

AWARD NUMBER: W81XWH-17-1-0206

TITLE: Novel Therapy Strategies for Mesenchymal Non-Small Cell Lung Cancer

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CONTRACTING ORGANIZATION: University of Texas MD Anderson Cancer Center  
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REPORT DATE: July 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;  
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<b>1. REPORT DATE</b> July 2018			<b>2. REPORT TYPE</b> Annual			<b>3. DATES COVERED</b> 1 Jul 2017 - 30 Jun 2018		
<b>4. TITLE AND SUBTITLE</b> Novel Therapy Strategies for Mesenchymal Non-Small Cell Lung Cancer						<b>5a. CONTRACT NUMBER</b>		
						<b>5b. GRANT NUMBER</b> W81XWH-17-1-0206		
						<b>5c. PROGRAM ELEMENT NUMBER</b>		
<b>6. AUTHOR(S)</b> Faye Johnson, MD, PhD  E-Mail: fmjohns@mdanderson.org						<b>5d. PROJECT NUMBER</b>		
						<b>5e. TASK NUMBER</b>		
						<b>5f. WORK UNIT NUMBER</b>		
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  University of Texas MD                      1515 Holcombe Blvd Unit 207 Anderson Cancer Center                      Houston, TX 77030-4017						<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>		
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012						<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>		
						<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>		
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited								
<b>13. SUPPLEMENTARY NOTES</b>								
We discovered that mesenchymal non-small cell lung cancer (NSCLC) cells were more sensitive to polo-like kinase (PLK1) inhibitors than epithelial cell lines. Additionally cMet and FAK were activated in resistant/epithelial NSCLC cell lines and inhibited in sensitive/mesenchymal lines following PLK1 inhibition. We hypothesize that mesenchymal NSCLC will undergo apoptosis following PLK1 inhibition <i>in vivo</i> but that activation of cMet will mediate resistance in epithelial NSCLC. We have made significant progress in testing this hypothesis. Treatment in four patient derived xenograft models demonstrated that the PLK1 inhibitor volasertib was more effective in the mesenchymal models. Likewise volasertib was more effective in the mesenchymal orthotopic NSCLC model than in its isogenic epithelial pair. We found a similar degree of epithelial to mesenchymal transition (EMT) within each NSCLC tumor but considerable intra-tumor EMT heterogeneity. We discovered that cMet was the upstream regulator of FAK that was driving resistance in the epithelial NSCLC. Combination studies with cMet and PLK1 inhibitors showed significant apoptosis in all NSCLC models tested. Overexpression of constitutively active cMet led to resistance to PLK1 inhibitors. In conclusion, our research supports our hypothesis, we are ahead of our planned schedule, and we anticipate that we will complete the project as proposed.								
<b>15. SUBJECT TERMS</b> Polo-like kinase 1 (PLK1) Non-small cell lung cancer (NSCLC)								
<b>16. SECURITY CLASSIFICATION OF:</b>				<b>17. LIMITATION OF ABSTRACT</b>	<b>18. NUMBER OF PAGES</b>	<b>19a. NAME OF RESPONSIBLE PERSON</b>		
<b>a. REPORT</b>	<b>b. ABSTRACT</b>	<b>c. THIS PAGE</b>	<b>USAMRMC</b>					
Unclassified	Unclassified	Unclassified	Unclassified		11	<b>19b. TELEPHONE NUMBER (include area code)</b>		

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## 1. INTRODUCTION

The successful completion of this project will validate polo-like kinase 1 (PLK1) as a potential therapeutic target for mesenchymal non-small cell lung cancer (NSCLC) and provide prospective biomarkers of response to PLK1 inhibitors that could be used to select patients for a future clinical trial (Aim 1). We may also identify rational drug combinations for clinical trial by testing anti-PD1 antibodies (Aim 2) and cMet inhibitors (Aim 3) with PLK1 inhibition.

## 2. KEYWORDS

polo-like kinase 1 (PLK1)  
non-small cell lung cancer (NSCLC)

## 3. ACCOMPLISHMENTS

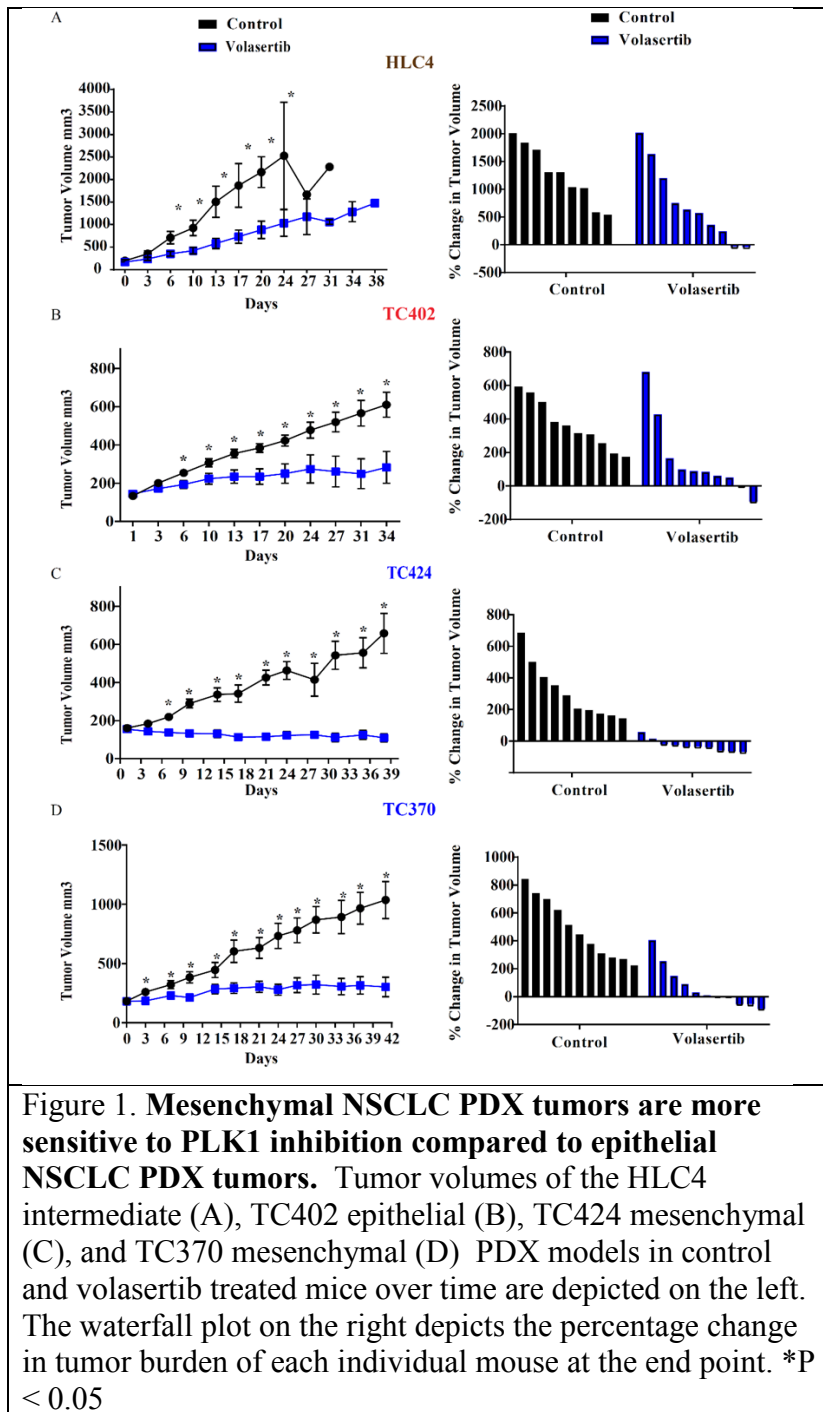
What were the major goals of the project

<b>Specific Aim 1: To validate mesenchymal biomarkers as predictors of PLK1 inhibition–induced apoptosis in NSCLC <i>in vivo</i> using patient derived xenografts (PDXs) and an immunocompetent, orthotopic model of NSCLC.</b>	<b>Timeline</b>	<b>Site 1</b>
<b>Major Task 1: Determination of biomarker expression and efficacy of volasertib in PDX models</b>	Months	
Milestone: Obtain HRPO approval	3	Dr. Johnson
Milestone: Obtain ACURO approval	3	Dr. Johnson
Implant 20 independent PDX models into 10 mice per group with one, independent biological replicate group. The number of mice needed for this experiment is: 5 mice per treatment group x 2 treatment groups x 2 biological replicates x 20 PDX models = 400 mice.	4-17	Dr. Johnson, 400 mice
Treat mice with volasertib or DMSO (n=5 mice per treatment group)	7-18	Dr. Johnson
Collect tissue for biomarkers	7-19	Dr. Johnson
Biomarker measurement and quantification via IHC staining.	19-21	Dr. Wistuba
Data analysis	21-24	Dr. Johnson
<b>Major Task 2: Efficacy of volasertib in NSCLC with epithelial and mesenchymal phenotypes (GEMM tumors)</b>		
Transfection of murine cells from Kras <sup>LA1/+</sup> p53 <sup>R172HΔG/+</sup> (KP) mice.	6-9	Dr. Johnson and Gibbons
Orthotopic implantation, treat with volasertib or DMSO. For each cell line, the tumor cells are implanted in the lungs of 20 mice. Five mice that express miR200a/b will be used for treatment, five mice that express ZEB1 will be used for treatment and ten for control. Thus, each independent model requires 20 mice. With 6	9-16	Dr. Johnson and Gibbons, 120 mice

cell lines, the total number of mice required for this aim will be: 20 mice per cell line x 6 cell lines = 120		
Biomarker measurement and quantification via IHC staining.	10-17	Dr. Wistuba
Data analysis	18-24	Dr. Johnson
<b>Specific Aim 2: To test the hypothesis that the combination of anti-PD1 immunotherapy and PLK1 inhibition will lead to tumor regression in mesenchymal NSCLC in vivo.</b>	<b>Timeline</b>	<b>Site 1</b>
<b>Major Task 3: Establish the degree of heterogeneity of EMT in NSCLC patient tumors</b>		
Measure the EMT score in 3 regions of 100 NSCLC tumors to estimate the intra-tumor heterogeneity of the EMT score.	1-12	Dr. Johnson, funded other sources (donors)
<b>Major Task 4: Test the combination of PLK1 inhibitors with PD1 inhibitors in syngeneic models of mesenchymal NSCLC</b>		
Three, independent isogenic mesenchymal cell lines from KP mice will be injected into 129Sv mice	9-12	Dr. Gibbons
Treat with murine anti-PD1 or control. Three mesenchymal models x 10 mice per treatment arm x 4 treatment groups = 120 mice.	12-18	Dr. Johnson, 120 mice
Biomarker measurement with TILs measured by flow cytometry.	17-19	Dr. Johnson and Dr. Wistuba
Data analysis	19-24	Dr. Johnson
<b>Specific Aim 3: To determine if FAK mediates PLK1-inhibitor induced apoptosis in NSCLC in vitro and in vivo.</b>	<b>Timeline</b>	<b>Site 1</b>
<b>Major Task 5: Test the combination of PLK1 inhibitors with FAK inhibitors</b>		
Test PLK1 and FAK (defactinib) inhibitors in vitro. Effects of the single agents and combinations on senescence, apoptosis, and cell cycle will be measured	1-9	Dr. Johnson
Determine if FAK overexpression leads to PLK1 inhibitor resistance via use of an inducible vector to conditionally express FAK in 3 mesenchymal (commercially, available) NSCLC cell lines.	10-17	Dr. Johnson
Data analysis	18-24	Dr. Johnson

**What was accomplished under these goals?**

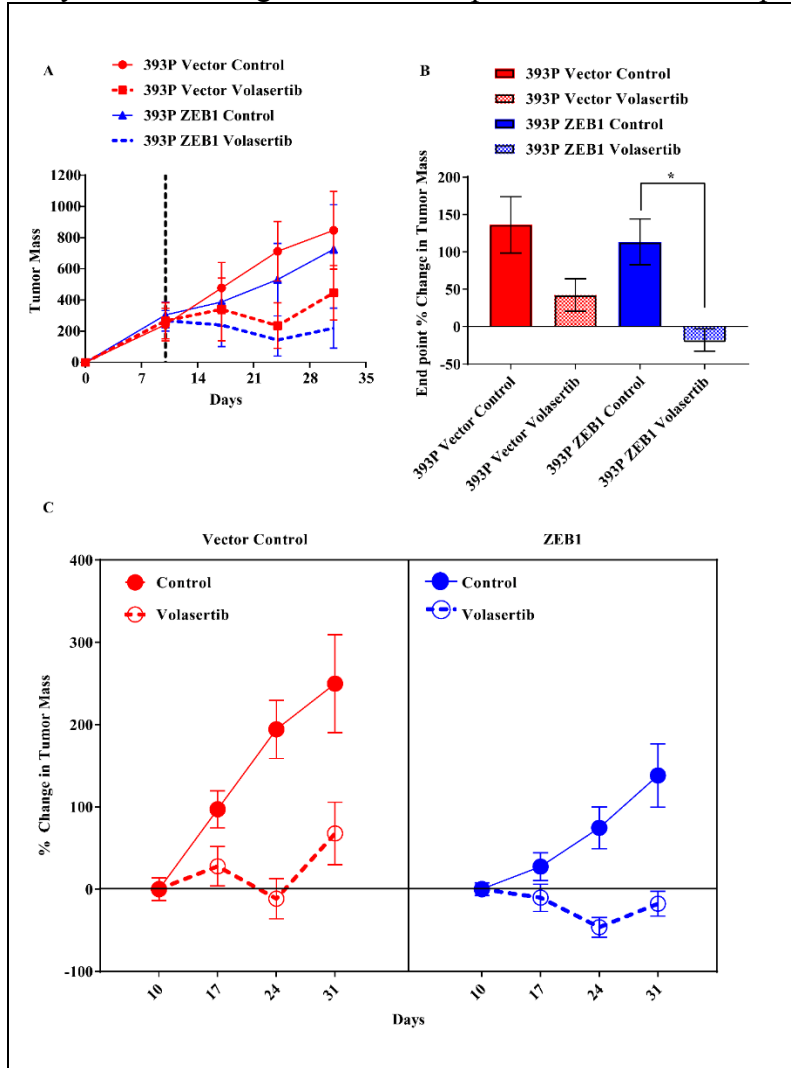
Under major task 1, both HRPO and ACURO approvals were obtained. Treatment in four patient derived xenograft (PDX) models has been completed. The PLK1 inhibitor volasertib was effective in all the PDX models with more efficacy in the mesenchymal models (Figure 1).



Under major task 2, the proposed experiments using KP mice with inducible ZEB1 have been completed. In the epithelial 393P vector model, volasertib led to a decrease in tumor mass on day 31 but the change was not statistically significant ( $p=0.08$ ) using Mann-Whitney t-test statistical method. The 393P cells with ZEB1 overexpression were slower growing *in vivo* in comparison to the 393P Vector cells. We observed a significant decrease in tumor mass in the 393P ZEB1 mice treated with volasertib in comparison to the 393P ZEB1 vehicle control mice ( $p=0.011$ ) as determined by one way anova (Figure 2). In the 393P vector mice treated with Volasertib, 3/10 tumors regressed while 7/10 tumors grew on therapy. In the 393P ZEB1 mice treated with Volasertib, 7/10 tumors regressed and 3/10 tumors grew on therapy. The overall percent change in tumor mass at day 31 in the four groups is depicted in Figure 2 which demonstrates that tumor regression was observed in the mesenchymal 393P ZEB1 mice treated with volasertib in comparison to the 393P Vector group where

only growth arrest of tumors was observed.

In order to quantitate the thoracic tumor burden in mice, we developed a novel method to analyze the CT images. A manuscript of this method is in preparation.



**Figure 2. Disease progression in 393P Vector and 393P ZEB1 orthotopic mouse model treated with vehicle control or volasertib.** A: The figure represents the tumor mass of mice in the 4 over groups over time. The dotted line represents the start of Volasertib or vehicle control treatment, i.e. day 10. B: The bar graph represents the percent change in tumor mass in the 4 groups at day 31. Tumor regression was observed in the 393P ZEB1 mice treated with Volasertib. C: The graph depicts the percent change in tumor mass in the 393P vector group treated with vehicle control or Volasertib and the 393P ZEB1 group treated with vehicle control or Volasertib. \*P < 0.05

We completed major task 3 that was recently published (donor funded):  
 W.C. Lee, L. Diao, J. Wang, J. Zhang, E.B. Roarty, S. Varghese, C.W. Chow, J. Fujimoto, C. Behrens, T. Cascone, W. Peng, N. Kalhor, C.A. Moran, A. Weissferdt, **F.M. Johnson**, W.N. William, Jr., S.G. Swisher, J.J. Lee, W.K. Hong, J.V. Heymach, Wistuba, II, P.A. Futreal, J. Zhang, Multiregion gene expression profiling reveals heterogeneity in molecular subtypes and immunotherapy response signatures in lung cancer, *Mod Pathol*, (2018).

This research showed that, “different regions of the same tumors demonstrated similar epithelial–mesenchymal transition scores for the most part [intraclass correlation coefficient of epithelial–mesenchymal transition score: 0.71, 95% CI (0.43, 0.90)]; however, considerable intra-tumor heterogeneity of epithelial–mesenchymal transition scores were observed in some tumors.”

We have not yet started major task 4.

We have completed major task 5. We discovered that cMet was the upstream regulator of FAK that was driving resistance in

the epithelial NSCLC cell lines (data not shown). Combination studies with cMet and PLK1 inhibitors (Figure 3) showed more efficacy than combinations with FAK and PLK1 inhibitors.

Overexpression of a constitutively active form of cMet led to resistance to PLK1 inhibitors (Figure 4). A manuscript of this work is in preparation.

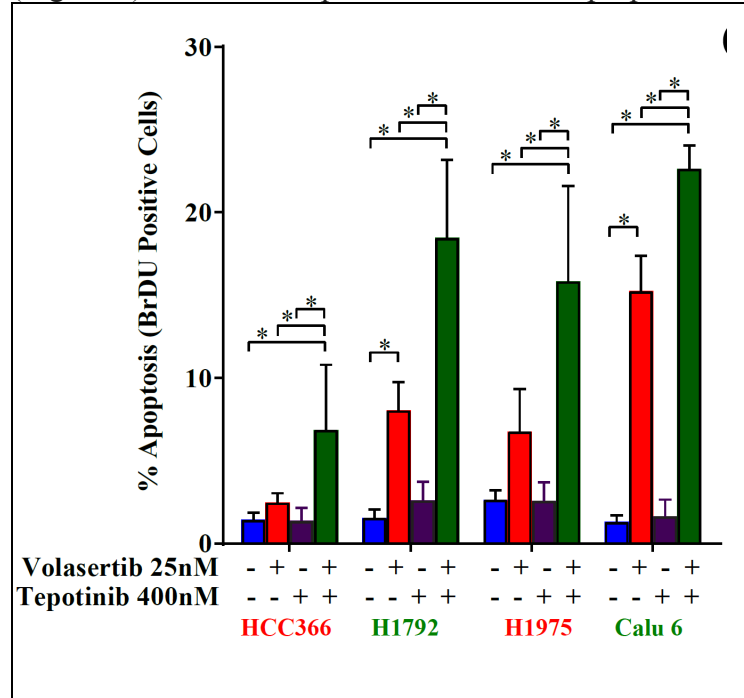


Figure 3. **The combination of cMet and PLK1 inhibition enhances apoptosis in NSCLC cell lines in vitro.** Four NSCLC cell lines were incubated with the PLK1 inhibitor volasertib or the cMet inhibitor tepotinib or both or vehicle alone for 48 hours. Apoptosis was measured using the TUNEL assay. \*P < 0.05

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

Two trainees were funded by this project. Dr. Singh and Ms. Viswanath both attended monthly journal clubs and weekly research seminars that were focused on signal transduction and lung cancer. Both also presented their work in this research seminar.

Dr. Singh attended the American Association of Cancer Research (AACR) annual meeting that included research presentations from multiple world-renowned cancer researchers. Dr. Singh presented his research at AACR.

Ms. Viswanath completed coursework and other requirements to successfully obtain a master's degree in biomedical sciences.

### How were the results disseminated

#### to the communities of interest?

Dr. Singh presented his research at AACR.

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

For major task 1, we are currently implanting more PDX tumors to complete this Aim. For major task 2, we have already completed one pilot study with the miR200-expressing KP model. Once these animal studies have been completed, the planned biomarker analyses will be initiated. We will initiate major task 4 within the next 4 months.

## 4. IMPACT

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

Nothing to report.

### 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

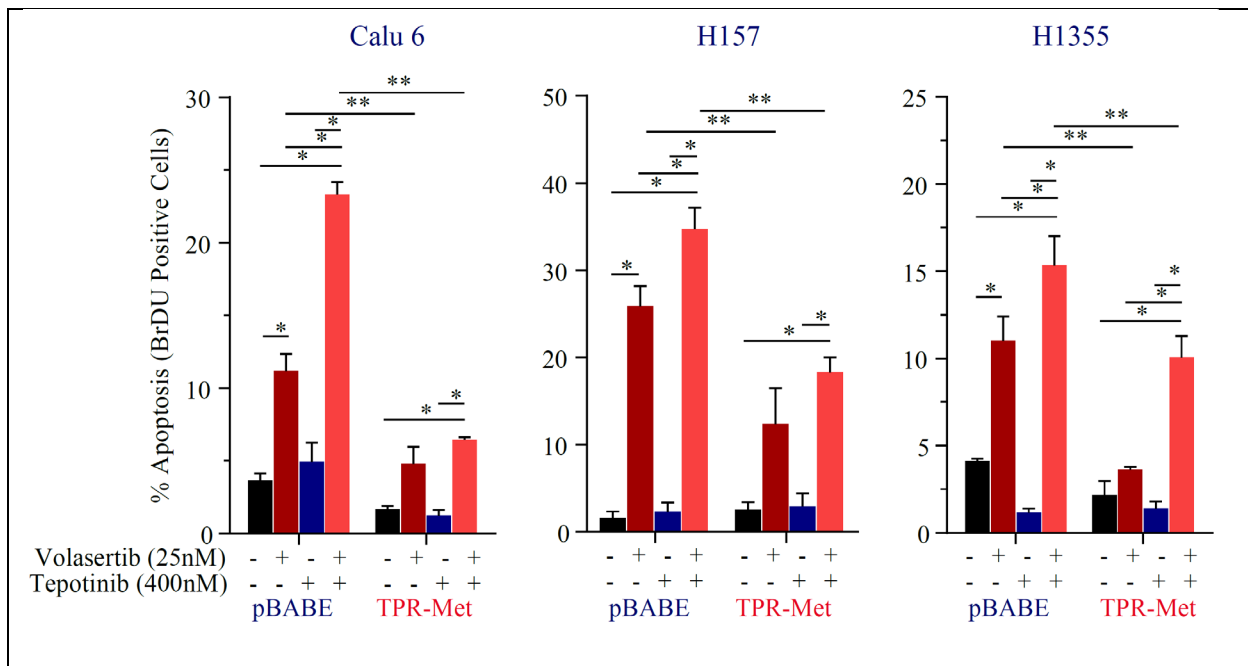


Figure 4. **The expression of constitutively active cMet leads to PLK1 inhibitor resistance.** Three NSCLC cell lines were transfected with a constitutively active cMet (TPR-Met) or vector control (pBABE). After confirming TPR-Met expression (not shown), cells were incubated with the PLK1 inhibitor volasertib or the cMet inhibitor tepotinib or both or vehicle alone for 48 hours. Apoptosis was measured using the TUNEL assay. \*P < 0.05

## 5. CHANGES/PROBLEMS

### Changes in approach and reasons for change

As noted above, we changed our focus in major task 5 from FAK to cMet because cMet was driving FAK activation. Combination studies with cMet and PLK1 inhibitors showed more promise than combinations with FAK and PLK1 inhibitors.

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

Nothing to report

### 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

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

Nothing to report

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

Nothing to report

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

Nothing to report

**6. PRODUCTS**

**Publications, conference papers, and presentations**

Singh S, Viswanath P, Peng S, Sambandam V, Shen L, Li L, Wang J, Fang B, Johnson FM. Noncanonical c-Met activation mediates de novo and acquired resistance to polo-like kinase 1 inhibitor-induced apoptosis in non-small cell lung cancer. American Association for Cancer Research Annual Meeting (AACR) - Chicago, IL (#895), 4/2018.

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

Nothing to report

**Technology or techniques**

Nothing to report

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

Nothing to report

**Other Products**

**7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

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

Name: Faye Johnson	Faye Johnson
Project Role:	Principal Investigator
Nearest Person Month Worked	2
Contribution to Project:	Dr. Johnson conceived and designed the proposed project and is responsible for overall scientific direction of the research, for reviewing and

	validating the data resulting from the research, and for supervising day to day experiments.
Funding Support:	Dr. Johnson's salary is provided in part by UT MD Anderson Cancer Center
Name:	Ratnakar Singh
Project Role	Post Doc Fellow
Nearest Person Month Worked	12
Contribution to Project:	Dr. Singh performs the bench experiments and the animal studies on this project.
Funding Support:	Funded 100% from this award
Name:	Pavitra Viswanath
Project Role:	Graduate Student
Nearest Person Month Worked	12
Contribution to Project:	Ms. Viswanath performs the bench experiments and the animal studies on this project.
Funding Support:	Funded 100% from this award

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

Changes to other support - Faye Johnson, MD, PhD – Principal Investigator

**Grants Closed** - PIQUR Therapeutics AG – “Dual PI3K and mTOR inhibition in HNSCC” - 05/06/2014 – 05/05/2017 - 1%, 0.12 calendar

Changes to other support – Don Gibbons, MD, PhD – Co-Investigator

**Grants Closed** - NIH/NCI (P50CA70907) – “Developing New Rationale, Personalized medicine for Lung Cancer” - 9/1/2016-8/31/2017 - 7%, 0.84 calendar

**New Active Grants -**

NIH/NCI – U01CA213273-01A1 – “Novel therapeutic approaches for enhancing anti-tumor immunity in SCLC” – 08/01/2017-07/31/2022 - 2%, 0.24 calendar

NIH/NCI (R01CA214609-01 – “The Role of Epithelial-Mesenchymal Transition in Re-Wiring KRAS Mutant Lung Cancer” – 10% 1.20 calendar – “Tumor Cell Epithelial-Mesenchymal Transition in Regulating Immunosuppression and Metastasis in Lung Cancer” - 3/1/2015-2/28/2018 – 20%, 2.40 calendar

**8. SPECIAL REPORTING REQUIREMENTS**

Not applicable

**9. APPENDICES: none**