AWARD NUMBER: W81XWH-16-1-0154

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

PRINCIPAL INVESTIGATOR: Hao Zhu, MD

RECIPIENT: University of Texas Southwestern Medical Center
                                        Dallas, TX 75390

REPORT DATE: October 2018

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
                                                    Distribution Unlimited

REPORT DOCUMENTATION PAGE		*Form Approved* *OMB No. 0704-0188*
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**		

1. REPORT DATE October 2018	**2. REPORT TYPE** Annual	**3. DATES COVERED** 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-16-1-0154
		5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S) Hao Zhu, Amit Singal, Adam Yopp, Daniel Siegwart, Akbar Waljee		**5d. PROJECT NUMBER**
		5e. TASK NUMBER
E-Mail: Hao.zhu@utsouthwestern.edu		**5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Texas 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 USAMRMC
a. REPORT Unclassified	**b. ABSTRACT** Unclassified	**c. THIS PAGE** Unclassified	Unclassified	28	**19b. TELEPHONE NUMBER** *(include area code)*

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

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 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 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 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 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		100% complete Oct 2018
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		50% complete Oct 2018
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?
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.

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.
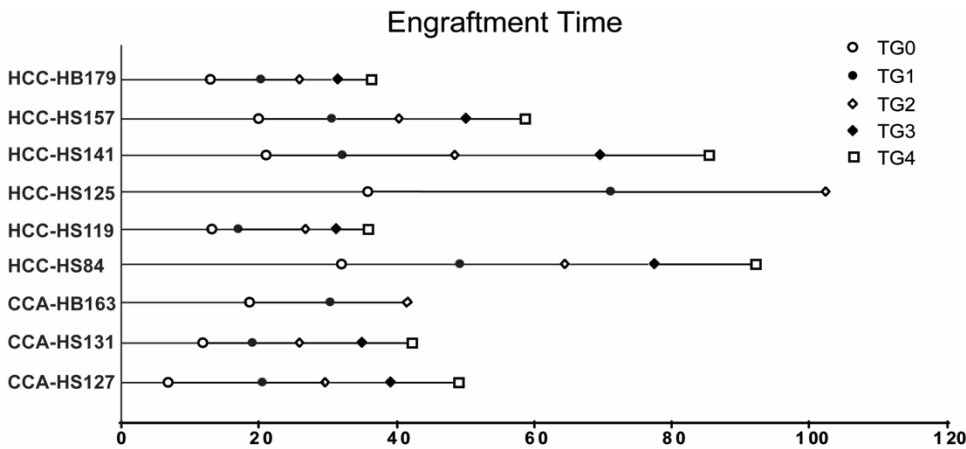

Subtask 4: Characterize tumor architecture, histology, growth, invasiveness, and paraneoplastic features of tumors that engraft, and determine if the grafts resemble or deviate from original tumors (surgical or biopsy specimens). We have found that 26 of 33 engrafted tumors have strong resemblance to 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.
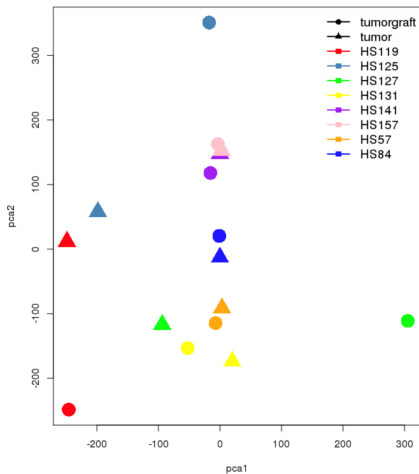

We also compared the DNA sequencing data of the patient tumors and the PDXs. We found many shared somatic mutations between patient tumor and PDX samples. The figure below shows the variant allele frequency of conserved mutations in patient tumors and their corresponding PDX models. We are still doing analysis for this question.
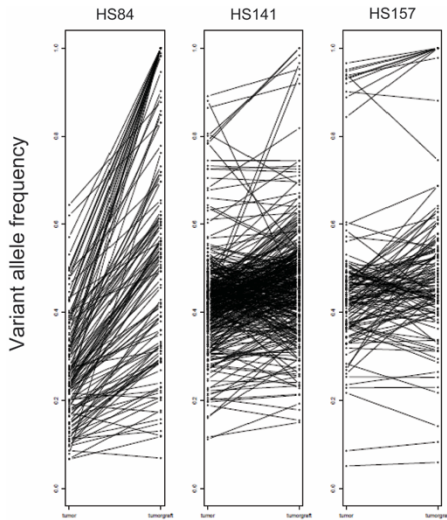
| | HS84 | HS141 | HS157 |

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.

6
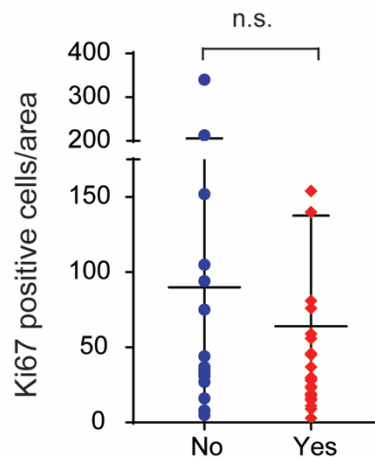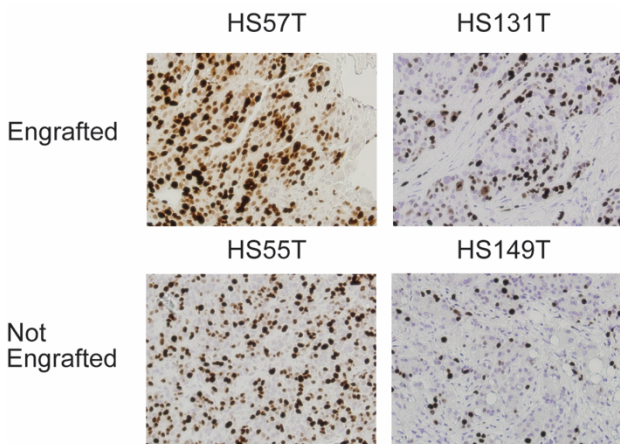
Type	Grade	implanted	PDX-yes	lymphoma	HCC-PDX	HCC-PDX /Total	transplantable HCC-PDX	ID
HCC Resection	N/A, well to moderate	5	2	0	2	40.00%	1	HCC-HS141
	moderate	43	12	3	9	20.94%	\	\
	moderate to poor	4	1	0	1	25.00%	1	HCC-HS157
	poor	17	9	4	5	29.41%	3	HCC-HS84 HCC-HS119 HCC-HS125
	total	69	24	7	17	24.63%	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 ($p\text{-value} < 0.05$). 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	0.76

with lasso	
(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.
Additional 5A2-SC8 synthesis has been performed and completed.

5A2-SC8 dendrimer lipid was re-synthesized in large scale. 20 grams of pure product was isolated and characterized by nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy (MS). These analyses demonstrated successful synthesis without impurities. We anticipate that this will be enough for 12-15 months of animal experiments.

Following large scale synthesis, the new batch of 5A2-SC8 was formulated into lipid nanoparticles (LNPs) containing 5A2-SC8/DSPC/Cholesterol/PEG2000-DMG (50/10/38/2, mol/mol) and siRNA against Factor VII to verify high activity in the liver. The results showed the expected nanoparticle diameter and >80% siRNA encapsulation. Both aliquots of LNPs were able to effectively silence Factor VII in mouse livers. This batch therefore passed quality control and was passed on for use in the future Subtasks below to be completed in the next period.
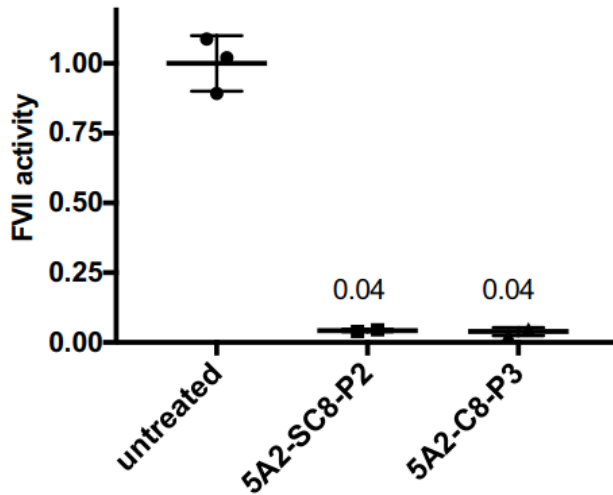
RNA LNP distribution
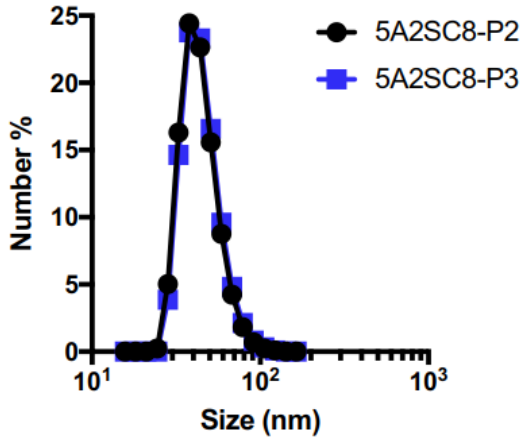
RNA binding
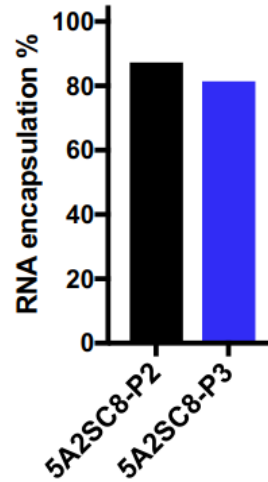
FVII knockdown with a dosage of 0.5 mg/kg

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
We did not work on this task during this period.

| Subtask 2: Define histological response and intermediate markers of tumor biology (Ki67, apoptosis, necrosis) |
| :--- |
| We did not work on this task during this period. |

Specific AIM 3: Define targeted therapy responders with HCC-PDX patient avatars and use to identify predictive biomarkers
We did not work on this task during this period.

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.

A. HS 84

B. HS 119

C. HS 157

D. HS 131

E. HS 127
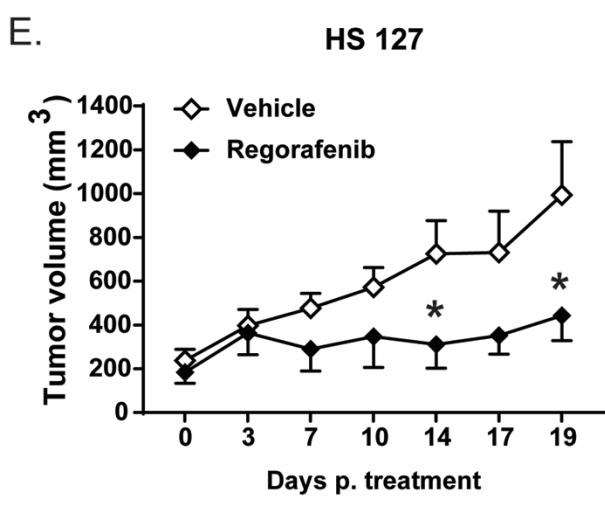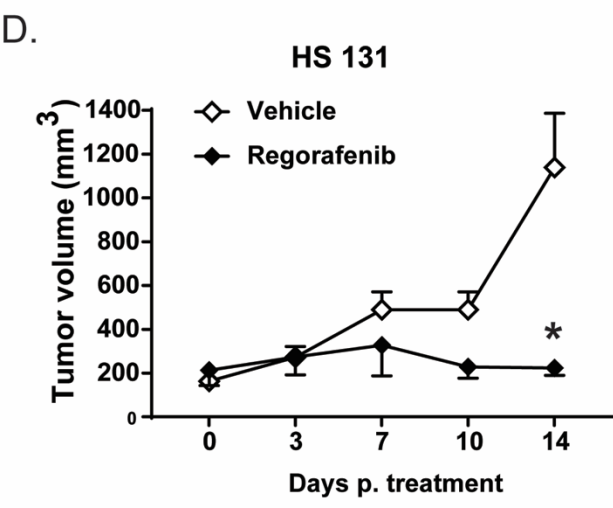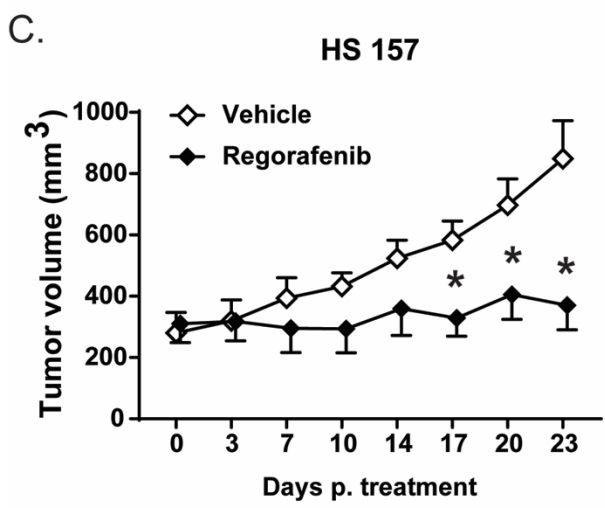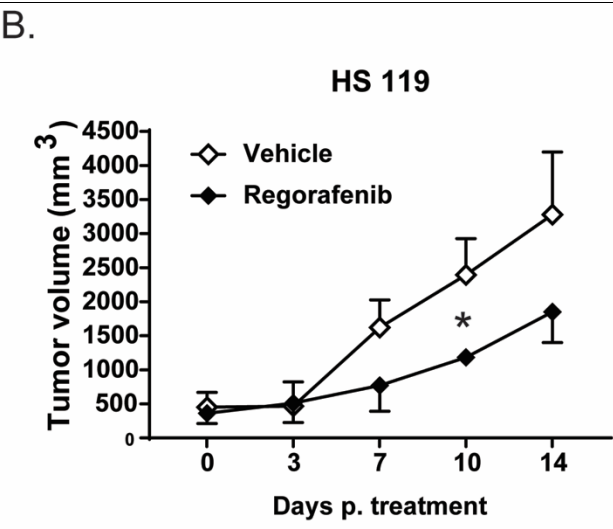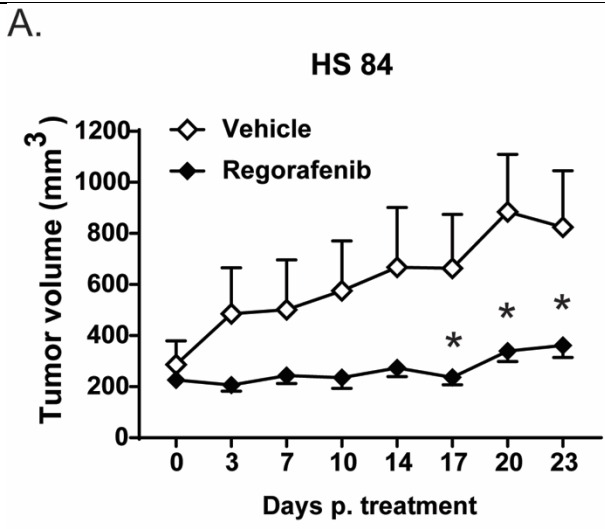
We are also examining other compounds such as sorafenib and other sorafelogs in comparision with regorafenib.

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 We are working on this.

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.

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?
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.

On April 30, 2018, Dr. Siegwart gave a presentation as a part of the American Cancer Society (ACS) Research Symposium at the Adolphus Hotel in Dallas. This event was organized and hosted by the Cattle Baron's Ball committee and sponsored by Mary Kay, Inc., Nancy C. and Richard R. Rogers, and the Deason Foundation. Dr. Siegwart talked about "Enhancing cancer drug selectivity to tumors" and mentioned DOD-funded work related to liver cancer PDXs. On August 7, 2018, Dr. Siegwart hosted ACS staff at UT Southwestern for a seminar, lab tour, and lunch. A presentation was given by Dr. Siegwart and three graduate students. They covered research and "life as a scientist", answered questions, and gave a comprehensive lab tour. Following that, the entire lab joined lunch and conversation with ACS staff and volunteers. Topics included "Development of nanoparticle carriers to deliver RNA drugs to tumors" and DOD-funded work related to liver cancer PDXs.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We 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?
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).

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?
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.

Knowledge learned from studying nanoparticle delivery to the liver has aided development of messenger RNA (mRNA) therapies for the liver. A manuscript was recently accepted for publication that acknowledged DOD support:

"Dendrimer-based Lipid Nanoparticles Deliver Therapeutic FAH mRNA to Normalize Liver Function and Extend Survival in a Mouse Model of Hepatorenal Tyrosinemia Type I." Qiang Cheng, Tuo Wei, Yuemeng Jia, Lukas Farbiak, Kejin Zhou, Shuyuan Zhang, Yonglong Wei, Hao Zhu, and Daniel J. Siegwart. ***Advanced Materials***, early view (**2018**), DOI: 10.1002/adma.201805308.

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 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
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.

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
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

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

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use 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:

- **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;*

1. "Dendrimer-based Lipid Nanoparticles Deliver Therapeutic FAH mRNA to Normalize Liver Function and Extend Survival in a Mouse Model of Hepatorenal Tyrosinemia Type I." Qiang Cheng, Tuo Wei, Yuemeng Jia, Lukas Farbiak, Kejin Zhou, Shuyuan Zhang, Yonglong Wei, Hao Zhu, and Daniel J. Siegwart. ***Advanced Materials***, early view (2018), DOI: 10.1002/adma.201805308.
2. "Design of synthetic materials for intracellular delivery of RNAs: From siRNA-mediated gene silencing to CRISPR/Cas gene editing." Jason B. Miller, Daniel J. Siegwart. ***Nano Research***, 11, 5310–5337 (2018).

other); acknowledgement of federal support (yes/no).

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).*

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.*

- **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.

  > CDMRP website as reported above.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

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

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  > Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen 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.*

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?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: 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: 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.

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

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."

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.

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
The major goals of this project are to determine roles for Lin28 and LET-7 in liver cancer development.

For Amit Singal, the following grants have become active:

U01 C230694 (Singal)   09/14/2018-07/31/2023   1.8 calendar
National Institutes of Health
Precision Risk Stratification and Screening for HCC Patients with Cirrhosis in the United States
To evaluate an imaging and biomarker-based precision medicine strategy for early detection of HCC in patients with cirrhosis tailored to individual patient risk and expected screening test performance.

R01-CA12008 (Singal)   08/01/2017-07/31/2022   2.4 calendar
National Institutes of Health
Harms of Hepatocellular Carcinoma Screening in Patients with Cirrhosis
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.

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:

R01 EB025192-01A1   (Siegwart)   7/1/18 – 6/30/23
National Institutes of Health
Title: "Defining the molecular interactions within nanoparticles that enable delivery of long nucleic acids"
The goal is to develop synthetic nanoparticles that can safely co-deliver Cas9 mRNA and targeted sgRNA to multiple organs in vivo.

SIEGWA18XX0   (Siegwart)   4/1/18 – 3/31/20
Cystic Fibrosis Foundation
Title: "Optimization of dendrimer lipid nanoparticles for CRISPR/Cas gene editing"
The goal is to develop dendrimer lipid nanoparticles for gene correction of mutations in CFTR.

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:
<u>*Organization Name:*</u>
<u>*Location of Organization: (if foreign location list country)*</u>
<u>*Partner's contribution to the project*</u> *(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.*

8. **SPECIAL REPORTING REQUIREMENTS**

   COLLABORATIVE AWARDS: N/A

   QUAD CHARTS: N/A

9. **APPENDICES:** N/A