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TITLE: Targeting Chemoresistance in Small Cell Lung Cancer

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CONTRACTING ORGANIZATION:

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14. ABSTRACT Small cell lung cancer (SCLC) is the deadliest form of lung cancer and the most strongly associated with smoking history. Unfortunately, chemotherapy remains the main treatment option for patients with SCLC. Although this cancer typically responds extremely well, relapse is fast and largely inevitable. There are no effective therapies for relapsed tumors. The goal of our proposed study is to find new targets for drug therapy against relapsed SCLC tumors and provide continued hope for these patients. In this regard, we have identified a protein called HEPACAM2, which demonstrates unique, high expression in SCLC cell lines and tumors, making it a very attractive target. Knockdown of HEPACAM2 leads to cell cycle arrest, followed by apoptosis. Tankyrase 1 (TNKS) has been shown to poly(ADP-ribosylate) HEPACAM2 and this modification is necessary for HEPACAM2 spindle localization and mitosis. Specific inhibitors of TNKS have been developed with little activity towards PARP1, a participant in the DNA damage response.

We hypothesize that HEPACAM2 represents a novel therapeutic target in SCLC and propose two specific aims: (1) to determine the expression and cellular localization of HEPACAM2 in SCLC, and (2) to determine if SCLC cells demonstrate increased sensitivity to TNKS inhibitors. HEPACAM2 expression will be tested at the mRNA and protein levels by qPCR and western blotting, respectively. Subcellular localization will be determined by confocal microscopy. Expression will be determined in multiple lung cancer cell lines, both SCLC and NSCLC, along with non-lung and normal cell lines as additional controls. Tumors will be investigated for expression, which will be correlated with survival. Inhibition of HEPACAM2 function will be tested by siRNA knockdown and small molecule inhibitors of TNKS using cell proliferation, migration and apoptosis assays. Synergism with other PARsylation inhibitors will also be explored.

This proposal will address the Area of Emphasis '*to understand predictive and prognostic markers to identify responders and non-responders'. HEPACAM2* expression could represent a predictive marker in this cancer showing sensitivity to TNKS inhibitors. In addition, its potential for variable expression in SCLC tumors could make its expression prognostic for survival or chemo-response.

15. SUBJECT TERMS
Small cell, lung cancer, HEPACAM2, poly-ADP-ribosylation, PARP, tankyrase

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	15	**19b. TELEPHONE NUMBER** *(include area code)*

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1. Introduction:

The goal of this study was to find new targets for drug therapy against relapsed SCLC tumors. In this regard, we focused on a protein called HEPACAM2, which appeared to be highly and specifically made only by SCLC tumors, making it a very attractive target. We sought to explore if HEPACAM2 was important for SCLC growth and, if so, how best to target it. Therefore, the aims of the study were to first determine the expression and cellular localization of HEPACAM2 in SCLC. HEPACAM2 expression would be tested at the mRNA and protein levels by qPCR and western blotting, respectively. Subcellular localization would be determined by confocal microscopy. Expression would be determined in multiple lung cancer cell lines, both SCLC and NSCLC, along with non-lung and normal cell lines as additional controls. Tumors would be investigated for expression, which would be correlated with survival. The second aim was to determine if HEPACAM2 could be used to specifically target SCLC cells using TNKS inhibitors. Tankyrase 1 (TNKS) has been shown to poly(ADP-ribosylate) HEPACAM2 and this modification is necessary for HEPACAM2 spindle localization and mitosis. Specific inhibitors of TNKS have been developed with little activity towards PARP1. Thus, SCLC may demonstrate particular sensitivity to TNKS inhibitors, leading to alterations in HEPACAM2 function measured by cell proliferation, migration and apoptosis assays. Synergism of TNKS inhibitors with other PARsylation inhibitors would also be explored

We have requested a no-cost extension for a year to allow us to continue pursuing these aims, thus this represents only an annual report. We describe below our progress for Year 1 as it relates to our SOW, some of the unexpected problems we have encountered and new, novel observations.

2. Keywords:

Small cell lung cancer, SCLC, HEPACAM2, HEPACAM, poly-ADP-ribosylation, PARP, tankyrase, TNKS, PARsylation

3. Accomplishments:

- **What were the major goals of the project? What was accomplished under these goals?** The goals are listed as the grant Specific Aims/tasks and are followed by relevant accomplishments.

<u>Specific Aim 1: To determine the expression and cellular localization of HEPACAM2 in SCLC</u>:

Major Task 1: To explore HEPACAM2 expression in SCLC and control cell lines (*ALL commercially available***: SCLC** H1694, H211, H1092, H1048, SHP77, H2141, H209, H82, DMS114, SW1271, H841, H1184; **NSCLC** A549, SW900, H2009, H1299, H520; **NORMAL** NL20)*:*

Subtask 1: Perform Taqman PCR.

Our initial excitement to study HEPACAM2 was based on data we extracted from the Cancer Cell Line Encyclopedia (CCLE) database (https://portals.broadinstitute.org/ccle/page?gene=RUNX1T1) demonstrating that HEPACAM2 expression was highly enriched in SCLC cell lines compared to NSCLC, as well as to all other cancer cell lines in the CCLE. Thus we began our study by using qPCR (Taqman) to validate the mRNA expression levels of both HEPACAM2 and HEPACAM among an assortment of SCLC, NSCLC, mesothelioma and normal lung cell lines. The results, shown on the left in Table 1, show that SCLC cell lines (red text) clearly express much higher levels of HEPACAM2 compared to NSCLC (blue text), mesothelioma (yellow text) and normal lung (black text) cell lines, and that the rank order of expression generally parallels that found by the CCLE dataset. We then performed qPCR for HEPACAM on the same samples and found, similar to the CCLE database, that HEPACAM mRNA was undetectable in SCLC and also very low in other thoracic cancer cells

(right side of Table 1). It should be noted that CCLE values are listed as log2, meaning that every 1.0 point change in value = two-fold change in mRNA expression. Our qPCR results are reported as the ratio HEPACAM2 mRNA normalized to that of β-actin. Taken together, these results confirm the highly specific expression of HEPACAM2 in SCLC and establish conditions to validate antibody results based upon the large difference in expression of HEPACAM2 between SCLC vs NSCLC.

Table 1: Validation of HEAPACAM2 mRNA expression by qPCR.

Hepacam2 q-PCR Basal Levels		Hepacam2 CCLE mRNA Levels			Hepacam q-PCR Basal Levels		Hepacam CCLE mRNA Levels	
H1694	4.318	H1694	13.5	SCLC	H520	2.15E-04	A549	4.73
DMS454	1.537	DMS454	12.61	NSCLC	NL-20	2.29E-05	H1869	4.68
H211	0.3383	H211	12.37	Meso	H2052	8.63E-06	H2029	4.62
H1092	0.2252	H69	12.36	Normal	Calu I	1.89E-06	H1299	4.61
H69	0.1761	H1092	12.01		H1694		H520	4.47
H2029	0.1574	H2171	11.34		DMS454		H211	4.36
H2171	0.115	H2029	11.16		H211		H209	4.34
H1048	0.0875	H1048	10.37		H69		SHP77	4.32
H526	0.0642	H526	9.91		H1092		Calu I	4.33
H209	0.0482	H209	8.44		H2171		H1975	4.32
SHP77	0.0095	H2141	7.77		H2029		DMS454	4.28
H2141	0.0057	SHP77	7.49		H1048		H1694	4.26
H1299	0.000166	H1869	3.78		H209		H2141	4.24
H520	0.000038	H1975	3.74		H2141		H1048	4.23
Calu I	5.17E-06	A549	3.72		H526		H2171	4.22
H1975	4.17E-06	H520	3.72		SHP77		H69	4.2
H2052	4.16E-06	H1299	3.69		H1299		H1092	4.2
NL-20	2.18E-06	Calu I	3.66		A549		H2052	4.15
A549	2.15E-06	H2052	3.58		H1869		H526	3.97
H1869	1.77E-06	NL-20	N/A		H1975		NL-20	N/A

Next, we wanted to obtain evidence that HEPACAM2 is highly and specifically expressed only in SCLC tumors. Thus, we compared RNAseq data of SCLC tumors (Rudin et al, 2012 dataset PMID 22941189) to that of others cancers found in the TCGA database, and again found that HEPACAM2 mRNA is highly enriched in SCLC tumors (see Figure 1), validating the results we obtained above for SCLC cell lines.

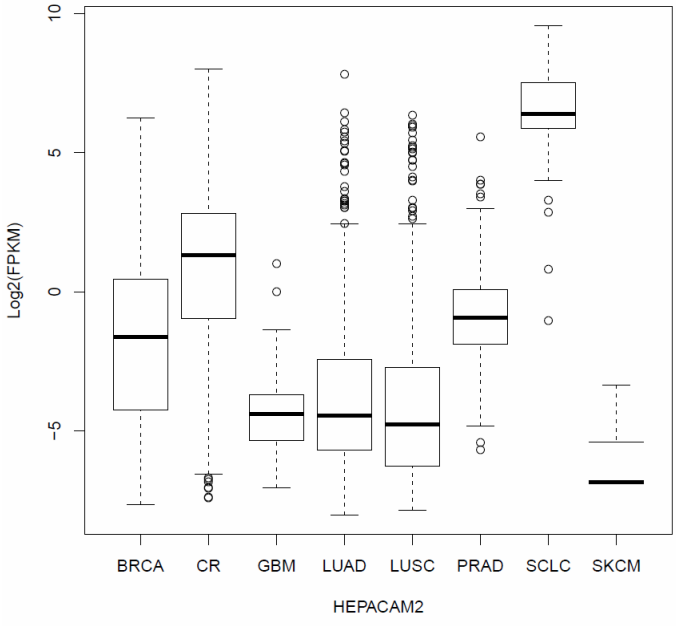

Figure 1: Boxplot of *HEPACAM2* mRNA expression in tumors. Determined from RNAseq data. BRCA: breast cancer, CR: colorectal cancer, GBM: glioblastoma, LUAD: lung adenoma NSCLC, LUSC: lung squamous NSCLC, PRAD: prostate adenoma cancer, SKCM: skin melanoma cancer.

Subtask 2: Perform western blots on protein lysates.

We next focused on finding an antibody that we could use for confocal analysis of cells as well as IHC of tumors, as these were major goals outlined for Aim 1. We chose an antibody from Bioss with reported utility in western blotting, immuno-fluorescence and immuno-histochemistry (IHC). We initially tested it by western blotting using SCLC cell lines having high levels of HEPACAM2 mRNA and obtained a relatively

clean band of about the expected size for HEPACAM2 = 52 kDa whose pattern of expression followed that of HEPACAM2 mRNA levels (see Figure 2). Encouraged by these results we began parallel experiments to use this antibody for immuno-fluorescent (IF) studies of cells and IHC of tumors. The results were disappointing. For IF, we stained DMS454 SCLC cells having high HEPACAM2 mRNA levels and NSCLC A549 cells as a negative control and obtained the same strong staining throughout the cells; no specific subcellular localization or SCLC specificity could be discerned despite multiple permutations to the staining protocol. In parallel we obtained IHC results of a TMA containing tissue cores of 22 SCLC and 12 NSCLC tumors, where again no specific staining of SCLC was observed relative to NSCLC. Thus, we abandoned further use of the Bioss antibody.

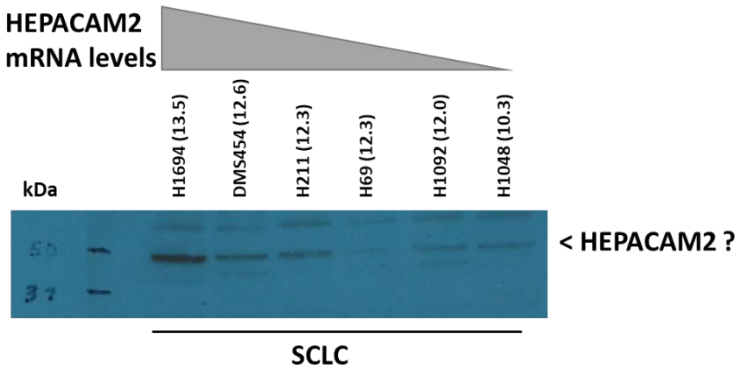

Figure 2: HEPACAM2 expression by western blotting: Western blotting results of SCLC cell line protein lysates using Bioss (#8417R) antibody. Cells are arranged, left to right, from highest to lowest HEPACAM2 mRNA levels. Arrow indicates putative HEPACAM2 band, which is reported to have a molecular weight of 52 kDa. The numbers in parentheses indicate mRNA expression values from the CCLE.

We next tried an antibody from Novus that was approved for western blotting. We hoped that it could also be useful for future IF and IHC experiments. When we western blotted an extended panel of cell lines with this antibody, a band of lower than expected size of HEPACAM2 was again observed whose expression roughly paralleled that of HEPACAM2 mRNA levels (see Figure 3). Although this lower band was similar in size to that visualized by the Bioss antibody, the results did not quite match, giving us some concern, as did the stronger band of higher molecular weight that did not correlate with HEPACAM2 mRNA expression (labeled NS in Figure 3).

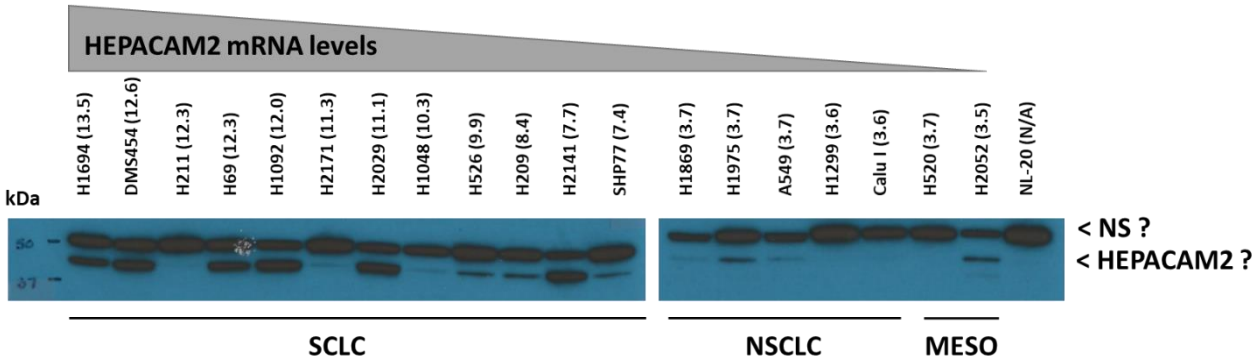

Figure 3: HEPACAM2 expression by western blotting: Western blotting results of different thoracic cell line protein lysates using Novus (#NBP1) antibody. Cells are arranged, left to right, from highest to lowest HEPACAM2 mRNA levels. Arrow indicates putative HEPACAM2 band, as well as a putative non-specific band (NS). MESO = mesothelioma. NL20 is a 'normal' lung cell line. The numbers in parentheses indicate mRNA expression values from the CCLE.

Subtask 3: Perform confocal microscopy:

See Major Task 1, subtask 2 immediately above. We did try with the Bioss antibody but there was no difference in staining intensity or location between SCLC (DMS454) and a NSCLC (A549) cells- contrary to our mRNA expression analysis of SCLC cell lines. We will try again when we have better validated antibodies (see below).

Major Task 2: To analyze HEPACAM2 expression in SCLC tumors:

Subtask 1: Optimize IHC detection of HEPACAM2 in SCLC tumors.

See Major Task 1, subtask 2 immediately above. We did try with the Bioss antibody on a mixed lung cancer TMA but there was no difference in staining intensity between SCLC and NSCLC- contrary to our mRNA expression analysis of SCLC cell lines and tumors. Thus, we judged this IHC to be unreliable and will await the outcome of experiments described below to obtain a better validated antibody.

Subtask 2: IHC stain 2-3 SCLC TMAs for HEPACAM2.

See Major Task 2, subtask 1 immediately above.

Subtask 3: Read & correlate IHC staining with clinical outcomes.

See Major Task 2, subtask 1 immediately above.

<u>Specific Aim 2: To determine if SCLC cells demonstrate increased sensitivity to TNKS inhibitors and synergism with other PARsylation inhibitors</u>:

Major Task 1: Determine efficacy of TNKS inhibitors in SCLC and control cells: (cells named above).

Subtask 1: Determine efficacy on cell growth & migration using IncuCyte ZOOM.

Independent of our experiments for Specific Aim 1 we began to look for effects of TNKS inhibitors on SCLC cell growth. Initially we choose the drug NVP-TNK S656 because of its high potency (IC50 6 nM) and selectivity (300-fold over PARP1/2). We choose three cell lines each with high (H1694, H2171, H1048) versus low (H446, SW1271, DMS114) HEPACAM2 mRNA levels. We proposed that SCLC cells with high HEPACAM2 expression would be most sensitive to growth inhibition by TNKS inhibitors.

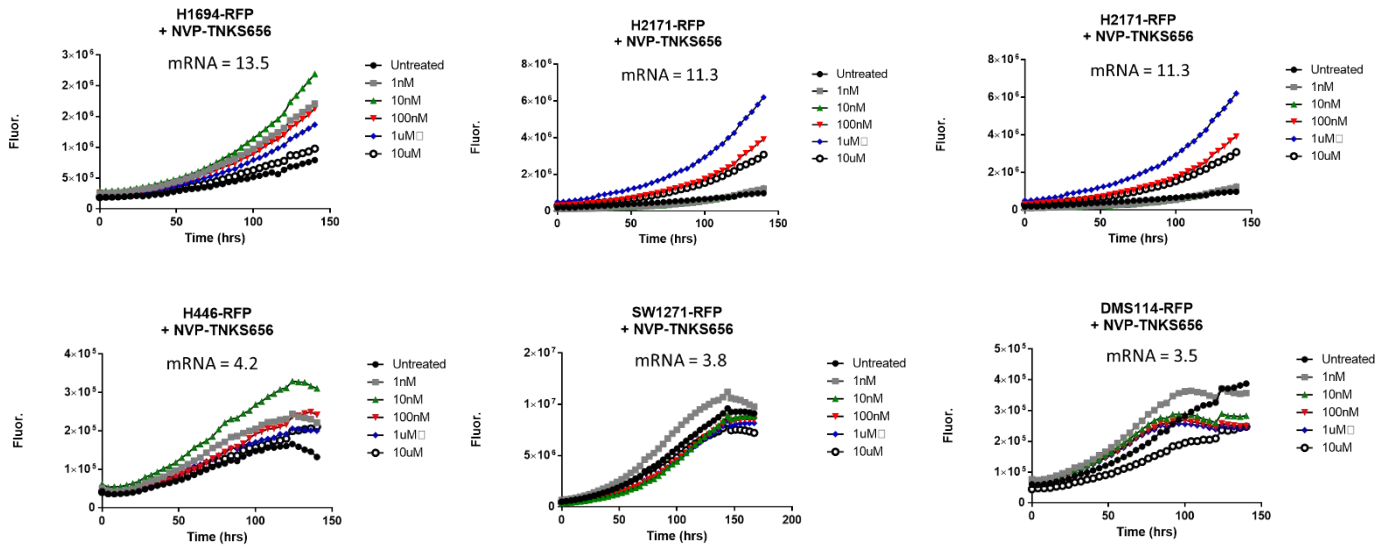

Figure 4: Effects of drug TNKS656 on SCLC cell growth: Real time measurement of RFP-labeled cell growth over 150-200 hr in the presence of five concentrations of drug (0–10 µM) using IncuCyte ZOOM. Top three cells express high and bottom three cells express low HEPACAM2 mRNA levels. Numbers for mRNA expression values from the CCLE.

Our results, shown in Figure 4, demonstrated that at a dose of 100 nM (red line), cells with high *HEPACAM2* mRNA exhibited *increased* cell growth, whereas growth in cells with low HEPACAM2 expression were relatively unaffected compared to untreated cells (black line). This result is counter to what we expected and will be expanded to include more TNKS drugs and SCLC/NSCLC cell lines in future experiments.

Subtask 2: Determine effects on subcellular localization, cell cycle arrest (FACS) and apoptosis induction by western blotting.

See Aim 1, Major Task 1, subtask 2 above for delay in subcellular localization studies. Because we observed increased cell growth by TNKS inhibition, this must be verified in more SCLC cell lines; but if true, it would rule out performing any apoptosis assays.

Major Task 2: Determine synergy between TNKS and PARP inhibitors: (cells named above).

Subtask 1: Determine synergism on cell growth & migration using IncuCyte ZOOM.

Nothing to report until a definitive answer is obtained for TNKS inhibitors in more cell lines.

Subtask 2: Determine synergistic effects on subcellular localization, cell cycle arrest (FACS) and apoptosis induction by western blotting.

See answers above in Aim 2.

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

Nothing to report.

- **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?**

Specific Aim 1: As outlined below, we hope to use all of the experimental tools we have at our disposal to validate results of antibody experiments such that we can confidently detect HEPACAM2 protein expression in SCLC cell lines and tumors.

Specific Aim 2: We plan on expanding these experiments to include more TNKS drugs and SCLC/NSCLC cell lines to establish if TNKS inhibition leads to *cell proliferation*, rather than cell inhibition as originally proposed. Using NSCLC cells will help establish the specificity of this drug effect. We will also explore assays on cell adhesion and migration because of our most recent observations with cells over-expressing HEPACAM2 (see below).

4. Impact:

- **What was the impact on the development of the principal discipline(s) of the project?**

Taken together, we have confirmed the specific expression of *HEPACAM2* mRNA in SCLC, but not in NSCLC or many other cancers; however, it remains unclear what the function of HEPACAM2 is in SCLC, let alone where

it is localized within SCLC cells. Thus HEPACAM2 remains an attractive therapeutic target in SCLC if we can discover more about its biological role in cancer.

- **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:

- **Changes in approach and reasons for change.**

We recently decided on a multi-prong approach to clarify the true HEPACAM2 band in western blots by: 1) purchasing three new antibodies for western blotting having matching blocking peptides to see what band(s) would disappear in western blots in the presence of peptide blocker, 2) making stable knockouts of endogenous HEPACAM2 using CRISPR to determine which band(s) disappears in western blots compared to parental controls, and 3) transiently over-expressing FLAG-tagged HEPACAM2 in HEK293T and other cells to determine its appropriate size and to serve as a positive control for other antibodies we have tested by western blotting for HEPACAM2. So far we have obtained the following results:

1) We purchased two antibodies from Aviva (epitope in N-terminal and mid regions of protein) and one from ProSci (epitope in C-terminal region). The Novus antibody also recognizes the exact same mid region of HEPACAM2 as Aviva.

2) We isolated pools of DMS454, H1694, H2029 and H69 cells with stable knockout (KO) of HEPACAM2 using CRISPR using three different guide RNAs = 12 new stable cell lines! The CRISPR guides were obtained from Genscript in lentiviral backbones, which we then packaged into viral particles and infected into each cell line. When we performed qPCR for *HEPACAM2* mRNA measurement in the CRISPR pools, however, little or no detectable KO was observed. Furthermore, we also could not detect HEPACAM2 KO on western blots using any of the five HEPACAM2 antibodies at our disposal. We have previously performed viral transduction of other genes into these same SCLC cells with no problem, so we believe the CRISPR KO should have worked. Occasionally, however, qPCR is unable to detect CRISPR KO because of the nature of the gene splicing event, so we are going to confirm if there is any effect of the CRISPR in our stable KO pools by taking the mRNA we have isolated, performing RT-PCR to generate PCR fragments containing the CRISPR guide site and using endonuclease digestion and nucleotide sequencing to determine the CRISPR efficacy.

We have also ordered siRNA to HEPACAM2 to attempt transient knockdown (KD) of the mRNA/protein. Using siRNA should allow us to definitively identify decreases in HEPACAM2 mRNA and thus allow us to probe protein lysates of treated cells with our new antibodies listed above. siRNA-treated cells can also be used in confocal and IHC validation experiments as negative controls.

3) We ordered a FLAG-tagged HEPACAM2 lentivirus from Genscript. We have used this to produce stable expression of HEPACAM2 in five cell lines with low putative HEPACAM2 protein expression (see Figure

3 above): HEK293T, H1299 and H1869 (both NSCLC) and H2171 and H211 (both SCLC). We are growing up these HEPACAM2 over-expressing cells now. We have noticed, however, that the adherent cell line H1299 cells are *very* difficult to trypsinize off the dish when we want to split them, and the mixed adherent/suspension cell line H211 cells have a greater percentage of adherent cells compared to their respective parental cells. This observation indicates that we should explore the effects of HEPACAM2 over-expression and knockdown on cell adhesion and/or migration, which we have added as the new Major Task 2 of Specific Aim 2, *replacing* the old one, which we do not think we will have time to pursue.

These new proposed experiments have been added as new Subtasks 1b-1d and 2b-2d in Specific Aim 1, Major task 1 of the revised SOW added to the Appendix. We have also replaced Major Task 2 of Specific Aim 2 to pursue our new experimental finding that HEPACAM2 may increase cell adhesion. This observation, if it holds true, would be novel and give us our first insight into the biological role of HEPACAM2. It would also suggest that HEPACAM2 does indeed reside on the cell surface and may therefore be a good therapeutic target.

- **Actual or anticipated problems or delays and actions or plans to resolve them.**

See answer to section 5 immediately above.

- **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.**

Nothing to report.

6. Products:

Nothing to report.

7. Participation & other collaborating organizations:

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

Name:	Afshin Dowlati, MD
Project role:	PI
Researcher identifier:	Orchid # 0000-0003-4535-6839
Nearest person months worked:	12
Contribution to project:	PI- oversight and direction.
Funding support:	This and grants listed below and University Hospitals of Cleveland research support.

Name:	Karen McColl, BS
Project role:	Lab manager
Researcher identifier:	N/A
Nearest person months worked:	12

Contribution to project:	Ms. McColl has worked on all aspects of this project.
Funding support:	This and University Hospitals of Cleveland research support.

Name:	Gary Wildey, PhD
Project role:	Program manager
Researcher identifier:	Orchid # 0000-0001-7105-1313
Nearest person months worked:	12
Contribution to project:	Research advise and report writing.
Funding support:	University Hospitals of Cleveland research support and grants listed below.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Yes, one new grant was funded, one was discontinued:

Active:

U24 (Rudin, PI) 02/20/17-01/31/22 0.6 calendar
NIH/NCI $75,000 (sub only)
Small-cell Lung Cancer (SCLC) Consortium: Coordinating Center
Subproject: *Construction of SCLC tissue micro-arrays*

Here we propose to construct multiple tissue micro-arrays from de-identified small-cell lung cancer tumor specimens and provide matching genomic profiling data on tumor specimens with sufficient tissue. Additional data on patient and tumor features will be provided for each tumor specimen placed in the TMA.
Role: Leader, tissue micro-array subproject.
Overlap: None- no funds are requested to make the TMAs used in the current application, as stated in the Budget Justification.

R21 (Dowlati, PI) 07/01/18-06/30/20 1.2 calendar
NIH/NCI $275,000
Identification and targeting of chemotherapy refractory small cell lung cancer

Here we propose to determine whether *RB1* mutation status and/or protein expression identifies chemo-refractory patients in small-cell lung cancer (SCLC). A secondary aim is to determine whether *RB1* mutation status and/or protein expression predicts sensitivity to CDK4/6 inhibitors in SCLC models.
Overlap: None.

Discontinued:

Concept Award (Dowlati, PI) 09/01/16-08/31/18 1.2 calendar
DOD < $60,000 (no cost extension)
RUNX1T1 amplification induces 'small cell' cancer

Here we examine the role of RUNX1T1 in small-cell lung cancer and the potential role it may play in non-small to small-cell lung cancer transformation.

Overlap: None.

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

Nothing to report.

8. Special reporting requirements:

Nothing to report.

9. Appendix:

Revised SOW.

REVISED STATEMENT OF WORK – 08/15/2018
START DATE Sept 01, 2017

Site 1: Case Western Reserve University
11100 Euclid Ave
Cleveland, OH 44106
PI: Afshin Dowlati

Specific Aim 1: **To determine the expression and cellular localization of HEPACAM2 in SCLC:**	**Timeline** **(Months)**	
Major Task 1: To explore HEPACAM2 expression in SCLC and control cell lines (*ALL commercially available*: **SCLC** H1694, H211, H1092, H1048, SHP77, H2141, H209, H82, DMS114, SW1271, H841, H1184; **NSCLC** A549, SW900, H2009, H1299, H520; **NORMAL** NL20)		
Subtask 1: Perform Taqman qPCR on: 1a. Endogenous HEPACAM2 1b. siRNA-treated SCLC to transiently KD HEPACAM2 1c. FLAG-tagged cells over-expressing HEPACAM2 1d. Cells with CRISPR KO HEPACAM2	1-18	Karen McColl
Subtask 2: Perform western blots on protein lysates from: 2a. Endogenous HEPACAM2 2b. siRNA-treated SCLC to transiently KD HEPACAM2 2c. FLAG-tagged cells over-expressing HEPACAM2 2d. Cells with CRISPR KO HEPACAM2	3-18	Karen McColl
Subtask 3: Perform confocal microscopy.	18-24	Karen McColl, Microscopy core
Major Task 2: To analyze HEPACAM2 expression in SCLC tumors:		
Subtask 1: Optimize IHC detection of HEPACAM2 in SCLC tumors. (*performed on cell line pellets or slides, named above*)	18-20	Histology core
Subtask 2: IHC stain 2-3 SCLC TMAs for HEPACAM2. (*in-house made TMAs*)	20-22	Histology core
Subtask 3: Read & correlate IHC staining with clinical outcomes.	22-24	Dr. Yang, Dr. Fu, Dr. Dowlati
Milestone(s) Achieved: IRB approval for TMA study	*02/28/2018 (most recent, good for 1 yr)*	
Milestone Achieved: HRPO/ACURO Approval:	*09/15/17 (original) 06/07/18 (continuing*	

	review approval)	
Specific Aim 2: **To determine if SCLC cells demonstrate increased sensitivity to TNKS inhibitors and synergism with other PARsylation inhibitors:**		
Major Task 1: Determine efficacy of TNKS inhibitors in SCLC and NSCLC cells as controls: (subset of cells named above)		
Subtask 1: Determine efficacy on cell growth, adhesion & migration using IncuCyte ZOOM.	6-24	Karen McColl
Subtask 2: Determine effects on subcellular localization.	6-24	Karen McColl, Microscopy core
Major Task 2: Determine effect of HEPACAM2 over-expression and knockdown: (subset of cells named above)		
Subtask 1: Determine effects on cell growth, adhesion & migration using IncuCyte ZOOM.	12-24	Karen McColl, Dr. Dowlati
Subtask 2: Determine effects on subcellular localization.	12-24	Karen McColl, Dr. Dowlati
Milestone(s) Achieved:		