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AWARD NUMBER: W81XWH-16-1-0155

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

PRINCIPAL INVESTIGATOR: Adam Yopp, MD

RECIPIENT: UT Southwestern Medical Center

REPORT DATE: Oct -2019

TYPE OF REPORT: Annual

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

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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)

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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.

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Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

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

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

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		100% complete April 2019

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	100% 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	100% 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	100% complete July 2019
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 Dec 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		100% Sept 2019
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		Incomplete, discontinued
Subtask 4: Therapeutically deliver siRNAs or microRNAs in dendritic nanoparticles to mice harboring these PDX models	12-36	Dr. Siegwart		20% complete July 2019
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		50% complete (performed in analogous models)
Subtask 2: Define histological response and intermediate markers of tumor biology (Ki67, apoptosis, necrosis)	12-36	Dr. Siegwart		50% complete (performed in analogous models)

Milestone #2: Co-author manuscript about therapeutic efficacy of small RNA therapy in HCC PDX models	24-36	Drs. Zhu and Siegwart		Not completed
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	100% complete July 2019
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	30% complete July 2019
Milestone #3: Co-author manuscript on HCC PDX treatments and predictive modeling results	24-36	Drs. Zhu, Yopp, and Singal	Dr. Waljee	50% complete July 2019

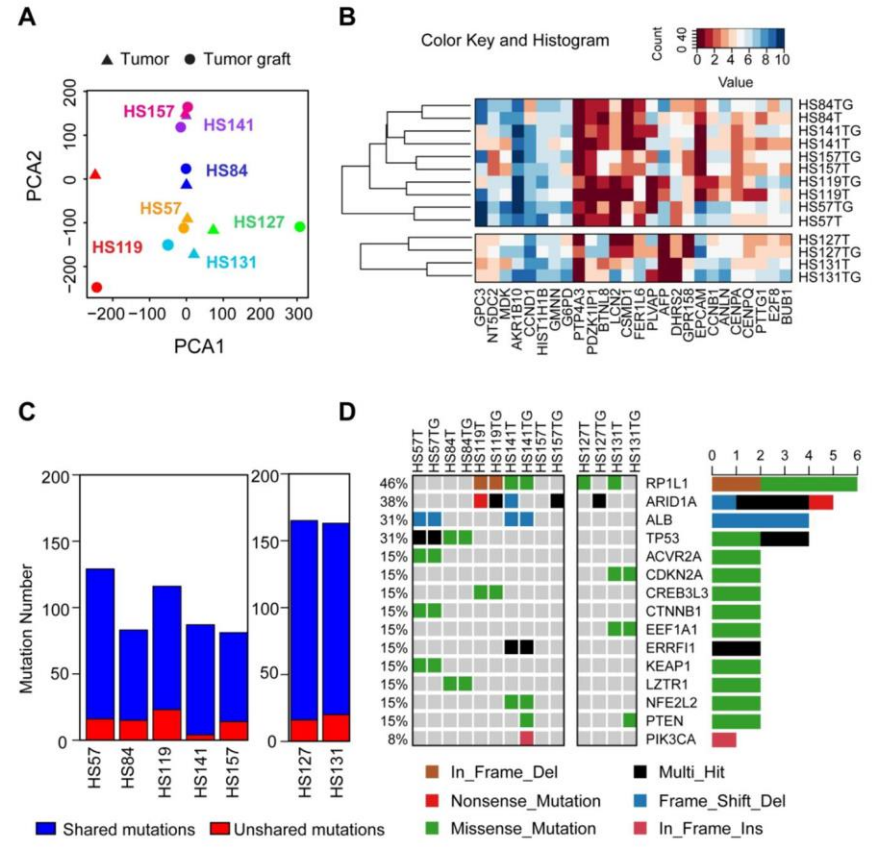
What was accomplished under these goals?

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.
Subtask 2: Continue to implant 25 biopsy samples from intermediate and advanced HCC cases in the subcutaneous space and livers of NSG mice. This subtask has been completed.
Subtask 3: Harvest primary PDX tumors, establish PDX bank, and passage into additional NSG mice. This task has been completed. We also thawed some PDX lines and found that most of the lines can be thawed from frozen stock and grow. Altogether we found that among 9 lines of

transplantable PDXs, 7 lines can be thawed and re-grown in mice. These lines are HS-84, HS-127, HS-131, HS-157, HS-119, HB-163, and HB-179. This makes it possible to stock the PDX lines in a bank and use them later to test therapies without constantly maintaining the lines in live mice. When thawed, the PDX lines can also be passaged and still maintain the histology of the patient tumors (Figure A & B). For HS-141, although we were not able to thaw the frozen stock successfully, the primary PDX tumor of HS-141 has been successfully passaged for 7 times and are still growing. Thus, 8 total lines can be used for drug testing. We will test the histology of the PDXs after multiple passages to see whether these PDXs still represent the patient tumor and maintain the biological features of their parent patient tumors.

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).
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 completed and analyzed in a more detailed way. Here we showed that the HCC PDXs retain the genomic expression and mutational profiles of parental tumors. A. Principal component analysis (PCA) of RNA-seq data from 7 pairs of parental tumors and PDX samples showed conservation of gene expression and B. We also performed hierarchical clustering of samples based on evaluation of highly expressed genes in HCC. C. Whole-exome sequencing in parental tumors and PDX models generally showed preservation of mutated genes. D. Oncoplot of mutations in parental tumors and PDXs. We focused on 35 genes commonly mutated genes in HCC based on the TCGA analysis. COSMIC mutations or exome mutations compared to 1000 genomes are shown. See below Figure.



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 completed under the supervision of Drs. Yopp, Singal, and Zhu.

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 aimed to determine if clinical variables such as tumor differentiation correlated with engraftment. Among 69 surgical HCC cases, 43 cases were moderately differentiated and 9 of these successfully engrafted (21%, see Supplementary Table 2). Seventeen were poorly differentiated and 5 engrafted (29%). When comparing the engraftment for "moderate", "moderate to poor", and "poor" HCCs, there was a non-significant trend of increasing engraftment from 21% to 25% to 29%. However, 3/17 poorly differentiated HCCs were serially transplantable while 0/43 moderately differentiated HCCs were transplantable (Fisher's exact test p=0.02). Surprisingly, the well differentiated HCC samples could also engraft, although the number of cases was not high enough to evaluate the engraftment rate. Previous reports of Asian HCC PDX models showed that engraftment correlated with tumor cell proliferation as measured by Ki-67. In our cohort, Ki-67 staining on 20 engrafting and 37 non-engrafting primary tumors showed no significant differences in the frequency of Ki-67 positive cells. In addition, clinical features such as ALT, AST, and sodium levels could not predict engraftment (data not shown). We think additional predictors are going to be difficult to find unless we have a much larger number of engrafting tumors. This task has been completed.

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 further studied how genomic data can predict engraftment by taking three important known clinical confounders into consideration. None of the three clinical confounders were not selected in our models for prediction. This may be due to small sample size. To determine if we could identify transcriptomic predictors of engraftment, we also performed deep RNA-seq on cohorts of parental tumors that either did or did not engraft in PDX assays (n = 17 and 19). However, we did not find gene sets that could distinguish between engrafting and non-engrafting cases using gene set enrichment analysis (GSEA). This task is complete.

Milestone #1: Co-author manuscript on biology and genomics of HCC PDX models
The manuscript has been favorably reviewed and is in 2nd review at Hepatology.

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

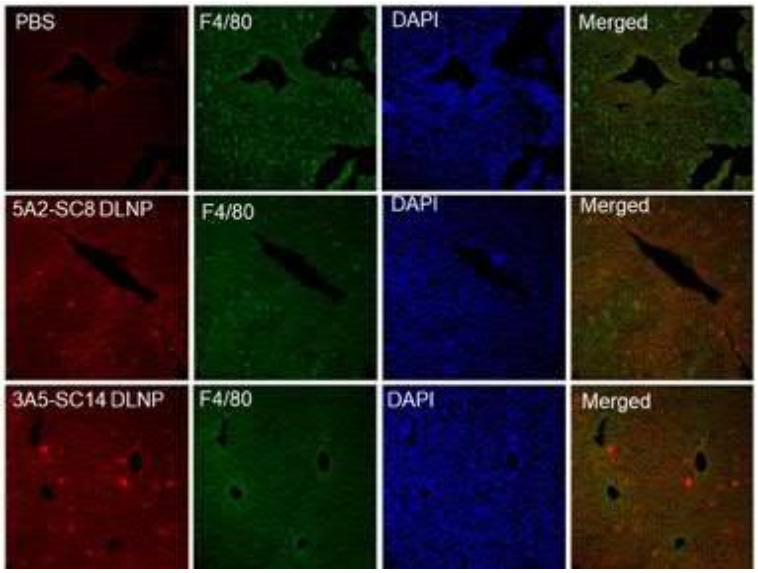
Subtask 1: Evaluate and optimize custom dendritic nanoparticle delivery to PDX tumors
This has been completed.

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.
This has been completed.

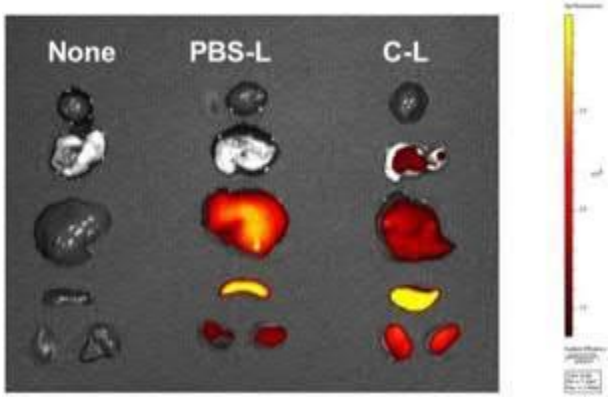
Subtask 3: Define HCC PDX models that overexpress MYC or LIN28B and those that suppress LET-7 family microRNAs
We did not find any PDXs that were overexpressing LIN28B, thus we have altered the targets for this subtask. Instead we will use an siRNA to target ANLN, which is a gene required for cytokinesis. We have previously shown in other work that this is a good therapeutic target for liver cancer.

Subtask 4: Therapeutically deliver siRNAs or microRNAs in dendritic nanoparticles to mice harboring these PDX models
In our previous report, we described our discovery of hepatocyte-specific and Kuppfer cell-specific dendrimer lipid nanoparticles (DLNPs). We made the exciting observation that dendrimer chemistry can control cellular tropism within the liver. Fortunately, the previously selected and validated carrier (5A2-SC8 dendrimer lipid nanoparticles (DLNPs)) with which we have obtained all prior tumor cell data continued to show efficacy in these new experiments. Thus, when comparing 5A2-SC8 DLNPs to 3A5-SC8 DLNPs, we found that 5A2-SC8 DLNPs continue to be ideal for carrying out the funded cancer studies.

We showed that 5A2-SC8 DLNPs can target cancer cells and hepatocytes in the liver, while 3A5-SC8 DLNPs instead target Kupffer cells. Building on this research, we have further confirmed these effects on the cellular level.



The above Figure shows differential uptake of DLNPs in the liver after delivery of siRNA-Cy5.5. Our previous data (from the last quarter report) was further confirmed. When we deleted Kuppfer cells in the liver, now the biodistribution of 3A5-SC8 DLNPs was significantly altered.



Depletion of Kupffer cells alters 3A5-SC14 DLNP biodistribution (3A5-SC14 DLNP 0.5 mg/kg siFVII-Cy5.5, 6 hours) from the liver Kuppfer cells to liver hepatocytes and splenic macrophages. This knowledge is continuing to aid our investigations of cancer therapy in PDX models. We have further expanded our models and nanoparticles in this past 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
Drs. Zhu, Yopp, and Siegwart are working on this now during the NCE period.

Subtask 2: Define histological response and intermediate markers of tumor biology (Ki67, apoptosis, necrosis)
To be completed after initiation of the small RNA therapeutics nanoparticle studies. We have obtained ANLN siRNAs from Alnylam to perform these experiments.

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 selected drug studies in the PDX models so this has been completed. We have also started testing experimental drugs that target telomere elongation. This is a drug developed by Jerry Shay at UTSW. So far, some PDXs have not responded to these drugs but we are continuing to add more PDX models to the testing scheme.
Subtask 2: Perform exome and RNA-expression sequencing for top responders and non-responders for each group to determine mechanistic basis of response This has not been completed because the numbers of PDXs and therefore responder/non-responder cohorts are too small for this type of analysis.
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 This has not been completed because we do not have enough responders and non-responders.
Subtask 2: Derive and internally validate predictive model using factors significantly associated with targeted therapy response This has not been completed and will be discontinued.
Milestone #3: Co-author manuscript on HCC PDX treatments and predictive modeling results This has not been completed. Other work involving PDX models will be pursued and published.

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

Nothing to report during this period.

How were the results disseminated to communities of interest?

Nothing to report besides the paper that we have submitted to Hepatology.

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

For the small RNA parts of the grant, we will shift focus to Anln siRNA in the place of Lin28-let-7 because there are potentially more PDX models which express and depend on Anln, a cytokinesis protein that is essential for cancer growth. This would help us considerably with the completion of AIM 2. Our goal is to test human *ANLN* siRNAs in human HCC PDX models. We will functionally assess the ability of either siRNA approach to enter and treat early HCCs. Because our previous siRNAs were designed against mouse ANLN, we will use optimized human ANLN siRNAs screened by our collaborator Alnylam. We will test delivery and efficacy in the distinct HCC PDX models that we have made (Zhu et al, *Hepatology, manuscript in revision*) and that we will continue to generate in a prospective fashion. All tumors will be grown until at least 200 mm^3 and then mice will be dosed with IV LNP-siRNA. Tumor volume will be followed over a 3-5 week period using the formula volume = $width^2$ x length/2. Completion of this goal will open up additional therapeutic development opportunities.

For Specific AIM 3 (Define targeted therapy responders with HCC-PDX patient avatars and use predictive modeling to identify prognostic biomarkers), we plan to treat these PDX lines with other drugs, including WNT inhibitors and other inhibitors from our collaborators. This would be valuable information for the development of WNT inhibitors.

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. We will be able to use these models to evaluate experimental therapeutics, in the form of small molecules or small interfering RNAs.

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

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

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.

None

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.

1. Zhu et al, Hepatology in revision.
2. "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, 2018, 30, 1805308.

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)**

Nothing to report

- **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: 36
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: 36
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: 36
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: 36
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: 36
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: 36
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: 36
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: 36
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: 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.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel

For Akbar Waljee, the following grant has become active:
UMHS-CGMH Waljee (Partner-PI) 06/01/2019-05/31/2020
Title: The genetic, environmental, and microbial determinant of IBD pathogenesis in Taiwan vs the U.S.
This joint project between CGMH and UMHS aims to better characterize IBD phenotypes and to identify potential risk factors by examining host genome, environmental exposures, and gut microbiota. Successful establishment of this pilot biorepository will provide the necessary preliminary data and available samples for an extramural grant application to perform more extensive analyses (e.g., host metatranscriptomics and serum metabolomics, fecal microbiota metagenomics/metatranscriptomics and metabolomics).

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.