**AWARD NUMBER: W81XWH-16-1-0158**

**TITLE: Defining Hepatocellular Carcinoma Subtypes and Treatment Responses in Patient-Derived Tumorgrafts**

**PRINCIPAL INVESTIGATOR: Akbar Waljee, MD, MSc**

**CONTRACTING ORGANIZATION: Veterans Education and Research Association of Michigan, Ann Arbor, MI 48105**

**REPORT DATE: October 2018**

**TYPE OF REPORT: Annual**

**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 |
|---|---|---|
| October 2018 | Annual | 30 Sept 2017-29 Sept 2018 |

**4. TITLE AND SUBTITLE**

Defining Hepatocellular Carcinoma Subtypes and Treatment Responses in Patient-Derived Tumorgrafts

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-16-1-0158

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Hao Zhu, Amit Singal, Adam Yopp, Daniel Siegwart, Akbar Waljee

E-Mail: amit.singal@utsouthwestern.edu; awaljee@med.umich.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

UT Southwestern Medical Center
5323 Harry Hines Blvd.
Dallas, TX 75390-9020

Veteran's Education and Research Association of Michigan (VERAM)
2215 Fuller Rd Rear
Ann Arbor, MI 48105-23032

**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**

Hepatocellular carcinoma (HCC) is the 6th most common cancer and 3rd leading cause of cancer-related death worldwide. We know that HCC subtypes exist because clear clinical, radiographic, and histological differences between patients with HCC are observed. In this study we proposed to investigate distinct subtypes of HCC using a mouse-human chimeric Patient Derived Xenograft (PDX) approach. So far, we have performed a large effort to implant 102 tumors from human HCC patients from Texas. We have established the protocol and the results have taught us that engraftment using a variety of transplantation techniques will result in a 25-30% engraftment efficiency for early stage surgical tumors. We have established 6 new human HCC PDX models that will be highly relevant for therapeutic and biological studies. These represent North American HCCs, including some patients with intermediate/advanced stage HCC, which is a unique resource for the field.

**15. SUBJECT TERMS**

HCC, patient derived xenografts, siRNA, mouse models of cancer.

| 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 | 22 | 19b. TELEPHONE NUMBER *(include area code)* |

**TABLE OF CONTENTS**

## 1. INTRODUCTION:

Hepatocellular carcinoma (HCC) is the 6th most common cancer and 3rd leading cause of cancer-related death worldwide. In the US, its incidence has doubled over the past two decades due to the growing number of patients with hepatitis C virus (HCV) and/or non-alcoholic steatohepatitis (NASH) (El-Serag, 2004, 2012). We know that HCC subtypes exist because clear clinical, radiographic, and histological differences between patients with HCC are observed (Yopp et al., 2015). In this study we proposed to investigate distinct subtypes of HCC using a mouse-human chimeric Patient Derived Xenograft (PDX) approach. We aim to analyze and functionalize early and advanced stage HCC tumors with a large and representative cohort of patient derived xenograft (PDX) models. Our hypothesis is that HCC is poorly understood because tissue has been obtained from early HCC but not advanced cases. Biological subclasses of HCCs that behave differently in terms of natural history, prognosis and treatment response have not been categorized and/or functionally analyzed. Our team will use human-mouse PDX models to uncover novel biology and establish a platform to study experimental therapeutics.

## 2. KEYWORDS:

HCC, patient derived xenografts, siRNA, mouse models of cancer.

## 3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

**What were the major goals of the project?**

For reference, the complete Statement of Work (SOW) is presented below with detail of Aims, Major Tasks, and Subtasks with Anticipated time lines. The column titled “Progress” indicates portion of the Major Task and related Sub-tasks completed.

| | | | |
|---|---|---|---|
| **Site 1:** | UT Southwestern Medical Center | **Site 2:** | Ann Arbor Veterans Affairs Healthcare System |
| | 5323 Harry Hines Blvd | | 2215 Fuller Rd |
| | Dallas, TX 75390 | | Ann Arbor, MI 48105 |
| | Initiating PI: Dr. Hao Zhu<br>Partnering PIs: Drs. Amit Singal; Adam Yopp; Daniel Siegwart | | Partnering PI: Dr. Waljee |

| **Specific AIM 1: Determine if early vs. advanced HCCs have distinct cell-intrinsic biology in PDX engraftment assays** | **Timeline in months** | **Site 1** (Initiating PI) | **Site 2** (Partnering PI) | **Progress (*Percent Complete or Completion Date*** |
|---|---|---|---|---|
| **Major Task 1:** Expand and characterize PDX models derived from surgical and biopsy HCC specimens | | | | |
| Pre-task: Allow time to receive the regulatory approval for animal use (IACUC and DoD ACURO) | 1-3 | Drs. Yopp, Singal, and Zhu | | 100 % complete November 2016 |
| Pre-task 2: Allow time to receive the regulatory approval for the Human Anatomical Substance use (IRB and DoD HRPO). | 1-3 | Drs. Yopp, Singal, and Zhu | | 100 % complete November 2016 |
| Subtask 1: Continue to implant 40 surgical HCC specimens in the subcutaneous space and livers of NSG mice | 0-12 | Drs. Yopp and Zhu | | 100% complete Sep 2018 |
| Subtask 2: Continue to implant 25 biopsy samples from intermediate and advanced HCC cases in the subcutaneous space and livers of NSG mice | 0-12 | Drs. Yopp, Singal, and Zhu | | 100% complete Sep 2018 |
| Subtask 3: Harvest primary PDX tumors, establish PDX bank, and passage into additional NSG mice | 6-18 | Drs. Yopp and Zhu | | 100% complete Oct 2018 |
| Subtask 4: Characterize tumor architecture, histology, growth, invasiveness, and paraneoplastic features of tumors that engraft, and determine if the grafts resemble or deviate from original tumors (surgical or biopsy specimens) | 6-24 | Drs. Yopp and Zhu | | 100% complete Sept 2018 |
| Subtask 5: Obtain genomic data from PDX grafts to determine if they resemble or deviate from original tumors (surgical or biopsy specimens) | 12-30 | Drs. Yopp, Singal, and Zhu | | 100% complete June 2018 |
| **Major Task 2:** Compare biological and genetic features (stage, survival, progression) of early vs. non-early HCCs | | | | |
| Subtask 1: Compare biological features of the tumors that engraft vs. those that do not, and determine if there is a difference between PDX made from early surgical or more advanced biopsy specimens | 6-24 | Drs. Yopp and Zhu | | 70% complete Oct 2018 |

| | | | | |
|---|---|---|---|---|
| Subtask 2: Compare patient clinical features (stage, survival, progression) of specimens that engraft versus not engraft and determine if engraftment can predict clinical outcomes | 6-18 | Drs. Singal, | Drs. Wajlee | 40% complete<br>October 2018 |
| Subtask 3: Analyze genomic data to survey genetic landscape of PDX population that successfully engrafts and identify genetic drivers of engraftment | 12-36 | Drs. Singal and Zhu | Drs. Wajlee | 80% complete<br>October 2018 |
| *Milestone #1: Co-author manuscript on biology and genomics of HCC PDX models* | 12-24 | Drs. Zhu, Singal, and Yopp | Drs. Wajlee | 60% complete<br>Oct 2018 |
| **Specific AIM 2: Determine the efficacy of small RNA therapeutics against the *LIN28B/LET-7* pathway in PDXs activating this oncogenic pathway** | **Timeline** | **Site 1** (Initiating PI) | **Site 2** (Partnering PI) | |
| **Major Task 1:** Identify and deliver small RNAs to target PDX populations | | | | |
| Subtask 1: Evaluate and optimize custom dendritic nanoparticle delivery to PDX tumors | 0-12 | Drs. Zhu and Siegwart | | |
| Subtask 2: Formulate and optimize siRNA and microRNA containing dendritic nanoparticles to ensure that successful modulation of LIN28B and or LET-7 is achieved in PDX models. | 6-24 | Dr. Siegwart | | |
| Subtask 3: Define HCC PDX models that overexpress MYC or LIN28B and those that suppress LET-7 family microRNAs | 6-24 | Drs. Singal and Zhu | | |
| Subtask 4: Therapeutically deliver siRNAs or microRNAs in dendritic nanoparticles to mice harboring these PDX models | 12-36 | Dr. Siegwart | | |
| **Major Task 3:** Define response to small RNAs in target PDX populations | | | | |
| Subtask 1: Determine response to small RNA therapies using luciferase and CT imaging | 6-30 | Dr. Siegwart | | |
| Subtask 2: Define histological response and intermediate markers of tumor biology (Ki67, apoptosis, necrosis) | 12-36 | Dr. Siegwart | | |
| *Milestone #2: Co-author manuscript about therapeutic efficacy of small RNA therapy in HCC PDX models* | 24-36 | Drs. Zhu and Siegwart | | |

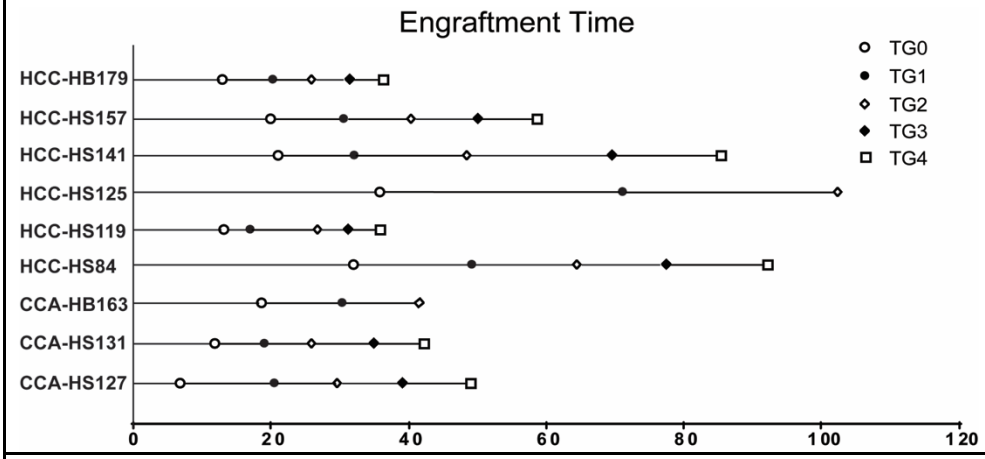
| **Specific AIM 3: Define targeted therapy responders with HCC-PDX patient avatars and use to identify predictive biomarkers** | **Timeline** | **Site 1** (Initiating PI) | **Site 2** (Partnering PI) | |
|---|---|---|---|---|
| **Major Task 1:** Define PDX models that show partial response, stable disease, and progressive disease to targeted therapies | | | | |
| Subtask 1: Characterize tumors for growth, histology, vascular invasion, metastasis, proliferation and apoptosis after treatment | 12-36 | Drs. Zhu, Yopp, and Singal | | 100% complete Oct 2018 |
| Subtask 2: Perform exome and RNA-expression sequencing for top responders and non-responders for each group to determine mechanistic basis of response | 18-36 | Drs. Singal and Zhu | Dr. Waljee | 50% complete Oct 2018 |
| **Major Task 2:** Establish predictive biomarkers for response to treatment | | | | |
| Subtask 1: Use machine learning methods to identify clinical and genetic factors associated with response to targeted therapies | 18-36 | Drs. Yopp, and Singal | Dr. Waljee | 100% complete Oct 2018 |
| Subtask 2: Derive and internally validate predictive model using factors significantly associated with targeted therapy response | 24-36 | Dr. Singal | Dr. Waljee | |
| Milestone #3: Co-author manuscript on HCC PDX treatments and predictive modeling results | 24-36 | Drs. Zhu, Yopp, and Singal | Dr. Waljee | |

**What was accomplished under these goals?**

| **Specific AIM 1: Determine if early vs. advanced HCCs have distinct cell-intrinsic biology in PDX engraftment assays** |
|---|
| **Major Task 1:** Expand and characterize PDX models derived from surgical and biopsy HCC specimens |
| **Subtask 1: Implant surgical HCC specimens in the subcutaneous space and livers of NSG mice.** This task has been completed, and Drs. Yopp and Zhu were responsible for this work. We have now implanted over 80 surgically obtained primary human HCC tumors into immunodeficient mice. This is a large number of patients and thus we can now make solid conclusions about the efficiency of PDX modeling for HCC. The data was shown in the previous progress report. |
| **Subtask 2: Continue to implant 25 biopsy samples from intermediate and advanced HCC cases in the subcutaneous space and livers of NSG mice.** This is the responsibility of Drs. Yopp, Singal, and Zhu. We have 6 engrafted biopsy samples that grew out from total of 26 implanted biopsies. The biopsy engraftment rate is less efficient than the surgical engraftment rate, but we cannot conclude that the advanced HCCs obtained via biopsy are less efficient engrafters than surgical samples because there is less tumor starting |

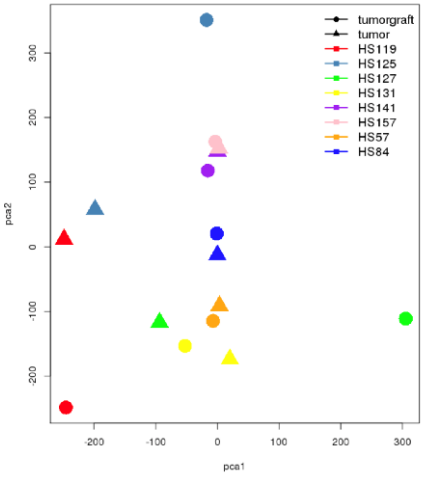
material. This subtask has been completed as of 7-1-18.

**Subtask 3: Harvest primary PDX tumors, establish PDX bank, and passage into additional NSG mice.** This has been performed by Min Zhu and Lin Li under the supervision of Drs. Yopp and Zhu. This task has been completed as was reported in the last progress report. Following is an updated table describing how long it takes from implantation to engraftment and passage. Enclosed are passageable lines.



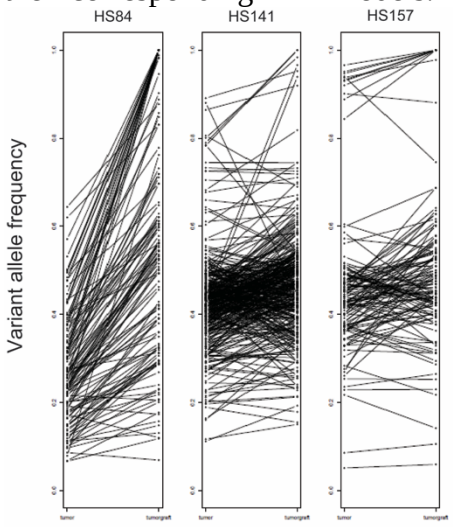

**Subtask 4: Characterize tumor architecture, histology, growth, invasiveness, and paraneoplastic features of tumors that engraft, and determine if the grafts resemble or deviate from original tumors (surgical or biopsy specimens).** We have found that 26 of 33 engrafted tumors have strong resemblance to the original HCC or cholangiocarcinoma tumors as based on Histology. Representative data was shown previously. This subtask is complete.

**Subtask 5: Obtain genomic data from PDX grafts to determine if they resemble or deviate from original tumors (surgical or biopsy specimens).** This has been performed under the supervision of Drs. Yopp, Singal, and Zhu. We have performed RNA-seq and whole exome DNA sequencing. There is good concordance between primary tumor and PDX tumor for RNA-seq, which measures the mRNAs expressed in the tumors vs. PDX models. See below for Principle component analysis showing that the primary patient tumors and PDX models general match in terms of mRNA expression. This confirms that there is gene expression program stability even after growth of tumors in immunodeficient mouse models.




We also compared the DNA sequencing data of the patient tumors and the PDXs. We found many shared somatic mutations between patient tumor and PDX samples. The figure

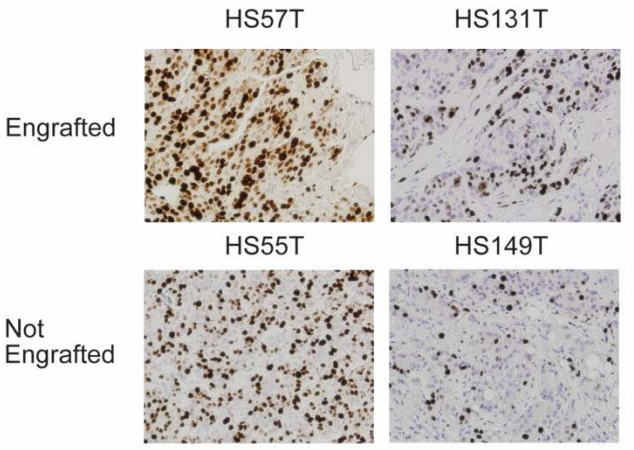
below shows the variant allele frequency of conserved mutations in patient tumors and their corresponding PDX models. We are still doing analysis for this question.

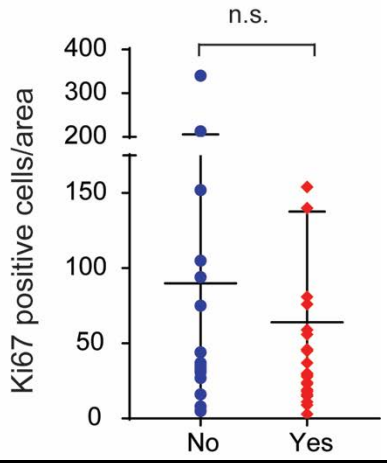



**Major Task 2:** Compare biological and genetic features (stage, survival, progression) of early vs. non-early HCCs

**Subtask 1: Compare biological features of the tumors that engraft vs. those that do not, and determine if there is a difference between PDX made from early surgical or more advanced biopsy specimens**

This has been performed under the supervision of Drs. Yopp, Singal, and Zhu. We have not identified a clear difference in Ki67 marked cell proliferation between the tumors that engraft vs. those that do not engraft. We also examined engraftment and tumor differentiation grade, which had previously been associated with engraftment in PDX models. The following is the table of comparing the engraftment rate among tumors with different grades. Biopsies and surgical samples were analyzed separately. No significant differences were observed, although admittedly there is a trend toward improved engraftment with higher grade and lower differentiation. We previously hypothesized that more advanced cancers (which are generally biopsied for tissue) might have higher egraftment or growth rates, but we found no evidence that biopsies have a higher engraftment rate than the surgical specimens. For surgical HCC samples, there seems to be a slightly higher engraftment rate for the poorly vs. moderately differentially tumors. The number of cases of well differentiated tumors is small so these numbers are not conclusive.



| Type | Grade | implanted | PDX-yes | lymphoma | HCC-PDX | HCC-PDX /Total | transplantable HCC-PDX | ID |
|---|---|---|---|---|---|---|---|---|
| HCC Resection | N/A, well to moderate | 5 | 2 | 0 | 2 | 40.00% | 1 | HCC-HS141 |
| | moderate | 43 | 12 | 3 | 9 | 20.94% | \ | \ |
| | moderate to poor | 4 | 1 | 0 | 1 | 25.00% | 1 | HCC-HS157 |
| | poor | 17 | 9 | 4 | 5 | 29.41% | 3 | HCC-HS84 HCC-HS119 HCC-HS125 |
| | total | 69 | 24 | 7 | 17 | 24.63% | 5 | |

| Type | Grade | implanted | PDX-yes | lymphoma | HCC-PDX | HCC-PDX /Total | transplantable HCC-PDX | ID |
|---|---|---|---|---|---|---|---|---|
| HCC Biopsy | N/A, well to moderate | 10 | 1 | 0 | 1 | 10.00% | 0 | |
| | moderate | 11 | 2 | 0 | 2 | 18.18% | 1 | HCC-HB179 |
| | moderate to poor | 1 | 0 | 0 | \ | \ | \ | |
| | poor | 2 | 0 | \ | \ | \ | \ | |
| | total | 24 | 3 | 0 | 3 | 12.50% | 1 | |

| Type | Grade | total cases implanted | PDX-yes | lymphoma | CCA-PDX | CCA-PDX /Total | transplantable CCA-PDX | ID |
|---|---|---|---|---|---|---|---|---|
| CCA Resection | well to moderate | 2 | - | 0 | 0 | \ | \ | |
| | moderate | 3 | 1 | 0 | 1 | 66.70% | 1 | CCA-HS127 |
| | poor | 1 | 1 | 0 | 1 | 37.50% | 1 | CCA-HS131 |
| | total | 6 | 2 | 0 | 2 | 33.30% | 2 | |

| Type | Grade | total cases implanted | PDX-yes | lymphoma | CCA-PDX | CCA-PDX /Total | transplantable CCA-PDX | ID |
|---|---|---|---|---|---|---|---|---|
| CCA Biopsy | moderate | 2 | 1 | | 1 | 50.00% | 1 | CCA-HB163 |
| | total | 2 | 1 | 0 | 1 | 50.00% | 1 | |

**Subtask 2: Compare patient clinical features (stage, survival, progression) of specimens that engraft versus not engraft and determine if engraftment can predict clinical outcomes**

Dr. Waljee's team performed explanatory analyses to find association between PDX engraftment results and several clinical features. We identified that one of the potential clinical predictors for engraftment is the size of the tumor with a coefficient of 0.2369, which was significant (p-value<0.05). Otherwise there were no clear correlations.

**Subtask 3: Analyze genomic data to survey genetic landscape of PDX population that successfully engrafts and identify genetic drivers of engraftment**

Dr. Waljee's team considered a variety of gene selection methods, including (1) logistic regression model with lasso regularization, (2) logistic regression model with elastic net regularization, (3) nearest shrunken centroid (NSC) method, and (4) adaptive hierarchically penalized NSC (AHP-NSC). The results are shown in table 1. The logistic regression model with lasso penalty method resulted in the highest accuracy rate of 76%. We also identified several genes that can potentially drive engraftment: SNORD15B; SNORA53; RP11−182J1.5; ZNF205; CX3CL1; RP5−837J1.1; MFSD9; SCARNA5; RAB3B

| Method | Accuracy rate |
|---|---|

| | |
|---|---|
| (1) logistic regression model with lasso | 0.76 |
| (2) logistic regression model with elastic net | 0.74 |
| (3) NSC | 0.60 |
| (4) AHP-NSC | 0.64 |

We will further study how genomic data can predict engraftment by taking three important known clinical confounders into consideration.

**Milestone #1: Co-author manuscript on biology and genomics of HCC PDX models**
The manuscript is being written and we plan on submitting to Hepatology.

**Subtask 1: Compare biological features of the tumors that engraft vs. those that do not, and determine if there is a difference between PDX made from early surgical or more advanced biopsy specimens**
We have not identified difference in Ki67 marked proliferation between the tumors that engraft vs. those that do not. We will examine differentiation and grade of tumors next.

**Specific AIM 2 (Determine the efficacy of small RNA therapeutics against the LIN28B/LET-7 pathway in PDXs activating this oncogenic pathway).**

**Major Task 1:** Identify and deliver small RNAs to target PDX populations

**Subtask 1: Evaluate and optimize custom dendritic nanoparticle delivery to PDX tumors**
This has been completed.

**Subtask 2: Formulate and optimize siRNA and microRNA containing dendritic nanoparticles to ensure that successful modulation of LIN28B and or LET-7 is achieved in PDX models.**
Ongoing 5A2-SC8 synthesis has been performed and completed. We anticipate that this will be enough for 9-12 months of animal experiments.

**Subtask 3: Define HCC PDX models that overexpress MYC or LIN28B and those that suppress LET-7 family microRNAs**
We did not do work on this task this period.

**Subtask 4: Therapeutically deliver siRNAs or microRNAs in dendritic nanoparticles to mice harboring these PDX models**
We did not work on this task during this period.

**Major Task 3:** Define response to small RNAs in target PDX populations

Subtask 1: Determine response to small RNA therapies using luciferase and CT imaging

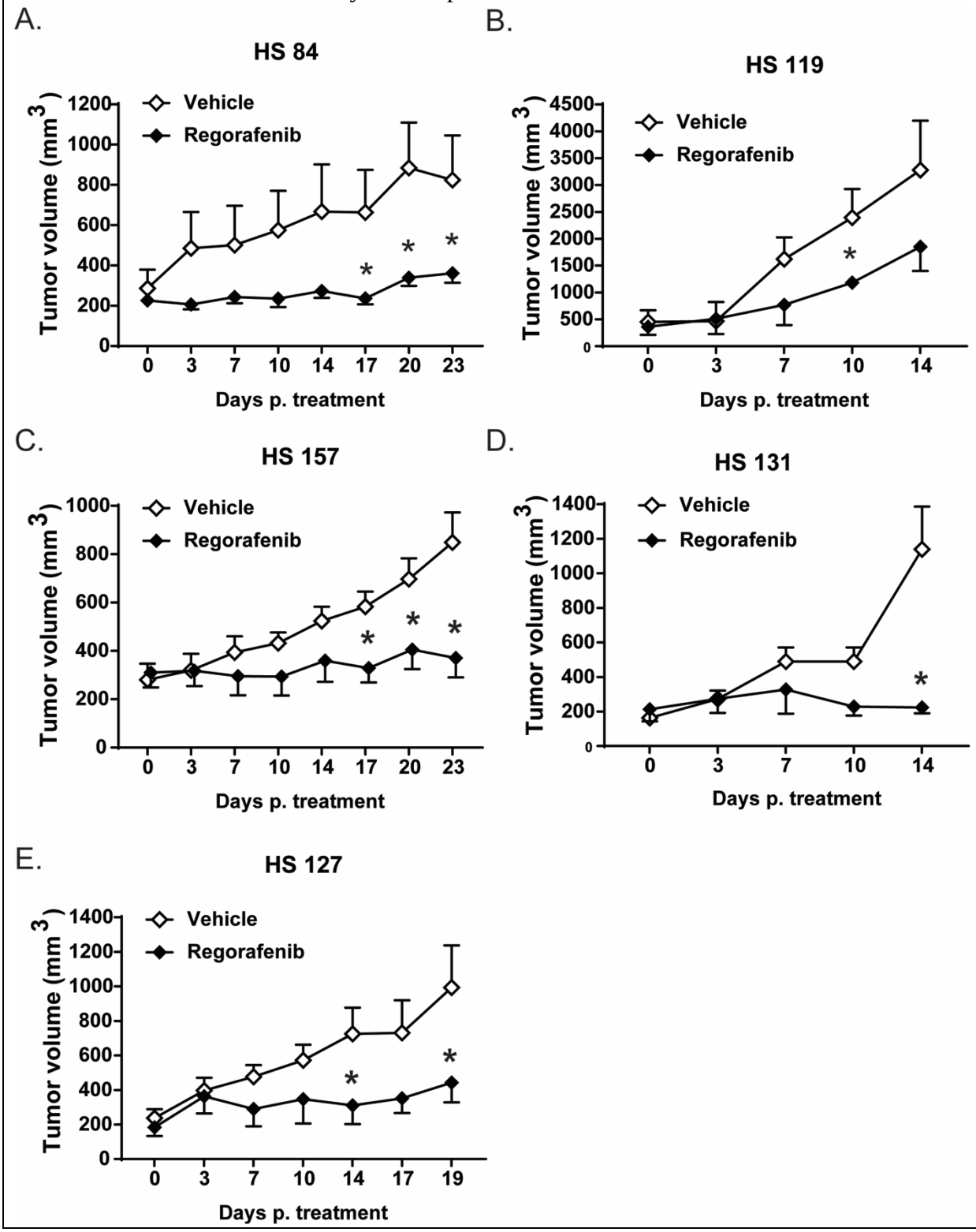
Subtask 2: Define histological response and intermediate markers of tumor biology (Ki67, apoptosis, necrosis)

**Specific AIM 3: Define targeted therapy responders with HCC-PDX patient avatars and use to identify predictive biomarkers**

**Major Task 1:** Define PDX models that show partial response, stable disease, and progressive disease to targeted therapies

Subtask 1: Characterize tumors for growth, histology, vascular invasion, metastasis, proliferation and apoptosis after treatment
We have performed drug studies in the PDX models that are growing well after passage into multiple recipient NSG mice. We started with using Regorafenib, a relatively new second line agent in HCC. This

drug is used after patients progress or cannot tolerate first-line therapy, i.e. sorafenib. We aimed to find out if there are subsets of HCCs that are more responsive to Regorafenib. Each PDX line was expanded in mice and separated into two groups. One group was treated with vehicle and one treated with Regorafenib. We found that 4 of the lines responded well after treating with Regorafenib. We plan to treat these PDX lines with other drugs and compare the results with Regorafenib. Interestingly, some of the most sensitive lines are cholangiocarcinomas that are not traditionally treated with multi-kinase inhibitors. This suggests that Regorafenib or other multikinase inhibitors could be effective in mixed type histology or biliary cancers. Notably, mixed type cancers that contain both HCC and cholangiocarcinoma components are difficult to treat and it is uncertain what the best systemic options are.



| |
|---|
| We are also examining other compounds such as sorafenib and other sorafelogs in comparision with regorafenib. |
| Subtask 2: Perform exome and RNA-expression sequencing for top responders and non-responders for each group to determine mechanistic basis of response |
| **Major Task 2:** Establish predictive biomarkers for response to treatment |
| Subtask 1: Use machine learning methods to identify clinical and genetic factors associated with response to targeted therapies |
| Subtask 2: Derive and internally validate predictive model using factors significantly associated with targeted therapy response |
| Milestone #3: Co-author manuscript on HCC PDX treatments and predictive modeling results<br>We are working on this. |

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

| |
|---|
| Min Zhu presented some of her work in our departmental retreat, where she gave a 20 minute presentation. |

**How were the results disseminated to communities of interest?**

| |
|---|
| Nothing to report |

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

We are going to use the data generated above to co-author a manuscript on biology and genomics of HCC PDX models from western populations. This could be informative because there are only three published experiences with HCC PDX models, all of which are derived from Asian non-cirrhotic hepatitis B patients who underwent curative resection (Huynh et al., 2007; Wei et al., 2014; Yan et al., 2013). These models do not represent the US HCC population, in whom >70% have HCV or NASH and >90% have cirrhosis. In this study, we have established protocols for HCC PDX development for a large number of American patients. We found that the engraftment and passageability of HCCs is relatively inefficient, but that certain recipient protocols can increase efficiency. We have also found that HCC biopsies can generated PDX models. These HCC ODXs represent the patient spectrum in the US. This knowledge will help elucidate mechanisms of treatment response to currently available as well as experimental therapeutics.

We will also be performing HCC organoid development to increase the approaches that we can use to generate functional human models of primary liver cancer. Please see below for how we addressed this.

We will also work more towards targeting the Lin28 let-7 pathway in HCC PDX models, which is a part of AIM 2.

For Specific AIM 3 (Define targeted therapy responders with HCC-PDX patient avatars and use predictive modeling to identify prognostic biomarkers), we did drug studies in the PDX models that are successfully growing. We started with using Regorafenib, which is a new second line agent in HCC. This drug is used after patients progress or cannot tolerate first-line therapy, i.e. sorafenib. We aimed to know if there are subsets of HCCs that are more responsive to Regorafenib. Each PDX line was expanded in mice and separated to two groups. One group was treated with vehicle and one treated with Regorafenib. We found that 4 of the lines responded well after treating with Regorafenib. We plan to treat these PDX lines with other drugs and compare the results with Regorafenib. We're in the process of treating the PDX lines with sorafenib and a novel derivative of sorafenib developed by our collaborator at Mt. Sinai.

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?**

The major impact at this point is that we have performed a large effort to implant 102 tumors from human HCC patients from Texas. We have established the protocol and the results have taught us how efficient this process will be. We have established 6 new human HCC PDX models that will be highly relevant for therapeutic and biological studies. These represent North American HCCs, including some patients with intermediate/advanced stage HCC, which is a unique resource for the field.

We have found that increasing the rate of engraftment with partial hepatectomy or mouse models of chronic liver disease helps to make the growth and engraftment of the tumors more efficient.

**What was the impact on other disciplines?**

Nothing to report

**What was the impact on technology transfer?**

Nothing to report

**What was the impact on society beyond science and technology?**

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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**

Site 2 (PI: Waljee)
•Due to limited server analytic capabilities on the VA network, an amendment to the analysis plan was reviewed and approved in April, 2018 by Ann Arbor R&D committee. This amendment would allow de-identified data from Site 1 to be analyzed on the University of Michigan servers. A Data Sharing and Use Agreement was initiated and executed on 5/2/2018 between UTSW and University of Michigan. Data was transferred from UTSW on 7/11/2018 via DropBox

The postdoc research fellow listed on the UM Subaward left for personal reasons in December 2017. We hired another postdoc research fellow in September 2018 to perform the work in 14 months, using 8 months at 100% effort (12 calendar months) and then back to 50% effort (6 calendar months) for the remaining 6 months. We are confident that this will not impact the achievement of aims stated in the Statement of Work.

**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.*

Site 1:
Given low PDX engraftment rates and long waiting times, we have not generated as many human cancer lines as we were hoping to. Thus, we have also been generating in vitro liver cancer organoid models in addition to in vivo PDX models. These are primary liver tumors grown in culture that can be passaged over time. In other tumor types, these have been shown to recapitulate the histology, gene expression, behavior of primary HCCs from patients. In theory, this could have increaseed the number of patients that could be represented in the laboratory environment and expand our ability to test hypotheses and reach conclusions. We have had some incomplete success with these organoids over the last 6 month period. We believe that we have established the appropriate procedures and protocols to generate organoids, but still the rate of passageable lies is less than 10%.

We have been examining why we have such a low rate of organoid and PDX engraftment/initiation. Recently, there was a Cell Reports paper showing that using a similar protocol as ours, they had 10/37 HCC biopsies grow successfully into organoids that are passageable. The only clinical variable that was predictive was Edmonson differentiation grade of 3. Their paper had 27/37 grade 3 or 4 HCCs (73%), and of the 27, only 10 grew out, indicating that if you are grade 3 or 4, you may not grow, but if you do form an organoid, then you definitely have to be grade 3 or 4. In our experiences with organoids, we had only 7 out of 50 patients were grade 3 or 4 (14%). As you might predict, this leads to a very low level of organoid formation or PDX engraftment. For us, maybe 2-3 of the 7 patients resulted in passageable organoids. This data could explain a lot about our efforts to functionalize human patient samples. It also might reflect our high level of indolent patients. This is quite interesting and important because indolence is a potential feature of HCC in the western world, and there has been little done to examine indolence in this population. Thus, we are both looking into indolence from a clinical research perspective, and we are focusing more on generating organoids from higher grade, lower differentiation patients.

**Changes that had a significant impact on expenditures**

Nothing to report

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

**Significant changes in use or care of human subjects**

Nothing to report.

**Significant changes in use or care of vertebrate animals.**

We renewed our animal protocol this October. It was essentially unchanged and it was approved.

**Significant changes in use of biohazards and/or select agents**

| |
|---|
| Nothing to report |

**6. PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  **Journal publications.**

| |
|---|
| Nothing to report |

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

| |
|---|
| Nothing to report |

  **Other publications, conference papers, and presentations**.

| |
|---|
| Nothing to report |

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

| |
|---|
| CDMRP website as reported above. |

- **Technologies or techniques**

| |
|---|
| These techniques have been described above and will be reported to the community when a manuscript is published. |

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

| |
|---|
| Nothing to report |

- **Other Products**

Data or databases: We continue to collect patient data in a clinical database.
Biospecimen collections: We have a human HCC biospecimen and PDX collection.
Research material: We have established live mice carrying human HCC PDXs.

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

Name: Hao Zhu
Project Role: Lead PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-8417-9698
Nearest person month worked: 24
Contribution to Project: Direct the project, design the experiments and objectives, organize personnel, report progress to the DOD.

Name: Lin Li
Project Role: Senior Research Associate)
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 24
Contribution to Project: implantation of HCC specimens, passage of engrafted PDXs, storage of PDX engrafts.

Name: Daniel Siegwart
Project Role: Co-PI
Researcher Identifier (ORCID ID): 0000-0003-3823-1931
Nearest person month worked: 24
Contribution to Project: Co-planned and co-directed research activities. Worked on 5A2-SC8 synthesis and purification. Worked on nanoparticle delivery optimization to liver tumors.

Name: Qiang Cheng
Project Role: Senior Research Associate
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 24
Contribution to Project: Developed nanoparticle delivery carriers with an improved ability to deliver RNAs to the liver. Assisted with 5A2-SC8 experiments.

Name: Adam Yopp
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 24
Contribution to Project: Design and conducted experiments, participated in co-PI conference calls to organize personnel and direct project.

Name: Min Zhu
Project Role: Senior Research Associate
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 24
Contribution to Project: implantation of HCC specimens, passage of engrafted PDXs, storage of PDX engrafts. inventory of HCC samples, preparation of genomic DNA libraries from HCC samples, data analysis, etc.

Name: Amit Singal

Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-1172-3971
Nearest person month worked: 24
Contribution to Project: Design experiments, participated in co-PI conference calls to organize personnel and direct project.

Name: Veronica Renteria
Project Role: Research coordinator
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 24
Contribution to Project: collection of HCC specimens

Name: Amanda Ellis
Project Role: Research assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2.4
Contribution to Project: Ms. Ellis has performed administrative duties such as organizing meetings, regulatory policies, and served as liaison between AAVA and UTSW.

Name: Gunwoong Park
Project Role: Statistician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.6
Contribution to Project: Implemented a cutting-edge prediction model in R that would handle data coming from a mixture of heterogeneous populations and he has also done numerous simulation studies comparing his implementation with several existing more traditional models.

Name: Xianshi Yu
Project Role: Statistician
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Will be helping predict engraftment using both clinical and various predictor genes.

See below for a chart that details the hours worked by staff at the University of Michigan site on this project for FY 18.

Award # W81XWH-16-1-0158

| **Name** | **Role** | **Quarter 1** | **Quarter 2** | **Quarter 3** | **Quarter 4** | **Totals FY18** |
|---|---|---|---|---|---|---|
| Akbar Waljee | Site PI | 7.8 hours | 7.8 hours | 7.8 hours | 7.8 hours | 31.2 hours |
| Rachel Lipson | Data Manager | 26 hours | 26 hours | 18 hours | 0 hours | 70 hours |
| Tony Van | Data Analyst | 0 hours | 0 hours | 8 hours | 26 hours | 34 hours |
| Amanda Ellis | RA | 104 hours | 104 hours | 104 hours | 104 hours | 416 hours |

Sub-Award to UM

| Name | Role | Quarter 1 | Quarter 2 | Quarter 3 | Quarter 4 | Totals FY18 |
|---|---|---|---|---|---|---|
| Akbar Waljee | Site PI | 7.8 hours | 7.8 hours | 7.8 hours | 7.8 hours | 31.2 hours |
| Ji Zhu | Co-I | 26 hours | 26 hours | 26 hours | 26 hours | 104 hours |
| Gunwong Park | Post Doc (Statistics) | 260 hours | 0 hours | 0 hours | 0 hours | 260 hours |
| Xianshi Yu | Post Doc (Statistics) | 0 hours | 0 hours | 0 hours | 160 hours | 160 hours |

**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."*

For Hao Zhu, the following grants have become active:
R01 DK111588-01A1 (Zhu)
NIH NIDDK
Title: Enhancing mammalian liver repair and regeneration
The major goals of this proposal are to understand the role of SWI/SNF complex components in liver disease and regeneration.

CPRIT New Investigator Award 9/1/2012 – 8/31/2016 No Effort Required
Cancer Prevention and Research Institute of Texas (Zhu)
The Lin28-LET-7 pathway in liver cancer
$455,000The major goals of this project are to determine roles for Lin28 and LET-7 in liver cancer development. AIM 1 of this grant has been completed and published. AIM 2 involves testing LET-7 therapeutic delivery in cancer models, and this is not discussed in the current R01.

For Amit Singal, the following grants have become active:
NIH R01CA12008-01A1 (PI: Singal) 07/01/2017-06/30/2022 3.0 calendar
Harms of Hepatocellular Carcinoma Screening in Patients with Cirrhosis
The goal of this proposal is to quantify HCC screening physical, financial, and psychosocial harms across 3 healthcare settings (academic tertiary care center, safety-net health system, and Veterans Affairs system).

R01-CA222900 (Singal) 01/01/2018-12/31/2022 1.8 calendar
National Institutes of Health
Precision Screening for Hepatocellular Carcinoma
To develop and evaluate a precision medicine strategy for early detection of HCC in patients with cirrhosis tailored to individual patient risk and expected screening test performance

For Amit Singal,and Adam Yopp the following grant has become active:
R01-MD12565 (Singal and Yopp) 04/03/2018-11/30/2022 1.8 calendar
National Institutes of Health
Multilevel factors for racial/ethnic and socioeconomic disparities in prognosis of HCC
To characterize the contribution of proximal, intermediate, and distal determinants to disparities to three measure of HCC prognosis in a large, racial/ethnically and socioeconomically diverse cohort of HCC patients

For Daniel Siegwart, the following grants have become active:
I-1855 (Siegwart) 6/1/2017 – 5/31/2020 1.0 calendar
Welch Foundation
“Design and synthesis of activatable pH-responsive water soluble dyes for biomedical imaging” The main objective of this proposal is to develop fluorecene probes capable of detecting cancer metastases.
RSG-17-012-01-CDD 07/1/2017-06/30/21 0.4 calendar
American Cancer Society (ACS)
“A functional polyester library for enhanced and selective miRNA delivery into patient-derived lung cancer cells for advanced cancer therapy”
This grant will identify materials that can preferentially deliver miRNAs to cancer cells, but not to normal cells using normal/tumor matched-pair screening approach.

**What other organizations were involved as partners?**

| |
|---|
| Nothing to report |

8. **SPECIAL REPORTING REQUIREMENTS**

**COLLABORATIVE AWARDS:** N/A

**QUAD CHARTS:** N/A

9. **APPENDICES:** N/A