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. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Thomas Jefferson University

2. **Reporting Period (start and end date of grant award period):** 1/1/2011-12/31/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Theodore F. Taraschi PhD

4. **Grant Contact Person's Telephone Number:** 215-955-3900

5. **Grant SAP Number:** 4100054872

6. **Project Number and Title of Research Project:** Project 1 – Network-Directed Therapy of Prostate Cancer

7. **Start and End Date of Research Project:** 1/1/2011-6/30/2013

8. **Name of Principal Investigator for the Research Project:** Lucia R. Languino, PhD

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:

   $ 388,087.48

   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	Position Title	% of Effort on Project	Cost
Languino	Professor	2% Yr 1,2,3	$13,048.40
Migneco	PostDoc	25% Yr 1	$13,236.72
Sayeed	Instructor	100% Yr 1, 38% Yr 2, 50% Yr 3	$145,878.15

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	Position Title	% of Effort on Project
None		

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
None		

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:

A NIH R01 application is planned for June 2015.

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

To develop a clinical trial to test inhibitors of the newly identified pathway in castrate-resistant prostate cancer.

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				
Female		1	1	
Unknown				
Total		**1**	**1**	

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

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

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

Yes_____X____ No_____

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

Dr Lucia Languino from University of Massachusetts and Dr Aejaz Sayeed from the California Pacific Medical Center Research Institute moved to Thomas Jefferson University for this project.

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.

Our team, which joined Thomas Jefferson University based on the availability of this award, brought in vivo expertise and mouse models of prostate cancer.

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____X_____ No_____

If yes, please describe the collaborations:

The collaboration with Dr Altieri at Wistar Institute was strengthened by these health research funds.

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.

Project Title and Purpose

Network-Directed Therapy of Prostate Cancer - Although considerable progress has been made in the understanding of cancer genes, only a handful of molecular therapies have become standard of care for cancer patients in the last thirty years. The management of prostate cancer remains challenging, and confusion still exists regarding the molecular networks that drive the disease, the reliability of biomarkers, and the appropriateness of treatment options. The present application did address these problems taking an innovative approach. This project is focused on a single network, the $\alpha_v\beta_6$ / survivin pathway, which plays a critical role in prostate cancer.

Project Overview

This project is focused on a single network, the $\alpha_v\beta_6$ / survivin pathway, which plays a critical role in prostate cancer. This pathway may be particularly important for prostate cancer progression, where survivin has been recognized as a marker of aggressive disease and a validated target for novel molecular therapies. This project integrated basic and translational studies to define the role of survivin as a metastasis-promoting gene in prostate cancer. Overall, this project provided a comprehensive and mechanistic map of a new pathway of tumor cell invasion. The specific aims were as follows: (AIM 1) To characterize the cellular requirements of survivin-directed tumor cell invasion. (AIM 2) To elucidate the mechanism of survivin-directed gene expression. (AIM 3) To dissect the role of survivin as a metastasis-promoting gene.

Background and significance

Backed by an unprecedented knowledge of cancer genes and their pathways, it is now possible to envision strategies to eradicate tumor cells, while leaving normal tissues unscathed. Unfortunately, this concept has proved feasible in only a small number of cancers. This may not only be due to the enormous heterogeneity of tumors, but also to a relatively narrow path to cancer drug discovery. Conversely, modeling cancer pathways in their globality as integrated signaling networks may lead to the identification of "hub" molecules as non-redundant integrators of multiple subnetworks. Different from traditional single-gene antagonists, pursuing "hub" molecules for cancer therapy may produce "pathway inhibitors" suitable to simultaneously disable multiple cancer signaling networks and thus bypass tumor heterogeneity.

Prototype "hub" molecules in cancer are survivin, a member of the Inhibitor of Apoptosis (IAP) family, and the $\alpha_v\beta_6$ integrin, which induces survivin expression. Strongly expressed in virtually every human tumor, but not in normal tissues, they promote metastasis and integrate multiple essential networks of cell division, checkpoint control, gene expression, and inhibition

of apoptosis. Disabling survivin and the $\alpha_v\beta_6$ integrin as a "hub" molecule in prostate cancer is expected to advance our understanding of cancer networks and achieve meaningful clinical gains in these patients.

Specific Aims
AIM 1. **To characterize the cellular requirements of survivin-directed tumor cell invasion**.
AIM 2. **To elucidate the mechanism of survivin-directed gene expression**.
AIM 3. **To dissect the role of survivin as a metastasis-promoting gene, <u>in vivo</u>**.

Summary of research completed
While the role of the $\alpha_v\beta_6$ and survivin as metastasis-promoting genes *in vivo* appears established at this point, this laboratory has continued to focus on the analysis of $\alpha_v\beta_6$ / survivin networks that promote cancer progression.

AIM 1. To characterize the cellular requirements of survivin-directed tumor cell invasion.
The molecular requirements of enhanced tumor cell motility induced by this network in prostate cancer cells were first investigated. Using a combination of molecular (dominant negative mutants) and genetic (short hairpin RNA –shRNA) antagonists targeting integrin $\alpha_v\beta_6$, we have shown that this pathway increases cell migration in response to ligand binding, promotes a cancer cell autonomous metastatic program in bone *in vivo*, and is a mediator of the response to TGFβ. We provide evidence that $\alpha_v\beta6$ is required for up-regulation of MMP2 by TGFβ1 through a Smad3-mediated transcriptional program in PrCa cells. The functional relevance of these results is underscored by the finding that $\alpha_v\beta_6$ modulates cell migration in an MMP2-dependent manner on an $\alpha_v\beta_6$-specific ligand, latency-associated peptide (LAP)-TGFβ. The data relevant to this study are published in the journal Cancer Research [1] and are In Press in Biochemical Journal [2]. Overall, our study describes a new TGFβ1 dependent and αvβ6-MMP2 dependent signaling pathway that, given TGFβ1's pro-metastatic activity, may have profound implications for PrCa therapy.

Since the androgen receptors Src and AKT are associated with increased cell motility and are known to promote survivin expression, we studied the localization of these molecules in intracellular compartments using immunofluorescence and subcellular fractionation approaches; we also studied Prostate-specific antigen (PSA) localization since it is known to transactivate AR. We found that PSA localizes to nuclei of androgen-stimulated PrCa cells and controls AR mRNA and protein levels. This regulatory loop is specific for PSA, does not involve known AR activators, such as Src and AKT, and may contribute to AR signaling under conditions of increasing PSA levels in patients. These data are published in The Prostate [3].

To characterize (AIM 1) the cellular requirements of survivin-directed tumor cell invasion, based on the knowledge that survivin is induced by Insulin-like Growth Factor Receptor (IGF-IR) stimulation, we investigated the downstream effectors of IGF-IR that mediate tumor cell invasion. The findings show that IGF-IR controls β_1, but not α_v integrin expression, and does so at the protein level by preventing β_1 proteasomal degradation. A series of new experiments demonstrate that in LNCaP prostate cancer cells, there is a significant decrease in expression of β_1, but not α_v integrins, upon IGF-IR down-regulation. It remained to be determined whether

IGF-IR or β_1 integrins are the primary regulators of survivin. Our new data indicate that re-expression of β_1 integrins rescues the effects observed upon IGF-IR down-regulation. A manuscript summarizing these data is published in J Cell Physiology [4] and a second one in PlosOne [5]. Finally, while analyzing new technical approaches to study metastasis, we have characterized a novel mechanism of metastatic dissemination mediated by a transmembrane molecule, Trop-2, which is frequently upregulated in human carcinomas. We searched for Trop-2 association with integrins and found that this molecule does not modulate migration on vitronectin (VN) nor associate with the major VN receptor, α_v integrin, but associates instead with β_1 integrins. A manuscript summarizing these data is published [6]. Overall, we conclude that the molecular requirements of survivin-directed tumor cell invasion comprise IGF-IR and β_1 integrins. Since these molecules promote metastasis, we conclude that inhibition of IGF-IR and β_1 integrins will ultimately inhibit survivin expression and prevent its ability to act as a metastasis-promoting gene *in vivo*.

AIM 2. To elucidate the mechanism of survivin-directed gene expression.
To elucidate the mechanism of gene expression (AIM 2) mediated by $\alpha_v\beta_6$ integrin and its downstream effector, survivin, we screened several downstream genes important in metastasis. We found that the $\alpha_v\beta_6$ integrin / survivin pathway induces expression of metalloprotease 2, a crucial effector that mediates bone metastasis. The data relevant to this study are published in Cancer Research [1] and are In Press in Biochemical Journal [2].

AIM 3. To dissect the role of survivin as a metastasis-promoting gene in vivo.
The studies in this aim are in progress and when completed will be used to apply for a new NIH grant. The mouse model we had planned to use (TRAMP) was not useful because significant variability in integrin expression was found. We had to switch to the PTEN-conditional knock out mouse model, which delayed the completion of the project. Tumor responses and target validation are being evaluated by molecular imaging, quantitative histopathology and molecular biomarker assessment.

All procedures used in this proposal have been extensively published by the principal investigator and described in the papers mentioned above.

Novel Materials and Methods developed with the support of the CURE grant funds (as published in 6) are as follows:

Cell Migration Assay
Cell migration assays have been performed as follows. Transwell chambers (12 mm pore diameter, Costar) were coated with FN (10 mg/mL) or VN (10 mg/mL) overnight at 4ºC. After cell detachment and trypsin inactivation, cells were seeded on coated transwell chambers at 37°C for 5 hours. After fixation with 3.7% paraformaldehyde (PFA), cells attached on both layers of the porous filter were stained with 1 mg/mL 4',6-diamidino-2-phenylindole (DAPI) and pictures of nuclei were acquired by fluorescence microscopy (Olympus IX71 or Nikon Eclipse TS-100 inverted microscopes equipped with fluorescence unit). Then, cells on the top layer were removed using a cotton swab, and pictures of nuclei from cells migrated to the bottom layer were

acquired. Five and twenty random fields were acquired for quantification of attached and migrated cells, respectively. Cell Profiler software (www.cellprofiler.org) was used for quantification of nuclei number, using 10-30 pixel units as range for discrimination between single nuclei and potential aggregates. The ratio between number of cells migrated onto the bottom layer and total (top + bottom) number of cells attached on the filter was calculated for each group of transfectants. The assays were repeated at least three times, and similar results were observed. Chi-Square test was used for statistical analysis.

Live Cell Microscopy Analysis of Cell Movements on FN

PC3-2 and LNCaP cell transfectants were seeded at 2×10^4 cells/mL and allowed to spread for 3 hours on FN in 24-well plates. Cell movements were monitored using a widefield live cell imaging system: images were acquired on a Zeiss live cell imaging system (Axiovert 200M, Carl Zeiss) using a 20x objective and a Coolsnap HQ CCD monochrome camera (Roper) at 10-minute intervals for 17 hours. A programmable X,Y,Z stage (Prior Proscan III) was used for acquisition of multiple positions and stitching of large areas. Cells were maintained at 37°C in a humidified 5% (v/v) CO_2 atmosphere during the time course using a dedicated environmental chamber (Okolab). Images were then converted to stacks using ImageJ, and migration tracks of non-dividing, non-clustered cells were manually obtained using the Manual Tracking (http://rsb.info.nih.gov/ij/plugins/track/track.html) and the Chemotaxis and Migration Tool (version 1.01; Ibidi GmbH, Martinsried, Germany; http://ibidi.com/software/chemotaxis_and_migration_tool/?x21b49=6f65b0702c4bf44855c8e20d1b340358) ImageJ plugins. Directionality (persistence) was determined by dividing the linear displacement of a cell after 17 hours by the total distance moved, where migration in a straight line equals to a directionality of 1. Migration paths in four randomly chosen fields were tracked in duplicate wells for each cell type. The significance of changes in directionality, velocity, total distance and linear displacement was determined using a Student's T test, as described.

Immunofluorescence (IF) and Confocal Microscopy

Cells were seeded on FN-coated glass coverslips for 1 hour at 37°C. Then, fixation with 3.7% PFA was performed for 15 minutes at room temperature, followed by quencing with 50 mmol/L NH_4Cl. Cells were permeabilized by incubation with PBS / 0.2% Triton X-100 for 5 minutes, and then incubated for 30 minutes at room temperature with the blocking solution (PBS / 5% BSA). Single staining and/or co-staining were performed incubating samples with primary Abs for 20 minutes at room temperature, followed by incubation with secondary Abs (Alexa Fluor 488-Rabbit anti mouse and/or Alexa Fluor 633-Donkey anti goat) for 20 minutes at room temperature. After three washes, coverslips were mounted on glass slides using Pro-Long anti-fade reagent (Invitrogen), and slides were analyzed on an inverted confocal microscope (LSM510, Carl Zeiss) using Plan-Apochromat 63x (1.4 NA) or Plan-Neofluar 100x (1.3 NA) lenses. Staining for visualization of talin in focal adhesions and membrane rims was performed on adhesive structures (cell ghosts), obtained by osmotic shock of cells seeded on FN, as described. Briefly, the FN-adherent cells were rinsed with PBS, then incubated in H_2O for 1 minute to osmotically disrupt the cells, and quickly flushed to shear away the dorsal membranes. The structures were then fixed in 3.7% PFA and processed for staining and IF analysis.

Internalization and Recycling Assay

Internalization of membrane receptors was induced by cooling cells on ice for 30 minutes, followed by stimulation at 37ºC for 30 minutes. Internalization was then stopped by placing cells back on ice for 30 minutes, and recycling to the plasma membrane was induced by re-incubation at 37ºC for 15 minutes. Cells were fixed at the various steps of internalization/recycling and examined by confocal microscopy. Co-localization of molecules trafficking vesicles was quantified using the "Pearson–Spearman correlation co-localization" ImageJ plugin. The Pearson's correlation coefficient (PCC) was calculated for each image, and then means ± SEM were calculated for each experimental group in order to score the extent of co-localization. In order to avoid overestimation, signals from membrane edges were excluded by drawing a mask surrounding only the cytoplasmic area.

References

1. Dutta, A., Li, J., Lu, H., Akech, J., Pratap, J., Wang, T., Zerlanko, B., FitzGerald, T.J., Jiang, Z., Birbe, R., Wixted, J., Violette, S.M., Stein, G.S., Lian, J.B., Languino, L.R. Integrin $\alpha_v\beta_6$ promotes a cancer cell autonomous osteolytic program through upregulation of MMP2. *Cancer Res.*, (2014) 74:1598-160.
2. Dutta, A., Li, J., Fedele, C., Sayeed, A., Violette, S.M., Manes, T.D., Altieri, D.C. and Languino, L.R. αvβ6 Integrin is required for TGFβ1-mediated matrix metalloproteinase2 (MMP2) expression. *Biochem J.* (2015), *In Press*.
3. Saxena, P, Trerotola, M (co-first authors), Wang, T, Li, J, VanOudenhove, J, Adams, DS, FitzGerald, TJ, Altieri, DC, Languino, LR. PSA is required for androgen receptor expression in prostate cancer cells. *The Prostate* (2011) 72:769-776.
4. Goel, H., Sayeed, A., Breen, M., Zarif, M.J., Garlick, D.S., Leav, I., Davis, R.J., FitzGerald, T.J., Morrione, A., Liu, Q., Dicker, A., Altieri, D.C., Languino, L.R. β1 integrins mediate resistance to ionizing radiation by inhibiting JNK. (2013) *J. Cell Physiol*. 228(7):1601-1609.
5. Sayeed, A., Fedele, C., Trerotola, M., Languino, L.R. IGF-IR Promotes Prostate Cancer Growth By Stabilizing α5β1 Integrin Protein Levels. *PlosOne*, 2013 e.76513.
6. Trerotola, M., Jernigan, D.L., Liu, Q., Siddiqui, J., Fatatis, A., Languino, L.R. Trop-2 promotes cancer metastasis by modulating β1 integrin functions. (2013) *Cancer Res*., 73:3155-3167.

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?

_____Yes
____X_No

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

_____Yes
__X___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?

_____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?

_____Number of subjects originally targeted to be included in the study
_____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:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____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.)

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, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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. αvβ6 Integrin is required for TGFβ1-mediated matrix metalloproteinase2 (MMP2) expression	Dutta, A., Li, J., Fedele, C., Sayeed, A., Violette, S.M., Manes, T.D., Altieri, D.C. and Languino, L.R.	Biochem J.	October 2014	☐Submitted ☐Accepted ☑Published
2. Integrin αvβ6 promotes an Osteolytic program in cancer cells by upregulatiing MMP2	Dutta, A., Li, J., Lu, H., Akech, J., Pratap, J., Wang, T., Zerlanko, B., FitzGerald, T.J., Jiang, Z., Birbe, R., Wixted, J., Violette, S.M., Stein, G.S., Lian, J.B., Languino, L.R.	Cancer Research	June 2013	☐Submitted ☐Accepted ☑Published
3. IGF-IR Promotes Prostate Cancer Growth By Stabilizing α5β1 Integrin Protein Levels	Sayeed, A., Fedele, C., Trerotola, M., Ganguly, K.K., Languino, L.R.	PlosOne	July 2013	☐Submitted ☐Accepted ☑Published
4. Trop-2 promotes cancer metastasis by modulating β1 integrin functions	Trerotola M, Jernigan DL, Liu Q, Siddiqui J, Fatatis A, Languino L.R.	Cancer Research	August 2012	☐Submitted ☐Accepted ☑Published
5. β1 integrins mediate resistance to ionizing radiation in vivo by inhibiting c-Jun amino terminal kinase 1	Goel, H.L., Sayeed, A., Breen, M., Zarif, M.J., Garlick, D.S., Leav, I., Davis, R.J., Fitzgerald, T.J., Morrione, A., Hsieh, C.C., Liu, Q., Dicker, A.P., Altieri, D.C., Languino, L.R.	J. Cell Physiol.	January 2013	☐Submitted ☐Accepted ☑Published
6. PSA regulates androgen receptor expression in prostate cancer cells	Saxena P., Trerotola M., Wang T., Li J., Sayeed A., Vanoudenhove J., Adams D.S., Fitzgerald T.J., Altieri D.C., Languino L.R.	Prostate	May 2011	☐Submitted ☐Accepted ☑Published

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

Yes ☑ No_____

If yes, please describe your plans:

I plan to submit a paper focused on results, which has been developed in part with the CURE program funds; the title of the paper will be: " αvβ6 Integrin regulates androgen receptor activity and prostate tumor progression."

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.

We have uncovered a novel pathway of prostate cancer progression centered on the signaling properties of the $\alpha_v\beta_6$ integrin/survivin network. Therapeutic targeting of this pathway using molecular and genetic disease models that approximate the human disease, combined with mechanistic dissection of its molecular requirements, may open concrete new prospects for the rational treatment of patients with advanced and metastatic prostate cancer.

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.

None.

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. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME Languino, Lucia R.	POSITION TITLE Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Bari School of Science, Bari, Italy	Laurea BS)	1981	Biochemistry
Negri Pharmacological Research Inst., Milan, Italy	Ph.D.	1984	Pharmacology
The Burnham Institute, La Jolla, CA	Postdoc	1987-1991	Biochemistry/Cell Biology

Positions and Honors

1984 – 1985 Research Fellow, Hopital de Bicetre, Paris, France.
1985 – 1986 Research Fellow, Mario Negri Institute, Milan, Italy.
1987 – 1991 Postdoctoral Fellow, Dr. E. Ruoslahti's Laboratory, The Burnham Institute, La Jolla, CA.
1991 – 1993 Senior Scientist, Dept of Cell Biology, RW Johnson PRI, San Diego, CA.
1993 – 1994 Principal Scientist, Dept of Cell Biology, RW Johnson PRI, San Diego, CA.
1991 – 1994 Visiting Scientist, Dept of Vascular Biology, The Scripps Research Inst., La Jolla, CA.
1994 – 2000 Assistant Professor, Dept of Pathology, Yale University, New Haven, CT.
2000 – 2002 Associate Professor, Dept of Pathology, Yale University, New Haven, CT.
2000 – 2004 NIH Study Section, Pathology B/TPM, Member.
2002 – 2010 Professor, Dept of Cancer Biology, Dept of Cell Biology, UMass Medical School, Worcester, MA.
2003 – Journal of Cellular Biochemistry, Editorial Board Member.
2005 – Editorial Board Member, Cancer Research.
2005 – 2008 NIH, National Cancer Institute, SubCommittee C, Member.
2005 – 2010 Professor, Dept of Radiation Oncology, UMass Medical School, Worcester, MA.
2008 Co-Chairman, NIH, National Cancer Institute, Discovery and Development Review Panel.
2008 – Associate Editor-in-Chief, American Journal of Translational Research.
2010 – Professor, Dept of Cancer Biology, Thomas Jefferson University, Philadelphia, PA.
2010 – Prostate Cancer Foundation, Scientific Advisory Board.
2011 Co-Chairman, NIH, National Cancer Institute, Discovery and Development Review Panel.
2012 – Editorial Board Member, PlosOne.
2013 – Editorial Board Member, Matrix Biology.

2013 Chairman, NIH, National Cancer Institute, "Provocative Questions" Review Panel.
2014 Young Investigator Retreat, Co-organizer, Prostate Cancer Foundation.
2014 – 2018 NIH Study Section, TPM, Member.

Selected Peer-Reviewed Publications (original and invited articles out of 111)

I. Original Articles (out of 81)

1. Mehrotra, S., **Languino, L.R.**, Raskett, C.M., Mercurio, A.M., and Altieri, D.C. IAP regulation of Metastasis. *Cancer Cell*, 17: 53-64 (2009). PMCID: PMC2818597
2. Sayeed, A., Alam, N., Trerotola, M., and **Languino, L.R.** Insulin-Like Growth Factor 1 Stimulation of Androgen Receptor Activity Requires β1A Integrins. *J. Cell Physiol.*, 227:751-758 (2012). PMCID: PMC3195902
3. Saxena, P., Trerotola, M. (co-first authors), Wang, T., Li, J., VanOudenhove, J., Adams, D.S., FitzGerald, T.J., Altieri, D.C., and **Languino, L.R.** PSA is required for androgen receptor expression in prostate cancer cells. *The Prostate*, 72:769-776 (2012). PMCID: PMC3404455
4. Trerotola, M., Li, J., Alberti, S., and **Languino, L.R.** Trop-2 inhibits prostate cancer cell adhesion to fibronectin through the β1 integrin-RACK1 axis. *J. Cell Physiol.*, 227:3670-3677 (2012). PMCID: PMC3369113
5. Garlick D.S., Li, J. (co-first authors), Sansoucy, B., Wang, T., Griffith, L., FitzGerald, T.J., Butterfield, J., Charbonneau, B., Violette, S.M., Weinreb, P.H., Ratliff, T.L., Liao, C.P., Roy-Burman, P., Vietri, M., Lian, J.B., Stein, G.S., Altieri, D.C., and **Languino, L.R.**. αvβ6 Integrin Expression is Induced in the POET and $PTEN^{pc-/-}$ Mouse Models of Prostatic Inflammation and Prostatic Adenocarcinoma. *Am. J. Transl. Res*., 4:165-174 (2012). PMCID: PMC3353537.
6. Chae, Y.C., Caino, M.C., Lisanti, S., Ghosh, J.C., Dohi, T., Danial, N.N., Villanueva, J., Ferrero, S., Vaira, V., Santambrogio, L., Bosari, S., **Languino, L.R.**, Herlyn, M., and Altieri, D.C. Control of tumor bioenergetics and survival stress signaling by mitochondrial Hsp90s. *Cancer Cell*, 22:331-344 (2012). PMCID: PMC3615709.
7. Trerotola, M., Jernigan, D.L., Liu, Q., Siddiqui, J., Fatatis, A., and **Languino, L.R.** Trop-2 promotes cancer metastasis by modulating β1 integrin functions. *Cancer Res.*, 73:3155-3167 (2013). PMCID: PMC3655712.
8. Goel, H., Sayeed, A., Breen, M., Zarif, M.J., Garlick, D.S., Leav, I., Davis, R.J., FitzGerald, T.J., Morrione, A., Liu, Q., Dicker, A., Altieri, D.C., and **Languino, L.R.** β1 integrins mediate resistance to ionizing radiation in vivo by inhibiting JNK. *J. Cell Physiol*., 228(7):1601-1609 (2013). PMCID: PMC3749928.
9. Caino, M.C., Chae, Y.C., Vaira, V., Ferrero, S., Nosotti, M., Martin, N., Weeraratna, A., O'Connell, M., Jernigan, D., Fatatis, A., **Languino, L.R.**, Bosari, S., and Altieri, D.C. Metabolic stress control of cytoskeletal dynamics and metastasis. *J. Clin. Invest.*, 123(7):2907-2920 (2013). PMCID: PMC3998961.
10. Chae, Y.C., Angelin, A., Lisanti, S., Kossenkov, A., Speicher, K., Wang, H., Powers, J.F., Tischler, A.S., Pacak, K., Fliedner, S., Michalek, R.D., Karoly, E.D., Wallace, D.C., **Languino, L.R.**, Speicher, D.W., and Altieri, D.C. Landscape of the mitochondrial Hsp90 metabolome in tumors. *Nature Communications*, 4:2139. (2013). PMCID: PMC3732457.

BIOGRAPHICAL SKETCH	
NAME Sayeed, Aejaz	POSITION TITLE Research Instructor
eRA COMMONS USER NAME SAS105	

EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
University of Kashmir, India	B.S.	1988-1991	Biology/Chemistry
University of Kashmir, India	M.S.	1992-1995	Biochemistry / Mol. Biology
Post Graduate Institute of Medical Education & Research, Chandigarh, India	Ph.D.	1996-2000	Experimental Medicine & Biotechnology.

PROFESSIONAL POSITIONS:

Sept 2010-current	Research Instructor, Cancer Biology, Thomas Jefferson University, Philadelphia,PA
Aug. 2005-2010	Research Associate Scientist, California Pacific Medical Center Research Institute, San Francisco, CA
2002-2005	Research Affiliate. Roswell Park Cancer Institute. Buffalo, New York
2000- 2002	Postdoctoral fellow, California Pacific Medical Center Research Institute, San Francisco, CA

PUBLICATIONS:

1. Anindita Dutta, Jing Li, Carmine Fedele, **Sayeed A**, Amrita Singh, Shelia M. Violette, Thomas D. Manes and Lucia R Languino. αvβ6 Integrin Is Required for TGFβ1-Mediated Matrix Metalloproteinase 2 Expression. Biochem J. (2015) Jan 6. PMID: 25558779.
2. **Sayeed A**, Carmine F, Trerotola M, Ganguly K and Lucia Languino. IGF-IR promotes prostate cancer growth by stabilizing α5β1 integrin protein levels, PLoS One: 8(10), 2013.
3. **Sayeed A**, Luciani G, Meng Z, Ling E and Dairkee SH. BST2 expression is a characteristic of high grade breast cancer and leads to endocrine resistance regardless of regardless of estrogen receptor status. PLoS One: 8(6), 2013.
4. **Sayeed A**, Hira Lal Goel , Michael Breen, Matthew J. Zarif, David S. Garlick, Irwin Leav, Roger J. Davis, Thomas J. FitzGerald, Andrea Morrione, Chung-Cheng Hsieh, Qin Liu, Adam P. Dicker, Dario C. Altieri and Lucia R. Languino. β_1 integrins mediate resistance to ionizing radiation in vivo by inhibiting c- Jun amino terminal kinase1. J Cell Physiology:228(7):1601-9, 2013.
5. **Sayeed A**, Alam N, Trerotola M and Languino LR. Insulin-like growth factor 1 stimulation of androgen receptor activity requires β1A integrins. J Cell Physiology: 227(2):751-8, 2012.

6. Parmita Saxena, Marco Treratola, Tao Wang, Jing Li, **Sayeed A**, Jennifer VanOudenhove, Dave S Adams, Thomas J FitzGerald, Dario C Altieri and Lucia R Languino. PSA regulates androgen receptor expression in prostate cancer cells. Prostate:72(7):769-76, 2012.
7. Goodson W, Luciani G, **Sayeed A**, Jafee I, Moore DH and Dairkee SH. Minimum concentrations of bisphenol-A(BPA) and methyl paraben (MP) induce pro survival functional changes in non malignant human breast epithelial cells. Carcinogenesis. 32(11): 1724-33, 2011.
8. Gloria Luciani, Junhee Seok, **Sayeed A**, Stacey Champion, William H Goodson, Stefanie S Jeffrey, Wenzhong Xiao, Michael Mindrinos, Ronald W Davis and Dairkee SH. Distinctive responsiveness to stromal signaling accompanies histologic grade programming of cancer cells. PLoS One: 6(5):2011.
9. **Sayeed A**, Ling E, Meng Z, Luciani G and Dairkee SH. Negative regulation of UCP2 by TGFβ signaling characterizes low and intermediate-grade primary breast cancer. Cell Death and Disease. Jul 15;1:e53, 2010 .
10. Shanaz Dairkee, **Sayeed A**, Gloria Luciani, Stacey Champion, Zenhang Meng, Lakshmi R Jakula, Heidi Feiler, Joe W. Gray, and Dan H. Moore. Immutable functional attributes of histologic grade revealed by context-independent gene expression in primary breast cancer cells. Cancer Research: 69(19), 2009.
11. Shanaz H. Dairkee, Junhee Seok, Stacey Champion, **Sayeed A**, Michael Mindrinos, Ronald W Davis and William H Goodson. Bisphenol A induces a profile of tumor aggressiveness in high risk cells of breast cancer patients. Cancer Research 68:(7),2008.
12. **Sayeed A**, Santi Konduri, Wensheng Liu, Sanjay Bansal, and Gokul Das. Estrogen Receptor alpha alleviates p53-mediated transcriptional repression: Implications for the regulation of Apoptosis. Cancer Research 67: (16), 2007
13. Dairkee SH, Nicolau M, **Sayeed A**, Champion S, Ji Y, Moore DH, Yong B, Meng Z, Jeffrey SS,. Oxidative stress pathways highlighted in tumor cell immortalization; Association with breast cancer outcome. Oncogene: 30 April, 2007
14. Zheng Li, Zhen Hang Meng, **Sayeed A**, Refaat Shalaby, Britt-Marie Ljung and Shanaz H. Dairkee. Genome –wide allelotyping of a new in-vitro model system reveals early events in breast cancer progression. Cancer Research: 62, 5980-598, 2002.
15. **Sayeed A**, Vohra H, Gupta A and Ganguly NK. Apoptosis and its molecular machinery. Current Science,80 No.3,349-360, 2001.