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

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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 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 found that expression of the most common mutant, K700E, led to alterations in RNA splicing and promoted hormone-independent proliferation of ER+ cells. Based on these initial observations, we are now studying the consequences of SF3B1 mutation on breast tumorigenesis, cancer progression, and sensitivity to spliceosomal targeted therapy.					
15. SUBJECT TERMS Antisense oligonucleotides, breast cancer, estrogen receptor, E7107, E7820, PIK3CA, RBM39, RNA splicing, SF3B1.					
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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 hormone-independent growth of breast cancer, (2) examine the effect of SF3B1 K700E mutation on 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. 80% 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. 33% 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. 25% 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 attempted to knock the *SF3B1* K700E allele into MCF7 by CRISPR. After transfecting Cas9, SF3B1 sgRNA and K700E repair template ssDNA into cells, we were able to detect knock in of K700E in a pool of cells (Fig 2A). However, we could not isolate single cell clones carrying the *SF3B1* K700E allele from this pool. There was preliminary evidence for selection against the allele in this context. We obtained MCF10A breast cells with heterozygous knock in of the *SF3B1* K700E from a collaborator (Fig 2B).

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).

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 37°C 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 *Sf3b1*^{K700E} *Pik3ca* double mutant mice, the *Sf3b1* ^{K700E} single mutant mice showed reduced mammary ductal branching (Fig 3B). Hastened death was observed in MMTV-Cre *Sf3b1*^{K700E/WT} *R26-Pik3ca*^{H1047R} double mutant mice (Fig 3C), where ER+/PR+, high Ki67 mammary gland adenocarcinoma developed (Fig 3D).

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 SF3B1^{K700E} 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.

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 AACR Annual Meeting in Atlanta.

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 validate key findings from RNA-seq analyses of MCF10A, MCF7, and T47D cells at the mRNA and protein level. We will also 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. We will also perform and analyze RNA-seq analyses of purified luminal (DAPI⁻ CD24^{low}) and basal (DAPI⁻ CD24^{high}) epithelial cells from the mouse models we have already generated to date.

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.

- 4. IMPACT:** *Describe 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 RNA splicing factors have enhanced sensitivity to drugs that further alter the RNA splicing process. This has led to a clinical trial of a novel RNA splicing inhibitor in patients with myeloid leukemia (clinicaltrials.gov identifier NCT02841540). More recently, 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.

Nothing to Report

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.

None.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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).*

None.

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, and T47D 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”.

<i>Name:</i>	<i>Omar Abdel-Wahab</i>
<i>Project Role:</i>	<i>PI</i>
<i>Researcher Identifier (e.g. ORCID ID):</i>	<i>None</i>
<i>Nearest person month worked:</i>	<i>.91</i>
<i>Contribution to Project:</i>	<i>Overseeing all experiments in conjunction with Dr. Chandarlapaty.</i>
<i>Funding Support:</i>	<i>US National Institutes of Health (NIH)-NHLBI grant R01 HL128239 & NIH-NCI grant R01 CA201247-03</i>

<i>Name:</i>	<i>Sarat Chandarlapaty</i>
<i>Project Role:</i>	<i>co-PI</i>
<i>Researcher Identifier (e.g. ORCID ID):</i>	<i>None</i>
<i>Nearest person month worked:</i>	<i>1.2</i>
<i>Contribution to Project:</i>	<i>Overseeing all experiments in conjunction with Dr. Abdel-Wahab.</i>
<i>Funding Support:</i>	<i>Breast Cancer Research Foundation (BCRF)</i>

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

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)

- *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.*

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).

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.

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

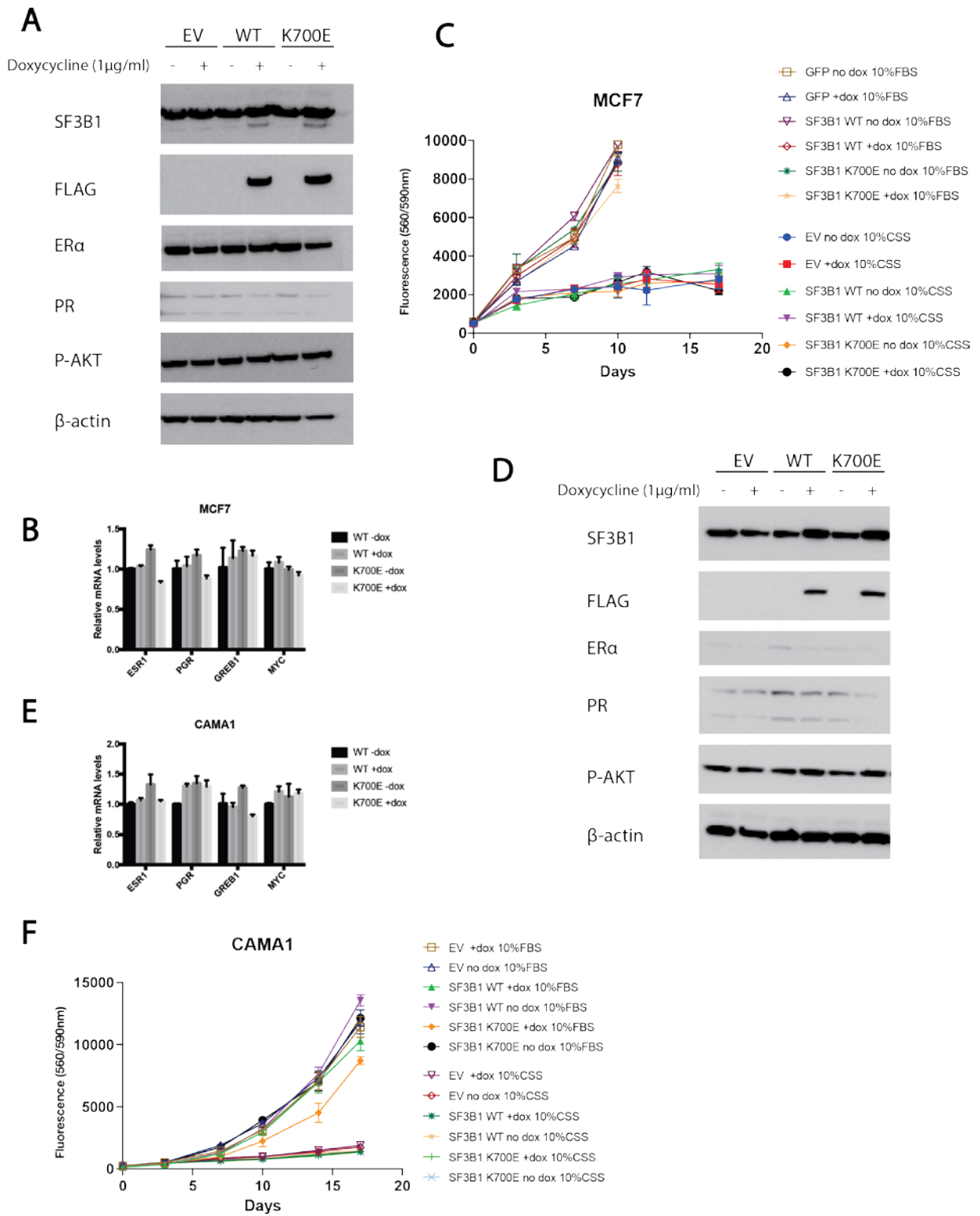


Figure 1.

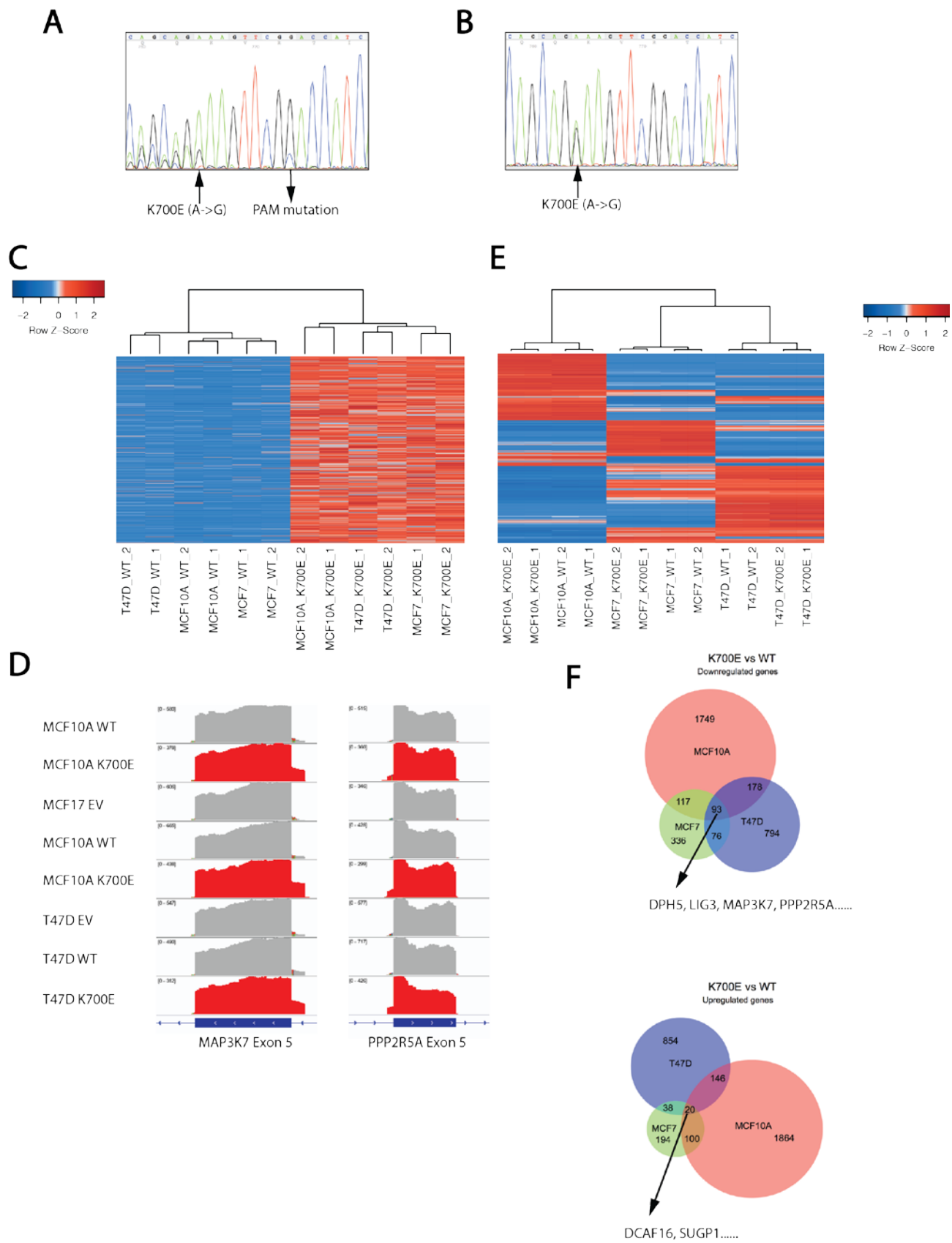


Figure 2

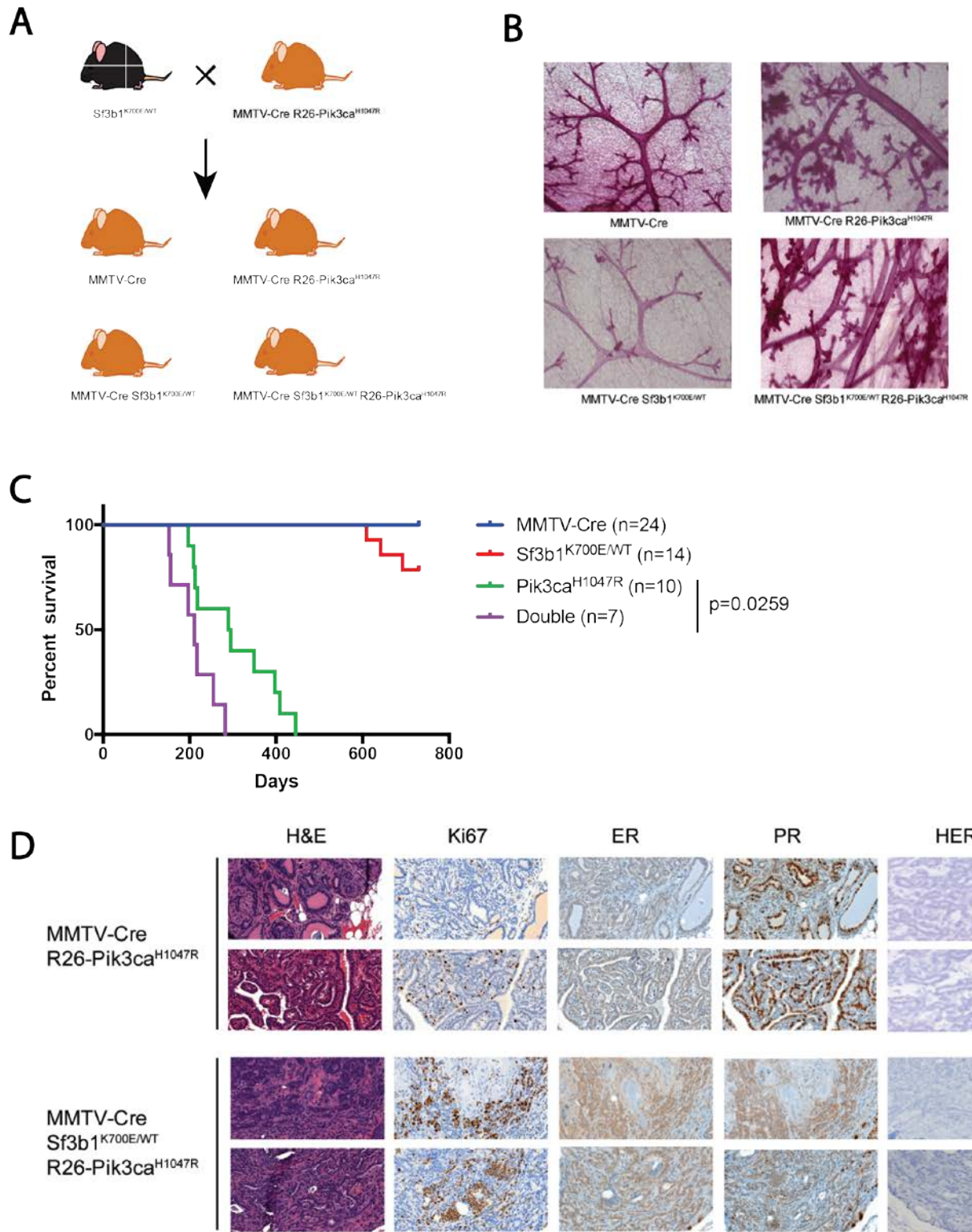
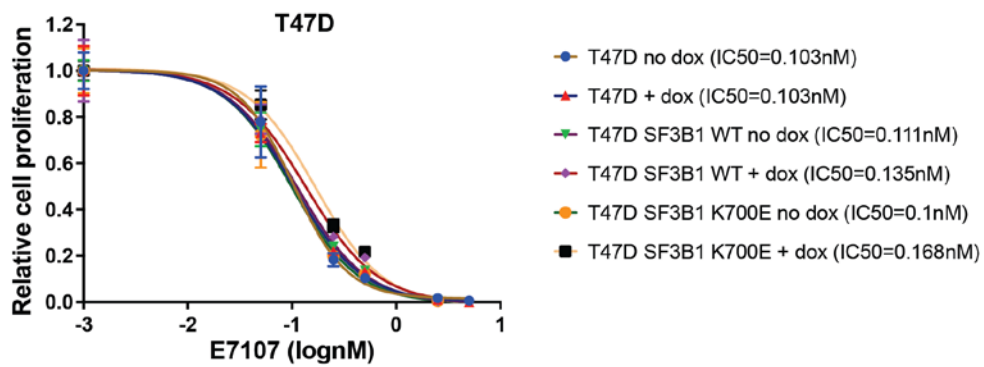
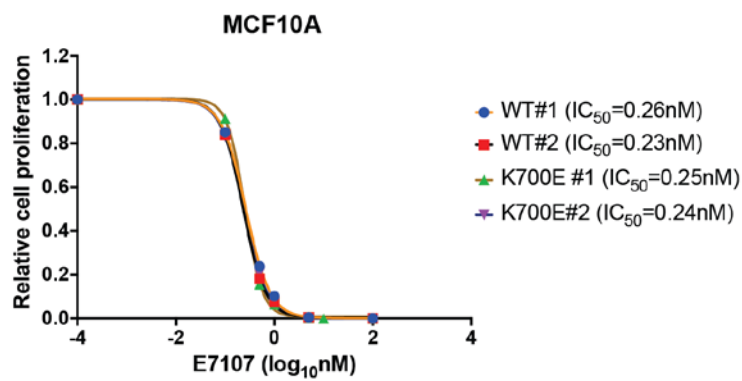
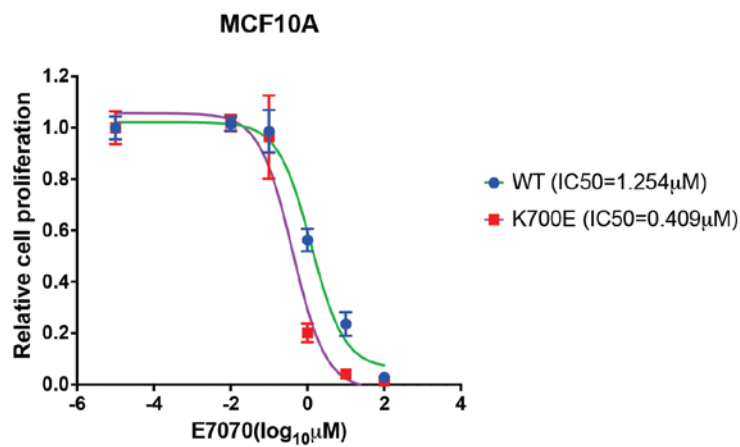


Figure 3

A**B****C****Figure 4**

OMAR I. ABDEL-WAHAB, M.D.

NEW ACTIVE SUPPORT

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 hormone-independent 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 CA233332-01 (PI: Abdel-Wahab) 3/7/2019 - 2/28/2025 0.46 calendar
NCI \$ 737,015

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 CA233290-01 (PI: Aghajanian / Lee / Morris) 3/6/2019 - 2/28/2025 0.60 calendar

/ Tallman / Zivanovic)

\$ 708,817

NCI

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

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- β 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

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

1R01HL145283-01 (PI: Landau)

2/1/2019 - 11/30/2022

0.20 calendar

NHLBI

\$ 87,279

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

GC238654 (PI: Shlush)

11/1/2018 - 10/31/2023

1.60 calendar

Leukemia and Lymphoma Society/ Rising Tide Foundation \$ 363,636

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

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

CONTINUING ACTIVE SUPPORT

W81-XWH-18-1-0265 (PI: Liu) 7/1/2018 - 6/30/2020 0.60 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

5 R01 HL138090-02 (PI: Geissmann) 8/16/2017 - 6/30/2021 0.60 calendar
NHLBI \$ 415,910

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 fraction 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 0.36 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 - 9/30/2020 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

I10-0074 (PI: Abdel-Wahab) 1/1/2017 - 12/31/2019 NCE 0.10 calendar
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

GC238545 (PI: Abdel-Wahab) 1/15/2017 - 1/14/2020 0.60 calendar
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 RNA-binding 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

1R01HL128239-04 (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 is 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

5 R01 CA201247-03 (PI: Abdel-Wahab / Park)

9/1/16 - 8/31/2021

1.81 calendar months

NCI

\$ 298,491

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 BRAFV600E 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 LAST REPORTING PERIOD:

GC235782 (PI: Abdel-Wahab) 9/1/2016 - 8/31/2019
Edward P. Evans Foundation \$ 181,818
Elucidating Critical Targets, Transcripts, and Collaborating Events in Spliceosomal-Mutant MDS
Goals & Specific Aims: 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

190454 (PI: Varmus) 9/1/2017 - 8/31/2019
Edward P. Evans Foundation \$ 159,207
The Roles of Mutant U2AF1 in the Initiation and Maintenance of MDS
Goals: Our work will improve our basic understanding of key genetic alterations in MDS & provide crucial insights into genetic targets for therapeutic intervention in MDS. Importantly, the majority of our proposed experiments will be conducted on primary human hematopoietic tissues from MDS patients or healthy donors, combining the pre--clinical and clinical expertise of the Varmus and Abdel--Wahab labs. Therefore, the knowledge gained will be directly relevant to MDS patients carrying U2AF1 mutations.
Specific Aims: Specific Aim I: Determine if mutant U2AF1 is required for MDS disease maintenance. Specific Aim II: Determine the molecular targets of U2AF1(S34F) responsible for MDS development.
Agency Contract: Michael Lewis, Ph.D. President,mdl@epefoundation.org 978 494-6009
Overlap: None

GC231058 (PI: Abdel-Wahab) 1/1/2018 - 12/31/2018
Histiocytosis Association \$ 50,000
Functional Evaluation of Immunologic Checkpoint Blockade in the Therapy of Histiocytosis
Goals: Thus, our proposal will provide greater insight into recurrent genomic alterations within the pediatric histiocytosis population as well as inform novel therapeutic approaches for children and adults with histiocytosis.
Specific Aims: The recent success of immune checkpoint blockade therapy in other cancer types combined with the expression of immune checkpoint antigens and abundant immune infiltrates in histiocytosis lesions suggests that such therapies may be effective for histiocytosis patients. We therefore seek here to (1) test the efficacy of checkpoint inhibitors (anti-CTLA4, anti-PD-1/PD-L1) alone or in combination with BRAF inhibition in in vivo models of LCH and (2) determine molecular correlates of PD-L1 expression.
Agency Contract: Grants Office, grants@histio.org, 856-589-6614
Overlap: None

1R56AG052501-01A1 (PI: Liu) 9/15/2017 - 8/31/2018
NIA \$ 7,007
Mutant p53 rejuvenates aged stem cells through modulating epigenetic regulators
Goals & Specific Aims: Milestone Task 1: Determine if MDS cells expressing mutant TP53 will accelerate the onset of MDS in NSG-SGM3 mice. MDS cells are difficult to engraft immunodeficient mice. Milestone Task 2: Determine whether mutant SRSF2 collaborates with gain-of-function mutations in TP53 in vivo.
Agency Contract: Grant Management Specialist: Eva Lawson-lipchin Email: lawsonlipchine@nia.nih.gov
Phone: 301-496-9350
Overlap: None

GA-2017-B0149 (PI: Park) 1/1/2018 - 12/31/2018
Hairy Cell Leukemia Foundation \$ 25,000
Investigating the Basis of Hairy Cell Leukemia and Its Response to Therapy Through Single-Cell Approaches

Goals & Specific Aims: The Abdel-Wahab lab will perform flow cytometric sorting of primary patient samples from HCL patients for single cell RNA sequencing studies. In addition, they will perform bulk cell RNA-sequencing in addition to mutational analysis by whole exome sequencing and/or MSKCC IMPACT targeted mutational analysis.

Agency Contract: Grants Management Specialist: Kwesi Wright Email: wrightnk@mail.nih.gov Phone: (301) 451-4789

Overlap: None

W81XWH-16-1-0059 (PI: Abdel Wahab) 5/1/2016 - 4/30/2019 NCE

Congressionally Directed Medical Research Programs \$ 180,000

Goals: Therapeutic targeting of spliceosomal mutant acquired bone marrow failure disorders

The purpose of the current DOD application is to test inhibitors of TGF-beta signaling pathway in preclinical mouse models as possible therapy for FA.

Specific Aims:

Agency Contract:

Overlap: None

GC231183(PI: Abdel-Wahab) 7/1/16 - 6/30/19

Pershing Square Sohn Cancer Research Alliance \$ 181,818

Identification of novel transcripts, pathways, and therapeutic strategies to target spliceosomal-mutant malignancies

Goals:

Specific Aims: Aim I. Determine the functionally relevant splice isoforms linking spliceosomal gene mutations to cancer development. Aim II: Explore novel therapeutic approaches for spliceosomal-mutant cancers through targeting altered RNA binding preferences and new spliceosomal inhibitors. Aim III. Investigate the intersection of spliceosomal mutations and epigenetic alterations in myeloid malignancies.

Overlap: None

Agency Contract:

*Effort commitment is not required per the sponsor.

1 R13 TR001695-01 (PI: Diamond) 8/18/16 - 8/17/17

NCATS \$ 10,000

Fourth International Medical Symposium on Erdheim-Chester Disease

Goals: Because of the rarity of the Erdheim-Chester disease (ECD), clinical experience is limited worldwide and international collaboration has proven vital to advancement in research and clinical care for these patients.

The proposed conference will be an international symposium to gather ECD specialists from the US and Europe to discuss clinical and research efforts, and specifically to derive a consensus approach to response criteria for implementation in ECD clinical trials.

Specific Aims: Aim 1. To bring together clinicians and investigators from an international setting who treat and study patients with Erdheim-Chester disease (ECD). Aim 2. To assess the current and updated collective experience regarding genomic alterations and targeted therapies for ECD. Aim 3. To define, by consensus approach of ECD experts, response criteria for therapeutic clinical trials for ECD.

Agency Contact: Leslie Le, Grants Management Specialist, (e) leleslie@mail.nih.gov, (P) 301-435-0856

Overlap: None

GC228236 (PI: J. Park) 7/1/14-6/30/17

Conquer Cancer Foundation \$62,466

A Phase II Study of the BRAF Inhibitor, Vemurafenib, in Patients with Relapsed or Refractory Hairy Cell Leukemia (HCL): Evaluating Clinical Efficacy and Molecular Mechanisms of BRAF Inhibition in HCL

Specific Aims: Aim 1: To determine the clinical efficacy of the BRAF inhibitor vemurafenib in patients with BRAFV600E+ relapsed or refractory HCL, as assessed by overall response rates, duration of response, kinetics

of minimal residual disease (MRD), disease-free and overall survival. Aim 2: To assess the effect of the BRAF inhibitor vemura.

Agency Contact: grants@conquercancerfoundation.org, 571-483-1700

Overlap: None

GC226666 (PI: Abdel-Wahab) 1/1/16 - 12/31/16
Histiocytosis Association \$ 50,000

MEK Inhibition in the Therapy of Histiocytic Neoplasms

Goals: We have recently completed a clinical trial of vemurafenib for the adult BRAFV600E-mutant patients and we now seek to identify novel therapies that will benefit those patients that are BRAFV600-wildtype. This grant would provide critical funding to (1) allow us to understand clinical responses in this clinical trial in greater depth and (2) provide critical support which will allow us to get additional data which will serve as the basis of a multi-PI R01 application with other already identified investigators on the biology and therapy of MAP2K1-mutant malignancies.

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: Functionally characterize the novel kinase mutations and fusions identified in histiocytic disorders without the BRAFV600E mutation and evaluate the in vitro efficacy of MEK inhibition in the activating mutants. Results of these studies will be compared to clinical outcome data generated by the cobimetinib trial.

Agency contact: Grants Office, grants@histio.org, 856-589-6614

Overlap: None

GC223032 (PI: Abdel-Wahab) 10/1/14-9/30/16
The V Foundation \$200,000

Understanding and Targeting Spliceosomal Mutations in Leukemia

Goals & Specific Aims: Aim 1: Determine how SRSF2 mutations alter SRSF2's normal roles in pre-mRNA splicing Aim 2: Utilize Srsf2 P95H mutant murine models to further identify transcriptional events and splicing changes critical to hematopoietic alterations driven by SRSF2 mutations Aim 3: Identify and test therapeutic strategies for targeting leukemic clones with spliceosomal gene mutations.

Agency contact: Carole C. Wegner, Director of Grants, 106 Towerview Court, Cary, NC 27513, 919-380-95005

Overlap: None

GC221801 (PI: Abdel-Wahab) 10/1/13-9/30/14
Erdheim-Chester Disease Global Alliance \$49,261

Somatic Genetic Alterations in the Pathogenesis and Therapy of Histiocytic Disorders

Goals: Histiocytic disorders, including Langerhans cell histiocytosis (LCH) and Erdheim Chester disease (ECD), represent a wide range of rare diseases with heterogeneous clinical course and prognoses. The relative rarity and protean clinical nature of the histiocytic disorders has made it difficult to clearly delineate the pathophysiology of these conditions.

Agency Contact: Kathy Brewer, President, support@erdheim-chester.org.

Overlap: None

5 U10 CA021115-39 (PI: Comis) 2/1/14 - 1/31/15
NIH \$ 48,548

Investigating the importance of MAP kinase signaling and novel mutations activating NOTCH signaling in CLL.

Goals: We propose to assess the status of somatic genetic abnormalities in CLL patient samples from the Integroup trial E2997 as performed in our pilot experiments.

Specific Aims: 1. Targeted resequencing and exome sequencing to identify novel somatic mutations that impact outcome and therapeutic response in CLL. 2. RNA-seq analysis to identify prognostically relevant expression

changes in CLL. 3. Epigenetic studies to determine the impact of epigenomic alterations on CLL prognosis and therapeutic response.

Agency contact: Debra Strandberg, Strandberg.debra@jimmy.harvard.edu

Overlap: None

1 U10 CA180827-01 (PI: Paietta)

4/14/14 – 2/28/15

NIH

\$ 45,885

Genetic and Epigenetic Determinants of response to therapy and outcome in patients with chronic lymphocytic leukemia

Goals: Perform targeted sequencing on a panel of ~300 recurrently mutated genes in genomic DNA from diagnostic CLL patient samples from E2997 clinical trial. Identification of samples with mutations activating MAP kinase signaling and NOTCH signaling will allow us to perform further functional analysis of the therapeutic and biological relevance of these 2 pathways in CLL.

Agency contact: Mary LaRocca, 111 East 210th Street, Bronx, NY 10467, mlarocca@montefiore.org

Overlap: None

5 K08CA160647-05 (Abdel-Wahab)

9/20/11 – 8/31/16

NIH/NCI

\$157,300

Clinical Scientist Research Career Development Award “Role of ASXL1 mutations in myeloid malignancies.”

Goals and Specific Aims: Aim #1: Identify target genes of ASXL1 loss and alterations in chromatin state due to ASXL1 loss in normal and leukemic hematopoietic cells using in vitro assays. Aim #2: Determine the in vivo effects of ASXL1 loss on hematopoiesis by targeted disruption of ASXL1. Aim #3: Determine the role of ASXL1 mutations in the pathogenesis of MPN and AML through studies in patient samples and in in vivo models in cooperation with known MPN and AML disease alleles.

Agency Contact: Esther Young, youngel@mail.nih.gov, 240-276-6325

Overlap: None

00003930 (PI: Janku)

1/1/16 - 12/31/16

Erdheim-Chester Disease Global Alliance

\$ 24,631

Understanding and targeting novel molecular alterations in Erdheim-Chester disease without the BRAFV600E mutation

Goals: We plan to extend our effort in furthering the treatment and understanding of histiocytic disorders through preclinical and clinical correlative studies to identify and target novel kinase alterations identified in BRAFV600E-wildtype ECD patients. These studies will be critical for (i) establishing diagnostic approaches to identifying the diverse, clinically actionable genomic alterations in ECD and (ii) promoting novel therapeutic studies for ECD patients.

Specific Aims: 1) Perform genomic analysis of Erdheim Chester Disease (ECD) tissue biopsy samples. 2) Functionally characterize the novel kinase alterations recently identified in systemic histiocytic disorder. 3) Determine the sensitivity of kinase mutations present in BRAFV600E-wildtype ECD to targeted therapeutics in preclinical models.

Agency contact: Jaime Farias, Assistant Director, OSP, 1515 Holcombe Boulevard, Unit 1676, Houston, TX 77030 (P) 713-792-3220

Overlap: None

GC227311 (PI: Abdel Wahab)

1/1/16-12/31/16

Hairy Cell Leukemia Foundation

\$ 58,000

HCL Grant Proposal 2015

Goals: To identify the spectrum of recurrent genomic alterations co-existing with BRAFV600E mutations in HCL and delineating their clinical relevance and functional contribution to HCL development

Specific Aims: Aim I: Identify genetic alterations co-existing with the BRAFV600E mutation in HCL patients and their clinical importance. Aim II: Identify the functional importance of recurrent mutations coexisting with the BRAFV600E mutations in HCL pathogenesis.

Agency contact: Michael Margolin, MD. President, 575 Madison Avenue, 10th Floor, New York, NY 10022

Overlap: None

PREVIOUS/CURRENT/PENDING SUPPORT

CHANDARLAPATY, SARAT

NEW ACTIVE

1 R01 CA235711-01A1 (PI: Iyengar) 4/3/2019 - 3/31/2024 0.12 calendar
NCI \$ 386,619

Phase 1a/1b Trial of Exercise Treatment in Hormone Receptor-Positive Metastatic Breast Cancer

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: Barbara Hodgkins Email: barb.hodgkins@nih.gov Phone: (240) 276-6294

Overlap: None

W81XWH-19-1-0078 (PI: Chandarlapaty) 3/1/2019 - 2/28/2022 1.20 calendar
Congressionally Directed Medical Research Programs \$ 90,000

A Novel Class of Antagonists for Robust Inhibition of Mutant Estrogen Receptor Action in Endocrine-Resistant Metastatic Breast Cancer

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.

Aims: 1) Design and Synthesize a Library of PTAs based on Our Novel Pharmacophore Model. 2) Evaluate the Potency and Efficacy of PTAs for Robust Inhibition of WT and Mutant ER Signaling in Cell-based Assays. 3) Evaluate the In Vivo Anti-tumor Efficacy of our PTAs in Orthotopic/ PDX xenografts with WT and Mutant ER

Contracting Officer: Jamie Shortall 301-619-2393, Jamie.a.shortall.civ@mail.mil

Overlap: None

W81XWH-18-1-0384 (PI: Chandarlapaty) 8/15/2018 - 8/14/2021 1.20 calendar
Congressionally Directed Medical Research Programs \$ 100,000

Programs

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 hormone-independent 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 R01 CA234361-01 (PI: Chandarlapaty / Solit) 12/5/2018 - 11/30/2023 0.91 calendar
NCI \$ 114,375

Defining mechanisms of resistance to hormonal therapy in breast cancer

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 Email: barb.hodgkins@nih.gov Phone: (240) 276-6294

Overlap: None

W81XWH-18-1-0562 (PI: Scaltriti) 9/30/2018 - 9/29/2021 0.60 calendar

Congressionally Directed Medical Research Programs \$ 166,666

NOXA loss as a major mechanism of intrinsic resistance to targeted therapies in breast cancer

Goals: 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 301-619-2393, Jamie.a.shortall.civ@mail.mil

Overlap: None

GC235518 (PI: Traina) 6/30/2019 - 6/29/2023 1.20 calendar

Breast Cancer Research Foundation, The \$ 501,208

Establishing AR and CDK4/6 as Therapeutic Targets in Triple Negative Breast Cancer

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.

Aims: 1. Conduct a randomized phase II trial of enzalutamide (E) versus chemotherapy in patients with metastatic TNBC to define the benefit of AR inhibition 2. Elucidate the molecular basis of AR dependence with pre-clinical patient-derived TNBC models; and 3. Devise biomarkers of AR dependence and sensitivity to AR and CDK4/6 inhibition, and validate these biomarkers using specimens from the randomized phase II trial proposed in Aim 1.

Contracting Officer: Lisa Risi Irisi@bcrf.org

Overlap: None

CONTINUING ACTIVE

5 R01 CA204999-03 (PI: Chandarlapaty) 1/1/2017 - 12/31/2021 2.04 calendar

NCI \$ 221,887

Therapeutic approaches to ER mutant breast cancer

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 potentially 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 in current use.

Aims: (1) Determine how different somatic *ESR1* mutations impact receptor conformation and activity to effect breast cancer growth. (2) Identify pharmacologic approaches to potentially 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

BCRF-17-185 (PI: Chandarlapaty) 10/1/2015 - 9/30/2019 1.20 calendar
Breast Cancer Research Foundation, The \$ 208,333
Understanding and Overcoming Resistance to Anti-Cancer Therapy
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@bcrcure.org, 646-497-2611
Overlap: None

5 P01 CA094060-15 (PI: Norton/ Rosen) 9/1/2015 - 6/30/2020 NCE 1.20 calendar
NCI \$ 311,955
Program Project in Models of Breast Cancer (Project 3: Development of Mechanism-based Strategies for the Treatment of Advanced Breast Cancer)
This proposal uses animal and laboratory models to dissect an interlinking network of molecular signals and pathways to lead to better prognostic and predictive clinical tests and more effective, less toxic treatments that target such aberrations.
Aims: Aim 1: Characterize feedback regulation of the PI3K pathway in breast cancer. Aim 2: Determine the biologic and therapeutic consequences of adaptations to PI3K pathway inhibitors. Aim 3: Development of therapeutic strategies based on combined inhibition of components of the PI3K pathway and of feedback reactivated proteins
Contracting Officer: Barbara Hodgkins Email: barb.hodgkins@nih.gov Phone: (240) 276-6294
Overlap: None

COMPLETED SUPPORT SINCE LAST REPORTING PERIOD

NIH/NCI P01 Program Project, 5 P01 CA094060-14
Role: Investigator (PI: Neal Rosen)
Dates: 9/16/2014-6/30/2019
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