Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 40. Oncogenic Mutations/Rearrangements in Patients with Advanced Adenocarcinoma Lung

7. **Start and End Date of Research Project:** 7/20/2012 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Chandra P. Belani, MD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 325,825

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Liu, Xin	Clinical Res Project Manager	100% (year 1) 50% (year 2)	$110,760
Rice, Shawn	Res Tech	100% (year 1) 50% (year 2)	$45,375

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Belani, Chandra	Professor/Deputy director	10% (yr1); 5% (yr 2
Zheng, Si-Yang	Assistant Professor	10% (yr1); 5% (yr 2)
Harouaka, Ramdane	Graduate Assistant	50% (yr1); 25% (yr 2)

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Centrifuge	Equipment used to spin whole blood tubes to separate blood components	$ 9,254.00
Biosafety Cabinet	Equipment used in day-to-day operations of the IPM Biorepository for the safe handling of human samples, tissues and fluids.	6,190
Ultralow freezer- participated in purchase	Freezer storage for human samples in the IPM Biorepository	16,506
Nanodrop	Instrument used for the measurement of sample DNA quantity and quality	22,055.87
Ultrasonicator- participated in purchase	State of the art equipment used for controlled fragmentation of DNA for NGS	67,208
Apollo Library Prep System- participated in purchase	State of the art robotic instrument that automates DNA library preparation for NGS	34,544
Thermal cycler,	Instrument used for the amplification and enrichment of DNA prior to sequencing	6,167
Water purification system	Manufactures ultrapure water for use in laboratory protocols	6,530.53
Deli case refrigerator / -20C freezer	Equipment used for storage of kits and reagents required in the process of Next Generation Sequencing (NGS)	8,369.00

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No__X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No___X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___X_____ No_____

If yes, please describe your plans:

We plan to apply for R21 or R01 funding from the NIH once analysis of our patient data from the overall project is complete.

12. Future of Research Project. What are the future plans for this research project?

We will get our manuscripts on this project published in high-quality peer-reviewed journals.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown			1	
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian				
Other				
Unknown				
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

Through this project, we have strengthened the research collaboration between Penn State Hershey Cancer Institute and Department of Bioengineering and Materials Research Institute at Penn State University Park, whose excellent research resources are invaluable for clinical cancer researchers at Penn State Hershey. The FMSA device provides a high throughput versatile platform capable of viable enrichment and analysis of CTC (circulating tumor cells) from clinically relevant volumes of whole blood. In addition, this collaborative project provides prognostic information and early indication of advanced NSCLC patient response to conventional therapy. This project has fostered collaborations between researchers from clinical departments and basic sciences departments at Penn State.

The project has helped establish the new Institute for Personalized Medicine Bio-repository and as an extension of that the Next Generation Sequencing capabilities of the institution. It has been a major initiative of the institution to establish a bio-repository of patient samples, such as blood, saliva and surgical tissues. We are currently consenting patients in fourteen clinical areas and routinely collecting saliva or whole blood samples from those patients. With the equipment purchased with grant funds we are able to safely prepare and handle the human tissues. It is crucial to properly house the patient samples for long-term storage and identification. We have purchased three ultralow freezers for the proper storage of samples, allowing us to aliquot those samples into three portions, from which one aliquot is promptly used for DNA isolation for potential downstream studies. Our three-freezer system allows for management of these valuable resources, and protects against a catastrophic event, by storing aliquots of specimens in more than one freezer. We have also included a large liquid nitrogen storage freezer, for the ultra-cold storage of samples to preserve DNA and RNA integrity for future studies.

The funds acquired through this grant also support the implementation and optimization of Next Generation Sequencing of patient DNA samples through whole genome sequencing, whole exome sequencing, and RNA sequencing. While the sequencing instrumentation itself is critical, proper pre-analytical sample preparation and enrichment is equally crucial to the quality of the sequenced material. The state of the art robotic equipment purchased for this purpose include the Covaris ultrasonicator, allowing for consistent fragmentation of DNA of multiple samples, and the Apollo Library Prep System that automates library preparation and allows for the high throughput of samples being analyzed. An ideal library consists of a well-balanced representation of the genomic areas of interest. Due to the complex nature of the sequencing protocols, a number of smaller support pieces of equipment are also important, such as the centrifuges, water system and thermal cyclers purchased with grant funds.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written

response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The primary objective is to determine the frequency of oncogenic mutations in patients with advanced adenocarcinoma of the lung. The clinical and mutational analysis will be used to determine the frequency of each mutation, its association with clinical features and outcome and its association with other mutations.

We hypothesize that approximately 50% of the patients with advanced adenocarcinoma of the lung will have the presence of one of the driver mutations/gene rearrangements that is related to clinical outcome (clinical features and overall survival). In addition, the study will characterize prognostic markers in adenocarcinoma lung and identify new drugable targets. This will subsequently lead to individualized approaches to treatment and overall increased efficacy.

Specific Aim 1: To determine the frequency of oncogenic mutation/gene arrangements in tumors from patients with advanced adenocarcinoma of the lung. The mutations of interest include those seen in EGFR, HER2, BRAF, MET, MEK1, EGFR, Ross1 and rearrangement of the EML4-ALK fusion gene.

Specific Aim 2: To correlate the presence of the oncogenic mutation/gene arrangements with clinical outcome. The clinical features associated with these mutations are not well characterized as the testing for these abnormalities has not been done in the current clinical setting. As some of these mutations/rearrangements are drugable, the ultimate goal will be to develop effective therapies with better understanding of the biology of lung cancer.

Specific Aim 3: To enumerate circulating tumor cells (EpCAM+CD45) from the same patients with adenocarcinoma of the lung and correlate with clinical outcome and detect the presence of the oncogenic mutations/gene rearrangements (e.g. ALK fusions) as outlined above. The tumor cell heterogeneity in the CTC population will be examined and compared with that seen in primary tumor samples.

We initiated a clinical/translational trial entitled "Oncogenic Mutations/Gene Rearrangements in Patients with Advanced Adenocarcinoma Lung" (PSHCI-12-064, IRB #40267EP) for this project. Target accrual was 40 patients and we have successfully enrolled 33 patients to the trial. 29 subjects were recruited from Penn State Hershey Cancer Institute. Research blood, FFPE-derived tumor tissues as well as clinical data were collected from these patients. In addition, four eligible patients' samples along with clinical data were requested from Lung Cancer Biospecimen Resource Network (LCBRN). 9 patients did not have enough tumor tissues for DNA extractions. Next generation sequencing technology has been used to detect oncogenic mutations in FFPE samples of tumor DNA from 24 patients with lung adenocarcinoma (*Specific*

Aim 1). Two samples were excluded for further analysis due to low tumoral cellularity (<20%). The association of common mutation status with clinical data was analyzed in these 22 patients (*Specific Aim 2*). Finally, circulating tumor cell (CTCs) counts were determined from the blood of ten patients over their courses of therapy (*Specific Aim 3*).

Specific Aim 1: To determine the frequency of oncogenic mutation/gene arrangements in tumors from patients with advanced adenocarcinoma of the lung. The mutations of interest include those seen in EGFR, HER2, BRAF, MET, MEK1, EGFR, Ross1 and rearrangement of the EML4-ALK fusion gene.

Material and Methods: The purpose of this study was to identify and quantify somatic mutations in archived FFPE tumor tissues from patients with advance-staged lung adenocarcinoma, utilizing Ion AmpliSeq™ Cancer panel in Ion Torrent platform. The Ion AmpliSeq™ Cancer Panel represents a fast and easy approach to generating target sequence for next-generation sequencing. It requires very little input DNA, and high read depths can be generated to support good sensitivity. In addition, Ion AmpliSeq™ technology is a new tool for enriching tens to thousands of genomic targets in a single tube from 10ng of input DNA. The Ion AmpliSeq™ Cancer Panel consists of a pool of 190 primer pairs that are designed to amplify mutation hotspot regions (including 739 known somatic mutations) across 46 genes. 20 FFPE-derived (from PSHCI) and 4 frozen resected (from LCBRN) lung adenocarcinoma samples of tumor DNA were amplified using this panel, and the products were then ligated with barcodes, subjected to emulsion PCR and sequenced on an Ion Torrent PGM using 316 chips (6 samples per chip).

Results: Estimated median tumoral cellularity of the selected samples was 60% (range 20% to 90%). Two samples were excluded for further analysis due to low tumoral cellularity (<20%). Among 22 lung adenocarcinoma patient specimens, we identified 39 genetic alterations in 21 genes by Ion Torrent variant caller software (Table 1). The sequencing analysis revealed frequent mutations in *TP53* (42%), *KDR* (29%), *KRAS* (21%), and *KIT* (13%) genes in these lung cancer patient samples. Moreover, distinctive patterns and the combination of mutations in various sets of genes, including *TP53, KDR, KRAS, KIT, SMO, MET, FGFR3, PTEN, ATM, PTPN11, APC, ABL1, HNF1A, SMARCB1, CDKN2A, STK11, ERBB4, ERBB2,RET* and *IDH1* were also identified. The range of somatic mutation frequencies was 4.39% to 98.99% and variant coverage ranges from 102× to 3249×. The average amplicon read length was 76 bp and the average read depth was 672; more than 85.7% of the amplicons had a read depth >200. In addition to the known sequence variants, a new nonsense mutation for gene *STK11* in patient LCBRN_V0120 was detected. Interestingly, 82% of specimens harbored at least one "druggable" alteration that has been linked to a target treatment or is currently being investigated in clinical trials. Those pairs of drug and targeted genes include AEE 788 to *KDR*; selumetinib to *KRAS*; sunitinib to *KIT;* vismodegib to *SMO;* Tivantinib to *MET*. These preliminary results indicate the necessity of targeted sequencing of key cancer related genes enabling identification of "druggable" mutations for individual lung adenocarcinoma patients.

Specific Aim 2: To correlate the presence of the oncogenic mutation/gene arrangements with clinical outcome. The clinical features associated with these mutations are not well characterized as the testing for these abnormalities has not been done in the current clinical setting. As some

of these mutations/rearrangements are drugable, the ultimate goal will be to develop effective therapies with better understanding of the biology of lung cancer.

Material and Methods: Two sample T test was used to analyze the potential association between the most common *TP53, KRAS, KDR* and *KIT* mutations and clinical factors including gender, age, race, disease stage, body surface area (BSA), ECOG performance status, tumor burden, systolic BP, diastolic BP, height, weight, pulse, respiration rate, temperature, alcohol assumption, smoking history, family lung cancer history, levels of LDH, albumin, alkaline phosphatase, bicarbonate, bilirubin, glucose, BUN, calcium, chloride, creatinine, RBC, WBC, ANC, platelets, and percentage of basophils (%), eosinophils (%), hematocrit (%), hemoglobin, neutrophils (%), WBC - Lymphocytes (%), WBC - Monocytes (%). In addition, the effect of *TP53, KRAS, KDR* and *KIT* mutations on overall survival in lung adenocarcinoma patients were determined by Kaplan–Meier survival curve.

Results: The tumor burden evaluated by RECIST is larger in those with *TP53* wild type tumors (11.51±1.62 cm) than in those with *TP53* mutated tumors (7.06±1.00 cm) (P= 0.038) (Figure 1). No clinical factor was found to be significantly associated with *TP53, KRAS, KDR* and *KIT* mutations (data not shown).

Specific Aim 3: To enumerate circulating tumor cells (EpCAM+CD45) from the same patients with adenocarcinoma of the lung and correlate with clinical outcome and detect the presence of the oncogenic mutations/gene rearrangements (e.g. ALK fusions) as outlined above. The tumor cell heterogeneity in the CTC population will be examined and compared with that seen in primary tumor samples.

Material and Methods: The dissemination of circulating tumor cells (CTCs) that cause metastases in distant organs accounts for the majority of cancer related deaths. CTCs have been established as a cancer biomarker of known prognostic value. The enrichment of viable CTCs for *ex vivo* analysis could further improve cancer diagnosis and guide treatment selection. We collaborated with Dr. Siyang Zheng, whose Micro & Nano Integrated Biosystem Laboratory at Penn State University Park designed a new flexible micro spring array (FMSA) device for the enrichment of viable CTCs independent of antigen expression (Harouaka RA, et al. **Clinical Chemistry**, 60:2, **2014**). CTCs were enriched and counted in blood samples obtained from ten lung adenocarcinoma patients during the course of their treatment with chemotherapy.

Results: FMSA device can enrich tumor cells with 90% capture efficiency, higher than 10^4 enrichment, and better than 80% viability from 7.5 mL whole blood samples in less than 10 minutes (0.5 cm^2 device). Varying trends were observed in CTC counts from these lung cancer patients over the course of therapy (Harouaka RA, et al. **Clinical Chemistry**, 60:2, **2014**). Baseline CTC counts were obtained prior to the initiation of therapy. Overall Survival was calculated based on measured baseline values for 10 patients undergoing therapy and followed up for 300 days. The patients were split into two groups: one with ≤ 5 CTCs detected at the baselines measurement (n=5), and another with ≥ 22 CTCs at baseline (n=5). Patients with ≤ 5 CTCs were observed to have a greater probability of survival beyond 200 days. (Figure 2) Interestingly, patient 01-005 initially showed a sharp decline in FMSA detected CTCs after the first cycle of treatment, but then CTC counts increased sharply and the patient died within two months of the final blood draw. Patients 01-001 and 01-004 with declining or comparatively low CTC counts over the course of therapy

are alive, and have survived at least 19 and 32 months respectively since diagnosis (Harouaka RA, et al. Clinical Chemistry, 60:2, 2014). Additional blood samples obtained during follow up visits were analyzed to monitor CTC counts during the course of therapy. Most patients with declining or comparatively low CTC counts over the course of therapy survived for 300 days after the baseline measurement, as indicated by the green lines in Figure 3. All patients except for one with an initial CTC count greater than 5 died within 300 days.

Table 1. Detection of somatic mutation in lung adenocarcinoma patient tumor samples.

patient #	GENE_NAME	Chro	Zygosity	POS	RESIDUE_REF	RESIDUE_ALT	TYPE
01-001	KRAS	12	Het	13	G	R	SNP
	TP53	17	Het	274	V	D	SNP
01-003	KIT	4	Hom	852	V	I	SNP
01-004	TP53	17	Het	173	V	L	SNP
01-005	KDR	5	Het	472	Q	H	SNP
	CDKN2A	12	Het	124	G	V	SNP
	KRAS	14	Het	12	G	A	SNP
01-006	KDR	4	Het	472	Q	H	SNP
	TP53	17	Het	215	S	G	SNP
01-008	APC	5	Het	1105	R	W	SNP
	KRAS	12	Het	12	G	C	SNP
	TP53	17	Het	277	C	W	SNP
	STK11	19	Het	163	G	V	SNP
01-009	KDR	4	Het	472	Q	H	SNP
	TP53	17	Het	215	S	G	SNP
01-010	N/A						
01-011	KDR	4	Het	472	Q	H	SNP
	ABL1	9	Het	266	K	R	SNP
	KRAS	12	Het	61	Q	L	SNP
01-013	MET	7	Het	359	R	Q	SNP
	KRAS	12	Het	12	G	C	SNP
01-016	N/A						
01-017	ERBB4	2	Het	362	I	M	SNP
	TP53	17	Het	245	G	V	SNP
01-021	N/A						
01-023	EGFR	7	Het	746	ELREA	.	Deletion
01-024	N/A						
01-025	RET	10	Het	649	S	L	SNP
01-026	IDH1	2	Het	110	E	K	SNP
	TP53	17	Het	248	R	Q	SNP
01-027	KRAS	12	Het	12	G	C	SNP
	KIT	8	Het	677	D	E	SNP

LCBRN_V0007	KDR	10	Het	472	Q	H	SNP
	MET	18	Het	162	F	L	SNP
	SMO	19	Het	404	V	M	SNP
	HNF1A	30	Het	202	N	D	SNP
	TP53	34	Het	242	C	W	SNP
	TP53	37	Het	105	G	C	SNP
	SMARCB1	40	Het	155	F	L	SNP
LCBRN_V0120	KDR	4	Het	472	Q	H	SNP
	SMO	7	Het	532	M	T	SNP
	TP53	17	Het	248	R	Q	SNP
	ERBB2	17	Het	862	T	A	SNP
	STK11	19	Het	48	K	*	Nonsense
LCBRN_W0024	IDH1	2	Het	115	K	E	SNP
LCBRN_W0076	ERBB4	2	Het	945	C	Y	SNP
	FGFR3	4	Het	699	G	S	SNP
	KIT	4	Het	566	N	S	SNP
	KDR	4	Het	1196	T	I	SNP
	PTEN	10	Het	173	R	H	SNP
	ATM	11	Het	3045	L	P	SNP
	PTPN11	12	Het	61	D	A	SNP
	TP53	17	Het	286	E	Q	SNP

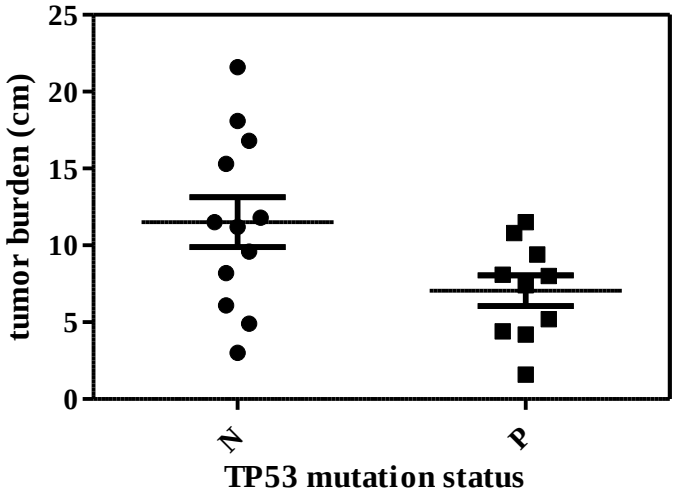

Figure 1. Effect of *TP53* mutations on tumor burden in lung adenocarcinoma. Tumor burden of patients with stage IIIB or IV lung adenocarcinoma was statistically significantly larger in those with *TP53* mutant tumors than in those with *TP53* wild-type tumors by unpaired T-test (P= 0.045). N=TP53 mutation negative, P=TP53 mutation positive.

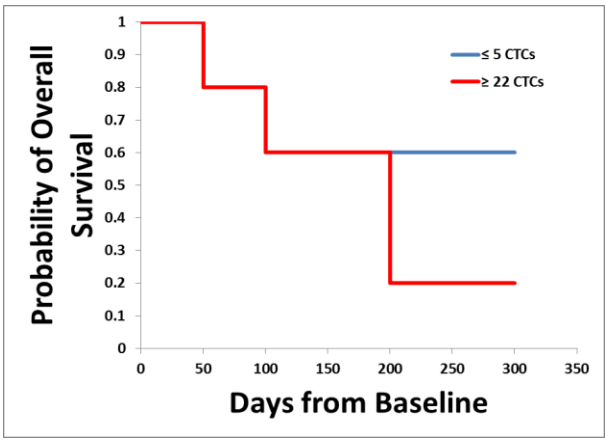

Figure 2. Kaplan-Meier estimates of Overall Survival probabilities for patients determined to have ≤ 5 CTCs at the baselines measurement (n=5) and for patients with ≥ 22 CTCs at the baseline measurement (n=5).

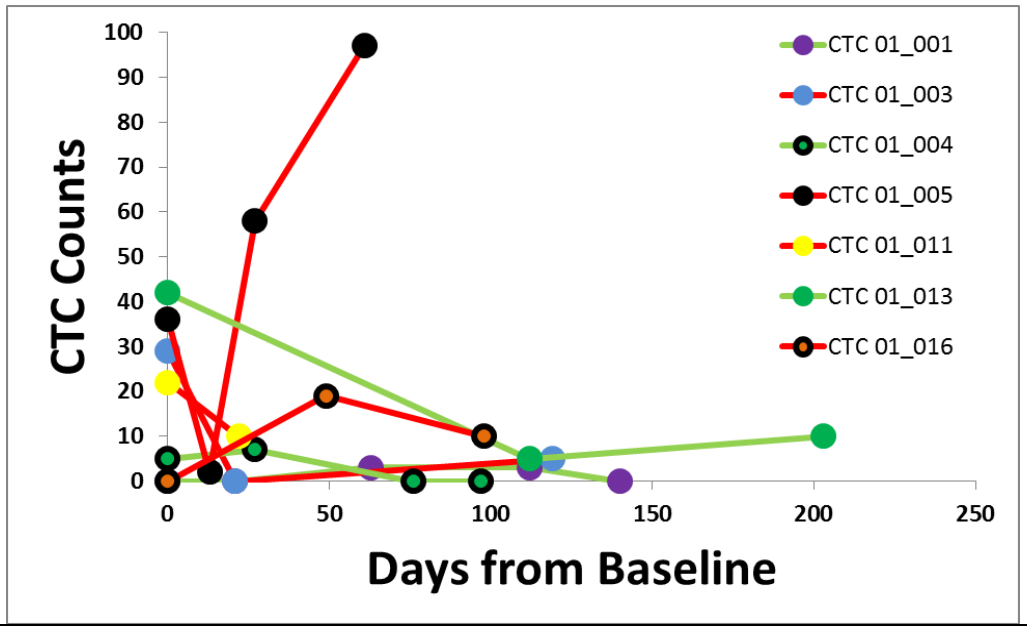

Figure 3. CTC counts are plotted for 7 patients through the course of therapy. Baseline counts obtained prior to initiation of therapy occurred at Day 0. Green lines represent patients that have survived 300 days from baseline, while red lines represent patients that have deceased.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_X___Yes
_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

__X__Yes
_____No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_12___Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_40___Number of subjects originally targeted to be included in the study
_33___Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
__14___Males
__19___Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
__33__Not Latinos or Hispanics
_____Unknown
Race:
_____American Indian or Alaska Native
_____Asian
__2__ Blacks or African American
_____Native Hawaiian or Other Pacific Islander
__31__White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

Dauphin

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X___No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

____ Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Flexible Micro Spring Array Device for High-Throughput Enrichment of Viable Circulating Tumor Cells.	Harouaka RA, Zhou MD, Yeh YT, Khan WJ, Das A, Liu X, Christ CC, Dicker DT, Baney TS, Kaifi JT, Belani CP, Truica CI, El-Deiry WS, Allerton JP, Zheng SY	Clin Chem.	October 2013	☐Submitted ☐Accepted XPublished

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes__X_____ No_____

If yes, please describe your plans:

1. One manuscript regarding clinical significance of CTCs in patients with advanced non-small cell lung cancer is in preparation and will be submitted to Clinical Cancer Research.
2. One manuscript describing oncogenic mutations in patients with advanced non-small cell lung cancer is in preparation and will be submitted to Clinical Cancer Research.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The research from this project has led to major discoveries that detection of CTCs by using a FMSA device that may be utilized in therapeutic response monitoring in advanced NSCLC patients and also served as a predictive factor for these patients' overall survivals. Progress in molecular biology and genomics is producing a revolution in cancer research and treatment. Indeed, our data showed that each patient with advanced NSCLC had a unique oncogenic mutation profiling, proving that the "one-size-fits-all" approach may not be appropriate in the current clinical settings; a tailored personalized therapy based on the tumor genetic profiling will be needed to cure.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance

of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2

NAME	POSITION TITLE
Chandra P. Belani, MD	Miriam Beckner Distinguished Professor of Medicine Deputy Director, Penn State Hershey Cancer Inst.
eRA COMMONS USER NAME (credential, e.g., agency login) belanicp	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Rajasthan, Jaipur, India	Pre-Med	06/72	Biology
Sawai Man Singh Medical College, Rajasthan, Jaipur	MBBS	10/78	Medicine
Sawai Man Singh Medical College, Rajasthan, Jaipur	MD	10/81	Internal Medicine
University of Maryland, Good Samaritan Hospital, Baltimore, MD	Int/Res	10/82-06/84	Internal Medicine
North General Hospital, Mt. Sinai School of Medicine, New York, NY	Intern	07/84-06/85	Internal Medicine
University of Maryland, Baltimore, MD	Fellowship	07/85-06/88	Hematology/Oncology

A. Personal Statement

As Deputy Director of the Penn State Hershey Cancer Institute and Associate Director of Clinical Research, I direct the clinical investigations across all programs. I foster collaborative efforts in the clinical/translational enterprise at the PSHCI. My role as a lung cancer researcher and translational investigator allows me to be active in developing personalized therapies and combined modality approaches utilizing translational novel targeted agents for lung cancer and thoracic malignancies. I have conducted pivotal clinical trials in the area of lung cancer that have resulted in changes in standard of practice for this disease and I have acquired an international reputation as a thought-leader and an expert in the field of lung cancer. My research has appeared in the most prestigious medical journals including the New England Journal of Medicine, Lancet, JAMA, JBC, CCR and the Journal of Clinical Oncology. More recently, my work is focused on determining the frequency of oncogenic mutations/gene rearrangements in tumors from patients with lung cancer with utilization of next generation sequencing for evaluation and correlation with clinical outcomes as outlined in the current proposal. In addition, we are also focusing on development of biomarkers for both early detection of lung cancer and progression by utilizing next generation sequencing and proteomic techniques. The ultimate goal is to develop personalized therapies with better understanding of the biology of lung cancer.

Positions and Honors

07/1/88 – 06/30/89 Instructor in Medicine, Department of Medicine, University of Maryland Medical School, Baltimore, MD

07/01/89 – 02/01/94	Assistant Professor of Medicine/Oncology, University of Maryland Cancer Center, Baltimore, MD
07/01/89 – 02/01/94	Director, Thoracic Oncology, University of Maryland, Baltimore, MD
07/01/89 – 02/01/94	Assistant Professor of Medicine/Oncology, University of Maryland Cancer Center, Baltimore, MD
07/01/89 – 02/01/94	Director, Thoracic Oncology, University of Maryland, Baltimore, MD
02/01/94 – 11/3098	Associate Professor of Medicine, University of Pittsburgh Department of Medicine, Division of Medical Oncology, Pittsburgh, PA
07/01/94 – 12/31/96	Co-Director, Experimental Therapeutics Program, University of Pittsburgh Cancer Institute, Pittsburgh, PA
01/01/97 – 12/31/02	Director, Community Oncology, University of Pittsburgh Cancer Institute, Pittsburgh, PA
01/01/97 – 06/30/07	Co-Director, Lung and Thoracic Malignancies Program, University of Pittsburgh Cancer Institute, Pittsburgh, PA
12/01/97 – 06/30/07	Professor of Medicine, University of Pittsburgh, Department of Medicine, Division of Medical Oncology, Pittsburgh, PA
07/01/07 – Present	Miriam Beckner Distinguished Professor of Medicine, Penn State Milton S. Hershey Medical Center, Department of Medicine, Hershey, PA
07/01/07 – Present	Deputy Director, Penn State Hershey Cancer Institute, Hershey, PA

Honors and Awards

1978	Fifth Position in the Sawai Man Singh Medical College at the Final Professional Examination with Honors in Ophthalmology and highest marks in OB/GYN
1981	Gold Medal for best performance at the M.D. (Medicine) Examination
1987 – 1988	MCP Award #INFCB-Supported by ACS Institutional Research Grant #IN-175F, MCP, University of Maryland
1990	Cora and John Davidson Foundation Award for Lung Cancer Research
2003	America's Top Physicians
1998 – 2009	Best Doctors in America
2001 - 2010	America's Top Doctors Award-Medical Oncology, Castle Connolly

B. Selected Peer-reviewed Publications

Most relevant to the current application

1. Ramalingam SS, Parise RA, Ramanathan RK , Lagattuta TF, Musguire LA, Stoller RG, Potter DM, Argiris AE, Zwiebel JA, Egorin MJ, **Belani CP** (2007). Phase I and pharmacokinetic study of vorinostat, a histone deacetylase inhibitor, in combination with carboplatin and paclitaxel for advanced solid malignancies. Clin Cancer Res 13(12):3605-10. PMID: 17510206
2. **Belani, C.P.**, Ramalingam, S., Perry, M.C., LaRocca, R.V., Rinaldi, D., Gable, P.S., Tester, W.J (2008). Randomized, Phase III Study of Weekly Paclitaxel in Combination with Carboplatin versus Standard every-3-weeks Administration of Carboplatin and Paclitaxel for Patients with Previously Untreated Advanced Non-Small-Cell Lung Cancer. J Clin Oncol 26(3), 468-73. PMID: 18202422

3. Ciuleanu, T., Brodowicz, T., Zielinski, C., Kim, J.H., Krzakowski, M., Laack, E., Wu, L., Bover, I., Begbie, S., Tzekova, V., Cucevic, B., Pereira, J.R., Yang, S.H., Madhavan, J., Sugarman, K.P., Peterson, P., John, W.J., Krejcy, K., **Belani, C.P.** (2009) Maintenance Pemetrexed Plus Best Supportive Care (BSC) Versus Placebo Plus BSC: A Phase III Study in Advanced NSCLC. Lancet, 374(9699):1398-400. PMID: 19767093
4. Ramalingam, S.S., Maitland, M.L., Frankel, P., Argiris, A., Koczywas, M., Gitlitz, B., Thomas, S., Espinoza-Delgado, I., Vokes, E.E., Gandara, D., **Belani, C.P**. (2010) Carboplatin and paclitaxel in combination with either vorinostat or placebo for first line therapy of advanced non-small cell lung cancer. J. Clin Oncol, 1:28(1):56-62. PMID: 19933908
5. Owonikoko TK, Ramalingam SS, Kanterewicz B, Balius TE, Belani CP, Hershberger PA. Vorinostat increases carboplatin and paclitaxel activity in non-small-cell lung cancer cells. Int J Cancer 2010 Feb 1;126(3):743-55. PMID: 20388843

Additional recent publications of importance to the field (in chronological order)

1. Gutheil, J.C., Hart, S.R., **Belani, C.P.**, Melera, P.W., Hussain, A. (1999) Alterations in Ca^{2+} transport ATPase and P-glycoprotein expression can mediate resistance to thapsigargin. J Biol Chem 269(11):7976-7981.
2. Gandara, D.R., Vokes, E., Green, M., Bonomi, P., DeVore, R., Comis, R., Carbone, D., Karp, D., **Belani, C.P.** (2000) Activity of docetaxel (Taxotere) in platinum-treated non-small cell lung cancer: Results of a phase II multicenter trial. J Clin Oncol 18(1):131-135.
3. **Belani, C.P.**, Einzig, A., Bonomi, P., Dobbs, T., Capozzoli, M.J., Earhart, R., Cohen, L.J. (2000) Multicenter phase II trial of docetaxel and carboplatin in patients with stage IIIB and IV non-small cell lung cancer. Annals of Oncology 11(6):673-678.
4. Sweeney, C.J., Zhu, J., Sandler, A.B., Schiller, J., **Belani, C.P.**, Langer, C., Krook, J., Harrington, D., Johnson, D. (2001) Outcome of patients with a performance status of 2 in Eastern Cooperative Oncology Group Study E1594. Cancer 92(10):2639-2647.
5. Schiller, J. Harrington, D. **Belani, C.P.**, Langer, C., Sandler, A., Krook, J., Junming, Z., Johnson, D. (2002) Comparison of four chemotherapy regimens in advanced non-small cell lung cancer. N Engl J Med, 346 (2) 92-98.
6. Kris, M.G, Natale, R.B., Herbst, R.S., Lynch, T.J., Jr., Prager, D., **Belani C.P**., Schiller, J.H., Kelly, K., Spiridonidis, H., Sandler, A., Albain, K.S., Cella, D., Wolf, MK., Averbuch, S.D., Ochs, J.J., Kay, A.C. (2003). Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA 290(16); 2149-58.
7. Fossella, F., Pereira, J.R., von Pawel, J., Pluzanska, A., Gorbounova, V., Kaukel, E., Mattson, K.V., Ramlau, R., Szczesna, A., Fidias, P., Millward, M., **Belani, C.P.** (2003) Randomi**zed, Multinational, Phase III Study of Docetaxel Plus Platinum Combinations Versus Vinorelbine Plus Cisplatin for Advanced Non-Small-Cell Lung Cancer: The TAX 326 Study Group. J Clin Oncol 21 (16): 3016-3024.**
8. **Belani, C.P.**, Barstis, J., Perry, M.C., LaRocca, R.V., Nattam, S.R., Rinaldi, D., Clark, R., Mills, G.M. (2003) Multicenter, randomized trial for stage IIIB or IV non-small cell lung cancer using weekly paclitaxel and carboplatin followed by maintenance weekly paclitaxel or observation. J Clin Oncol 21(15):2933-2939.
9. **Belani, C.P.**, Pereira, J.R., von Pawel, J., Pluzanska, A., Gorbounova, V., Kaukel, E., Mattson, K.V., Ramlau, R., Szczesna, A., Fidias, P., Millward, M., Fossella, F. (2006) TAX 326 study group. Effect of chemotherapy for advanced non-small cell lung cancer on patients' quality of life. A randomized controlled trial. Lung Cancer, 3(2):331-7.
10. Ramalingam, S., Barstis, J., Perry, M.C., LaRocca, R.V., Nattam, S.R., Rinaldi, D.,

Clark, R., Mills, G.M., **Belani, C.P**. (2006) Treatment of Elderly Non-small Cell Lung Cancer Patients with Three Different Schedules of Weekly Paclitaxel in Combination with Carboplatin: Subanalysis of a Randomized Trial, J Thorac Onc 1(3):240-4.

D. Research Support

Ongoing Research Support

CA099168-1-0 Belani (PI) 03/1/13-02/28/14 .3 calendar
Phase I Clinical Trials of Novel Anticancer Agents
To develop, implement and conduct early phase I trials of novel agents and combination therapy with these agents

N01-CM-62209 Gandara (PI) 03/01/11-12/31/15 .12 calendar
Early Therapeutic Development with Phase II Emphasis
The main goals of this project are to develop clinical trials of novel anticancer agents and/or combinations in specific malignancies

Completed Research

4100050904 Belani (PI) 07/20/12-12/31/13 .24 calendar
Oncogenic Mutations/Rearrangements in Patients with Advanced Adenocarcinoma Lung
To determine the frequency of oncogenic mutation/gene rearrangements in tumors from patients with advanced adenocarcinoma of the lung
PA Tobacco Settlement Fund (TSF) / Direct Cost: $149,000