mRNA splicing in HSCs and MDS cells. Dr. Abdel-Wahab will provide assistance with analysis of splicing in hematopoietic cells with or without mutations in p53. AIM 2. Determine the impact of inhibition of EZH2 and the spliceosome on MDS cells with TP53 mutations. Dr. Abdel-Wahab will test several therapies targeting RNA splicing in vitro and in vivo in primary human MDS and AML cells with or without mutations in TP5
Agency Contract: Director of Research Administration, 3 International Drive, Suite 200, Rye Brook, New York 10573, researchprograms@lls.org
Overlap: None

W81XWH-18-1-0383 (PI: Abdel-Wahab) 8/15/2018 - 8/14/2021 0.91 calendar
Congressionally Directed Medical Research Programs $ 100,000
Mechanistic and Therapeutic Implications of Spliceosomal Gene Mutations in ER+ Breast Cancer
Goals: Our grant aims to (1) identify what drives breast cancer growth; determine how to stop it and (2) revolutionize treatment regimens by replacing them with ones that are more effective, less toxic, and impact survival.
Specific Aims: (1) Determine the mechanism by which the SF3B1 K700E mutation promotes hormoneindependent growth of breast cancer, (2) Examine the effect of SF3B1 K700E mutation on mammary tumorigenesis in vivo, (3) Determine the therapeutic benefit of modulating RNA splicing in genetically defined breast cancers with and without spliceosomal gene mutations.
Agency Contract: Grants Specialist: Jodi Cardoza Phone: 301-619-2693 Email: jodi.l.cardoza.civ@mail.mil
Overlap: None

1 UG1 CA233332-02 (PI: Levine) 3/7/2019 - 2/28/2025 0.46 calendar
NCI $ 736,976
ECOG-ACRIN Integrated Leukemia Translational Science Center (LTSC)
Advances in the treatment of hematologic malignancies have been disappointing. The ECOG-ACRIN
Goals: Leukemia Translational Science Center will create, support, and synergize partnerships between clinical and laboratory investigators and foster the continuous, near-term translation of state-of-the art laboratory studies into the clinical context to improve outcomes for leukemia patients.
Specific Aims: 1. To identify the molecular signatures which define clinical, prognostic, and therapeutically distinct leukemia subtypes, and can be used to develop risk-stratified therapeutic protocols and/or to therapy with mechanism-based therapies for biomarker-defined disease subsets. 2. To evaluate the mechanism of action, therapeutic efficacy, and predictors of response/resistance of mechanism based therapies for leukemia patients. 3. To perform dynamic, biomarker based molecular assessment of response to leukemia therapies which can be used to monitor disease response, inform mechanisms of sensitivity and resistance to leukemia therapies, and to design clinical trials which incorporate dynamic biomarker profiling into clinical trial design and clinical care.
Agency Contract: Grants Management Specialist: Barbara Hodgkins Email: barb.hodgkins@nih.gov Phone: (240) 276-6294
Overlap: None

I12-0063 (PI: Abdel-Wahab) 1/1/2019 - 12/31/2020 0.24 calendar
Starr Cancer Consortium $ 75,000
Identification of transcriptional determinants of asparaginase sensitivity in leukemias
Goals: Asparaginase, which selectively depletes asparagine from serum, kills asparagine-dependent leukemia cells and is a first line chemotherapeutic agent. However, the precise mechanisms underlying the asparagine response of leukemic cells and whether there are other cancer types dependent on particular nutrients remain poorly understood.

Specific Aims: Aim 1: Determine the precise mechanism by which ZBTB1 enables ALLs to survive under asparagine depletion. Aim 2: Examine the clinical significance of ZBTB1 in ALL and other lymphoid malignancies. Aim 3: Map the amino acid dependencies of blood cancer cell lines using DNA-barcoding technology.
Agency Contract: Sylvie Le Blancq, PhD, Executive Director, Sloan Kettering Institute, 1275 York Avenue, New York, NY 10065, lesblanc2@mskcc.org, 646-888-3773
Overlap: None

1 UG1 CA233290-02 (PI: Aghajanian / Lee / Morris / Tallman / Zivanovic) 3/6/2019 - 2/28/2025 0.60 calendar
NCI $ 9,615
Network Lead Academic Participating Site: Memorial Sloan Kettering Cancer Center
Goals: This goal will be achieved through the continued successful development and execution of definitive, randomized, clinical treatment and advanced imaging trials across a broad range of diseases and diverse patient populations.
Specific Aims: 1. Scientific Leadership in Development of National Clinical Trials Network (NCTN) Trials 2. Scientific Leadership in the Activities of the NCTN and NCI Scientific Steering Committees 3. Patient Accrual on NCTN Trials 4. Mentoring Junior Investigators in Clinical Trial Research
Agency Contract: Grants Management Specialist: Barbara Hodgkins Email: barb.hodgkins@nih.gov Phone: (240) 276-6294
Overlap: None

DRG2018 (PI: Figueroa) 9/1/2018 - 8/31/2020 0.24 calendar
Edward P. Evans Foundation $ 90,909
Determining the Contribution of MDS-associated RNA Splicing Factor Mutations to Altered DNA Methylation
Goals & Specific Aims: As part of this collaboration, our lab will perform analyses to integrate DNA methylation and hydroxy-cytosine methylation data with RNA splicing data. We will also attempt to understand how alterations in DNA methylation alter splicing through focused studies of splicing and expression of potential regulators of DNA methylation. In addition, we will perform locus-specific biochemical experiments to understand how alterations in the epigenome impact splicing.
Agency Contract: Michael Lewis, Ph.D. President,mdl@epefoundation.org 978 494-6009
Overlap: None

5R01HL145283-02 (PI: Landau) 2/1/2019 - 11/30/2023 0.20 calendar
NHLBI $ 96,396
The role of DNA methylation modifiers in shaping the hematopoietic differentiation topology
Goals & Specific Aims: The Abdel-Wahab will generate and maintain the murine models used in Aims 1-2 as well as perform purification of mouse and human cell populations in all aims of the grant. The Abdel-Wahab lab will also perform the cell transduction and differentiation experiments described in Aim 2.
Agency Contract: Grants Management Specialist: Laurel Katherine Kennedy Email: laurel.kennedy@nih.gov Phone: 301-82704777
Overlap: None

713327 (PI: Shlush) 11/1/2018 - 10/31/2023 1.60 calendar
Leukemia and Lymphoma Society/ Rising Tide Foundation $ 36,364
Early diagnosis and treatment of pre-leukemia
Goals: Successful completion of our aims could have major ramifications on how subjects with ARCH are evaluated and treated, which could, in turn, have a major impact on development of MDS and AML.
We now propose to utilize this knowledge to treat high-risk individuals with ARCH, at a time point before they have developed disease, by targeting the driving alterations most associated with AML development.

Specific Aims: Aim 1. Develop novel efficient strategies to identify individuals with preleukemia. Aim 2. Determine the molecular and functional consequences of SMMs in human preleukemia. Aim 3. Identify targeted therapies effective at eliminating preL-HSPCs carrying SMMs.
Agency Contract: Director of Research Administration, 3 International Drive, Suite 200, Rye Brook, New York 10573, researchprograms@lls.org
Overlap: None

5 R01 HL138090-04 (PI: Geissmann) 8/16/2017 - 6/30/2021 0.60 calendar
NHLBI $ 396,910
Lineages and pathophysiology of clonal histiocytic disorders
Goals: This project aims to characterize at the molecular, cellular and organismal levels the pathophysiology of clonal histiocytic disorders, exemplified by Langerhans Cell Histiocytosis (LCH) and Erdheim-Chester Disease (ECD).
Specific Aims: We will (Aim 1) investigate the pathological, molecular and cellular mechanisms that underlie the development of the brain neurodegenerative disease we observe in our genetic model Csf1rMeriCreMer; BRAFV600E; Rosa26LSL-YFP in which we introduce a BRAFV600E allele in a small number of EMPs. Next (Aim 2) we will determine the pathological consequences of targeting BRAFV600E outside the brain and in particular test whether environmental factors can influence the pathogenesis of histiocytosis in the liver. We will also investigate the extent to which lineage(s) of origin of histiocytoses determines pathological outcomes using a murine model to conditionally target BRAFV600E expression to a small faction of HSCs. Finally (Aim 3) we will investigate the efficiency of BRAF inhibitor administration to prevent or treat the neurodegenerative disease in our models.
Agency Contract: Renee Livshin, livshinr@nhlbi.nih.gov, 301-435-0174
Overlap: None

LLS-02 (PI: Nimer) 10/1/2017 - 9/30/2022 0.20 calendar
Leukemia and Lymphoma Society $ 77,500
Interventional Epigenetics in Myeloid Malignancies
Goals: Our ultimate goal is to define the mechanistic epigenetic basis for the development and progression of myeloid malignancies and to develop novel and effective epigenetic-focused therapies for patients with these disorders.
Specific Aims: Major Task 1: Determine genome-wide localization of ASXL1 versus ASXL2. Major Task 2: Characterize the effects of ASXL2 loss in the setting of AML1-ETO. Major Task 3: Determine the effect of p300 inhibition in AML1-ETO cells with or without Asxl2 loss. Major Task 4: Test LSD1 inhibition in ASXL1/2-deficient AML cells. Major Task 5: Identify genes which are synthetic lethal with ASXL1-mutant cells by performing functional genomic screens.
Agency Contract: Director of Research Administration, 3 International Drive, Suite 200, Rye Brook, New York 10573, researchprograms@lls.org
Overlap: None

6533-18 (PI: Park) 10/1/2017 - 9/30/2021 NCE 0.30 calendar
Leukemia and Lymphoma Society $ 180,018
Chemotherapy-Free Targeted Therapeutic Approaches for New and Relapsed Hairy Cell Leukemia
Goals: This proposed study has a potential to challenge and change the current standard treatment practice of frontline HCL and offer chemotherapy-free, rationally targeted therapeutic options in HCL.
Specific Aims: Aim 1: Determine the efficacy of combined RAF inhibition and anti-CD20 immunotherapy as frontline therapy for classical hairy cell leukemia (cHCL). Aim 2: Determine the safety and efficacy of ERK inhibition in relapsed/refractory (R/R) cHCL and hairy cell leukemia variant (HCLv).
Agency Contract: Director of Research Administration, 3 International Drive, Suite 200, Rye Brook, New York 10573, researchprograms@lls.org
Overlap: None

GC238545 (PI: Abdel-Wahab) 1/15/2017 - 1/14/2021 0.12 calendar NCE
Henry & Marilyn Taub Foundation $ 181,818
Understanding and manipulating pathologic splicing preferences and nonsense-mediated mRNA decay (NMD) in spliceosomal-mutant MDS
Goals: Here we aim to develop two novel mechanism-based approaches targeting spliceosomal-mutant MDS based on: (1) selectively targeting the pathologic RNA-binding preferences of mutant spliceosomal proteins; and (2) restoring the expression of key transcripts targeted for nonsense-mediated decay (NMD) due to aberrant splicing. Understanding the mechanistic link between NMD and spliceosomal gene mutations will be critical for this latter aim.
Specific Aims: Aim I. Determine the precise mechanism by which mutant SRSF2 promotes NMD. Aim II. Utilize antisense techniques to restore expression of mRNAs pathologically degraded by NMD in spliceosomal-mutant MDS cells. Aim III. Determine the therapeutic potential of oligonucleotide decoys that exploit the RNAbinding preferences of WT versus mutant SRSF2 in SRSF2-mutant malignant cells.
Agency Contract: Erin Johnstone, Program Officer, The Medical Foundation, ejohnstone@hria.org
Overlap: None

5 R01 CA201247-04 (PI: Abdel-Wahab / Park) 9/1/16 - 8/31/2021 1.81 calendar months
NCI $ 289,536
Origins of BRAF-mutant hematologic malignancies and their therapeutic resistance
Goals: We expect our studies to elucidate mechanisms that underlying the development of these disparate blood cancers driven by a shared mutation, provide the first description of the genetic events that likely cooperate with the BRAFV600E mutation to drive these disorders and therapy resistance.
Specific Aims: Aim 1. Determine the mechanistic basis for the divergent phenotypes of BRAFV600E mutant hematopoietic disorders. Aim 2. Determine whether recurrent mutations co-occurring with the BRAFV600E mutation HCL and SH alter the disease phenotype. Aim 3: Define mechanisms of vemurafenib resistance in HCL using patient samples and murine models of BRAFV600E-mutant hematopoietic malignancies.
Agency Contract: Funmi Elesinmogun, Grants Management Specialist, elesinmf@mail.nih.gov, 240-276-6313
Overlap: None

2314-17 (PI: Abdel Wahab) 7/1/2016 - 6/30/2021 0.0 calendar months*
Leukemia and Lymphoma Society $ 104,762
Investigating and Targeting Diverse Kinase Alterations Driving Systemic Histiocytic Neoplasms
Goals: The discovery of BRAFV600E mutations in 50% of patients with systemic histiocytoses provided the first molecular target in these disorders. More recently, MAP2K1, ARAF, and N/KRAS mutations as well as kinase fusions have been identified in BRAFV600 wildtype patients. Here we propose to understand the clinical and biological importance of diverse kinase alterations in BRAFV600E wildtype histiocytic neoplasms.
Specific Aims: Aim 1: Determine the clinical activity of cobimetinib in adult systemic histiocytic disorder patients as measured by radiologic (RECIST) response criteria, metabolic (FDG-PET) response criteria, and longitudinal assessment of somatic mutation burden in cell-free DNA (cfDNA). Aim 2: Identify the biological importance of mutations outside of BRAFV600E on histiocytosis pathogenesis and the molecular determinants of response to MEK inhibition.
Agency Contract: Director of Research Administration, 3 International Drive, Suite 200, Rye Brook, New York 10573, researchprograms@lls.org
Overlap: None
**This grant provides salary support only. Effort is not required per the sponsor.*

COMPLETED SINCE PREVIOUS REPORTING PERIOD

7018-19 (PI: Cleveland) 10/1/2018 - 9/30/2023 0.60 calendar
Leukemia and Lymphoma Society $ 178,333
Regulation and Targeting of Inflammatory Circuits in Myelodysplastic Syndromes
Goals & Specific Aims: Aim 1: Determine the mechanistic basis for hyper-activated innate immune signaling in spliceosome-mutant MDS bearing mutations in RNA splicing factors. Aim 2: Define the therapeutic

potential for MAP3K7, p38/MAPK, and TGF-□ pathway inhibition in spliceosome mutant MDS. Aim 3: Define the mechanistic basis for preferential dependency on IFN-JAK-STAT signaling in SF3B1 mutant cells.
Agency Contract: Director of Research Administration, 3 International Drive, Suite 200, Rye Brook, New York 10573, researchprograms@lls.org
Overlap: None

I10-0074 (PI: Abdel-Wahab) 1/1/2017 - 12/31/2019 NCE
Starr Cancer Consortium $ 197,500
Dissecting micro-environmental contributions to the pathogenesis of myelodysplastic syndromes
Goals: Our proposal aims at highlighting the molecular alterations in the HMEV of MDS patients that could serve as novel therapeutic targets.
Specific Aims: Specific Aim 1: Identify the functional impact of molecular alterations in the microenvironment of MDS patients on hematopoiesis. We will investigate the mechanisms through which molecular alterations identified in our gene expression analysis of mesenchymal subsets in MDS patients contribute to MDS pathogenesis.
Agency Contract: Sylvie Le Blancq, PhD, Executive Director, Sloan Kettering Institute, 1275 York Avenue, New York, NY 10065, lesblanc2@mskcc.org, 646-888-3773
Overlap: None

W81-XWH-18-1-0265 (PI: Liu) 7/1/2018 - 6/30/2020 0.56 calendar
Congressionally Directed Medical Research Programs $ 12,136
Understanding and Targeting Mutant p53 in Myelodysplastic Syndromes
Goals and Specific Aims: Task 1. We will assist in the generation of patient-derived xenografts from patients with myelodysplastic syndromes (MDS). Task 2. We will generate p53R248W/+SRSF2P95H/+ -Mx1-Cre+ mice.
Agency Contract: Grant Specialist: Karen L. Petrore Phone: 301-619-3590 Email: Karen.l.petrore.civ@mail.mil
Overlap: None

1R01HL128239-05 (PI: Bradley) 8/1/15 - 5/31/2020 2.26 calendar
NIH $ 181,189
Genetic and molecular basis for SRSF2 mutations in myelodysplasia
Goals: Measure the therapeutic efficacy of drugs that inhibit RNA splicing, and test whether drug-induced splicing inhibition specifically affects SRSF2 mutation-responsive exons versus causing widespread splicing failure.
Specific Aims: Perform functional studies to determine the effect of specific mis-spliced isoforms on myeloid differentiation. Test the hypothesis that a wildtype copy of SRSF2 s necessary for hematopoietic cell survival and maintenance of functional splicing. Determine whether SRSF2 mutations do not co-occur with other spliceosomal mutations because they are functionally redundant, or whether multiple spliceosomal mutations are deleterious to the cell. Test the hypothesis that commonly co-occurring SRSF2 and epigenetic factor mutations are synergistic, leading to more aggressive disease and/or more extreme splicing changes. Measure the therapeutic efficacy of drugs that inhibit RNA splicing, and test whether drug-induced splicing inhibition specifically affects SRSF2 mutation-responsive exons versus causing widespread splicing failure. Generate cells to be utilized in CRISPR/Cas9 screen and perform validation of sgRNAs from the screen.
Agency Contract: Kelly Stewart, Fred Hutchinson Cancer Res. Center, kstewart@fredhutch.org, 206-667-6925
Overlap: None

Notes
If necessary because of pending grants being funded, Dr. Abdel-Wahab will reduce his effort on his active awards to not exceed 12.00 calendar months.

CHANDARLAPATY, SARAT

NEW ACTIVE SUPPORT

1 R01 CA245069 01 (PI: Taylor)
NCI
Role: Co-Investigator
Dates: 3/1/2020 - 2/28/2025
Project Title: Defining the impact of mutant oncogene zygosity
Level of Funding: $ 228,750
Time Commitment: 0.36 calendar
Goals: Mutant oncogenes are central to the initiation, progression, and response to therapy of many human cancers. Yet, the significance of mutant oncogene zygosity whereby changes in the dosage and stoichiometry of oncogenic driver mutations leads to selective growth advantages and therapeutic vulnerabilities remains largely unknown. To overcome this challenge, we propose an innovative and multidisciplinary translational genomic approach to establish the prevalence, evolutionary origin, biological, and therapeutic impact of changes in mutant oncogene zygosity to optimize the treatment of advanced cancer patients.
Contracting Officer: Ian M. Fingerman, fingerma@mail.nih.gov
Overlap: None

1 R01 CA244812-01 (PI: Chandarlapaty)
NCI
Role: PI
Dates: 2/15/2020 - 1/31/2025
Project Title: Implications and Vulnerabilities of APOBEC Mutagenesis in Breast Cancer
Level of Funding: $ 489,699
Time Commitment: 0.90 calendar
Goals: Metastatic breast cancer is one of the leading causes of death among women in the United States with mortality linked to the disease becoming resistant to hormonal therapies. Analyses of breast cancers that are metastatic reveal the presence of pathogenic DNA mutations originating from the mutagenesis process induced by the activity of the APOBEC family of antiviral enzymes.
Contracting Officer: Sudhir B. Kondapaka, kondapas@mail.nih.gov
Overlap: None

GC240658 (PI: Wang)
National Science Foundation
Role: Principal Investigator
Dates: 1/2/2020 - 1/1/2021
Project Title: Assessing an innovative Microfluidic Tissue Array device for applications in drug development and precision medicine
Level of Funding: $ 27,840
Time Commitment: 0.36 calendar
Goals: For this subcontract with VivoZ Labs, Inc, the Chandarlapaty Lab will provide PDX cancer samples, and supervise the experiments related to the processing and drug treatment of PDX cancer samples and the Jiang Lab will oversee and supervise the pathological and cell death analysis of cancer tissues.
Contracting Officer: Not Available
Overlap: None

1 P50 CA247749 01 (PI: Powell)
NCI
Role: Co-Investigator
Dates: 8/13/2020 - 7/31/2025
Project Title: MSK SPORE in Genomic Instability in Breast Cancer
Level of Funding: $341,131

Time Commitment: 0.60 calendar
Goals: Project 03: Diagnosis and Treatment of APOBEC Mutagenesis in Metastatic Breast Cancer
In this highly collaborative project, we leverage our team's collective expertise in genomic characterization of cancer, advanced modeling and treatment of endocrine resistance, and APOBEC biochemistry and genetics to meet these challenges and develop a rational strategy for targeting APOBEC mutagenesis in the clinic.
Contracting Officer: Joyann Courtney, joyann.rohan@nih.gov
Overlap: None

CONTINUING ACTIVE SUPPORT

The Breast Cancer Research Foundation, BCRF-17-185
Role: PI
Dates: 10/1/2015-9/30/2020
Project Title: Understanding and Overcoming Resistance to Anti-Cancer Therapy
Level of Funding: $208,333
Time Commitment: 1.2 calendar months
Goal: The goal of this proposal is to determine molecular mechanisms of resistance to various therapies in breast cancer including PI3K and HER2 targeted therapies so as to eliminate these impediments to cure in the future.
Aims: To study (1) acquired resistance to CDK4 inhibitors in ER+ breast cancer and (2) mechanisms of resistance to neratinib in HER2-mutant breast cancer
Contracting Officer: Margaret Flowers, Associate Director, BCRF, mflowers@bcrfcure.org, 646-497-2611
Overlap: None

NIH/NCI R01 Research Grant, 5 R01 CA204999-03
Role: PI
Dates: 1/1/2017-12/31/2021
Project Title: Therapeutic approaches to ER mutant breast cancer
Level of Funding: $221,887
Time Commitment: 2.04 calendar months
Goal: The major goals of this proposal are to characterize the biochemical and biologic implications of *ESR1*mutations to identify unique vulnerabilities of ER mutant cancers, establish therapeutic strategies to potently antagonize different ER mutants using next generation selective estrogen receptor degraders (SERDs), and ascertain the mechanisms of resistance that are likely to emerge in ER mutant cancers treated with SERDs incurrent use.
Aims: (1) Determine how different somatic *ESR1* mutations impact receptor conformation and activity to effect breast cancer growth. (2) Identify pharmacologic approaches to potently antagonize mutant ER activity. (3) Elucidate mechanisms of acquired resistance to next generation ER antagonists.
Contracting Officer: Rogers Gross, Grants Management Specialist, NCI, rogers.gross@nih.gov, 240-276-5360
Overlap: None

NIH/NCI R01 Research Grant, 1 R01 CA234361-01 (PIs: Solit/Chandarlapaty)
NCI
Dates: 12/5/2018 - 11/30/2023
Defining mechanisms of resistance to hormonal therapy in breast cancer
Level of Funding: $75,000 to Chandarlapaty Lab
Time Commitment: 0.91 calendar
Goals: Hormonal therapy remains a mainstay of the systemic treatment of breast cancer patients but responses vary and intrinsic and acquired resistance are common. This project seeks to define the landscape and timing of molecular changes that arise under the selective pressure of hormonal therapy in breast cancer patients.
Aims: Aim 1. Define mechanisms of intrinsic and acquired resistance to hormonal therapy and the timing

with which they arise in patients with ER+ breast cancer. Aim 2. Define mechanisms of intrinsic and acquired resistance to anti-HER2 therapy in patients with
ERBB2-mutant, ER+ breast cancer. Aim 3. Develop combinatorial therapies for patients with ER+ breast cancer with mutations in *NF1*.
Contracting Officer: Barbara Hodgkins, Grants Management Specialist, barb.hodgkins@nih.gov, (240 276-6294
Overlap: None

BC171737P1, W81XWH-18-1-0383 (Partnering PI: Chandarlapaty)
Congressionally Directed Medical Research Programs
Role: PI
Dates: 9/30/2018 - 9/29/2021
Project Title: Mechanistic and therapeutic implications of spliceosomal gene mutations in ER+ breast cancer
Level of Funding: $100,000
Time Commitment: 1.20 calendar
Goal: Determine if breast cancer cells with SF3B1 mutations and/or MYC overexpression are preferentially sensitive to H3B- 8800 or E7070 and E7820 as well as the mechanistic basis for their sensitivity.
Aims: Aim 1. Determine the mechanism by which the *SF3B1* K700E mutation promotes hormone-independent growth of breast cancer. Aim 2. Examine the effect of *Sf3b1*K700E mutation on mammary tumorigenesis *in vivo.* Aim 3. Determine the therapeutic benefit of modulating RNA splicing in breast cancers with and without spliceosomal gene mutations.
Contracting Officer: Jamie Shortall, Grants Officer, jamie.a.shortall.civ@mail.mil, (301) 619-2393
Overlap: None

BC180833P1, W81XWH-19-1-0078 (Partnering PI: Chandarlapaty)
Congressionally Directed Medical Research Programs
Dates: 2/1/2019 - 1/31/2022
Project Title: A Novel Class of Antagonists for Robust Inhibition of Mutant Estrogen Receptor Action in Endocrine-Resistant Metastatic Breast Cancer
Level of Funding: $ 90,000
Time Commitment: 1.20 calendar
Goals: Design and synthesize a library of ER ligands that possess high binding affinity to ER, and functionalize them with suitable linkers and reactive groups for coupling to degrons.
Specific Aims: Specific Aim 1 Design and Synthesize a Library of PTAs based
on Our Novel Pharmacophore Model Specific Aim 2 Evaluate the Potency and Efficacy of PTAs for Robust Inhibition of WT and Mutant ER Signaling in Cell-based Assays Specific Aim 3 Evaluate the In Vivo Anti-tumor Efficacy of our PTAs in Orthotopic/ PDX xenografts with WT and Mutant ER
Contracting Officer: Jodi Cardoza, Contract/Grants Specialist, Jodi.l.cardoza.civ@mail.mil, (301) 619-2693.
Overlap: None

BC171535P1, W81XWH-18-1-0562 (Partnering PI: Scaltriti)
Congressionally Directed Medical Research Programs
Role: Co-Investigator
Dates: 9/1/2018 - 8/31/2021
Project Title: NOXA loss as a major mechanism of intrinsic resistance to targeted therapies in breast cancer
Level of Funding: $ 166,666
Time Commitment: 0.60 calendar
Goal: Characterize the miRNA4728/ER/NOXA axis in HER2-amplified breast cancers and its role in intrinsic resistance to HER2i.Characterize the role of the ER-NOXA axis in responses to anti-estrogens in ER+ positive breast cancers in vitro and in clinical specimens.
Aims: Aim 1: Characterize the miRNA4728/ER/NOXA axis in *HER2*-amplified breast cancers and its role in intrinsic resistance to HER2i. Aim 2: Characterize the role of the ER-NOXA axis in responses to anti-estrogens in ER+ positive breast cancers in vitro and in clinical specimens. Aim 3: Assess the efficacy of dual HER2 and

MCL-1 inhibition in diverse *HER2* amplified breast PDX models and dual ER and MCL-1 inhibition in diverse ER+ breast PDX models.
Contracting Officer: Jamie Shortall, Grants Officer, jamie.a.shortall.civ@mail.mil, (301) 619-2393
Overlap: None

1 R01 CA235711-01 (PI: Iyengar)
NCI
Role: Co-Investigator
Dates: 4/1/2019 - 3/31/2023
Project Title: Phase 1/2 Trial of Exercise Treatment in Hormone Receptor-Positive Metastatic Breast Cancer
Level of Funding: $ 485,003
Time Commitment: 0.12 calendar
Goals: We will identify the precise dose of exercise that is both feasible and has anti-tumor effects for patients with MBC using rigorous standards adapted from oncology drug development trials in order to improve outcomes for patients with MBC.
Specific Aims: AIM 1: Part 1 – Dose Finding (Phase 1): To identify the recommended phase 2 dose (RP2D) of exercise. AIM 2: Part 2 – (Phase 2): To determine biological and clinical antitumor activity of the exercise RP2D.
Contracting Officer: Rogers Gross, Grants Management Specialist, NCI, rogers.gross@nih.gov, 240-276-5360
Overlap: None

GC235518 (PI: Traina)
The Breast Cancer Research Foundation
Role: Co-Investigator
Dates: 6/30/2019 - 6/29/2023
Project Title: Establishing AR and CDK4/6 as Therapeutic Targets in Triple Negative Breast Cancer
Level of Funding: $ 515,051
Time Commitment: 1.20 calendar
Goals: In this proposal, we will utilize tumor samples from our ongoing anti androgen studies to create patient derived models of AR+ TNBC and to develop a biomarker which will help identify patients most likely to benefit from enzalutamide. We will conduct a randomized phase II study comparing the activity of enzalutamide to standard of care chemotherapy, allowing us to also validate the predictive biomarker in secondary analyses.
Contracting Officer: Margaret Flowers, Associate Director, BCRF, mflowers@bcrfcure.org, 646-497-2611
Overlap: None

COMPLETED SINCE PREVIOUS REPORTING PERIOD

NIH/NCI P01 Program Project, 5 P01 CA094060-15
Role: Investigator (PI: Neal Rosen)
Dates: 9/16/2014-6/30/2020 (NCE)
Project Title: Development of mechanism-based strategies for the treatment of advanced breast cancer with PI3K pathway inhibitors.
Level of Funding: $311,955
Time Commitment: 1.2 calendar months
Goal: See aims
Aims: (1) Characterize feedback regulation of the PI3K pathway in breast cancer. (2) Determine the biologic and therapeutic consequences of adaptations to PI3K pathway inhibitors. (3) Development of therapeutic strategies based on combined inhibition of components of the PI3K pathway and of feedback reactivated proteins.
Contracting Officer: Sarah Scharf, Grants Specialist, sarah.scharf@nih.gov, 240-276-5472
Overlap: None