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):** 01/01/2009-12/31/2012

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

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

5. **Grant SAP Number:** 4100047652

6. **Project Number and Title of Research Project:** 2 **-** Prolactin and Growth Factor Signaling in Breast Cancer

7. **Start and End Date of Research Project:** 1/1/2009 - 12/31/2012

8. **Name of Principal Investigator for the Research Project:** Hallgeir Rui, MD, Ph.D.

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:

   $676,617.46

   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
Rui	Professor	21% Yr 1,2,3	$79,398.41
Utama	Instructor	50% Yr 1, 2	$39,151.77
Johnson	Post Doc Fellow	100% Yr 1	$ 5,680.70
Peck	Laboratory Research Mgr	100% Yr 1,2,3	$57,618.38
Tran	Post Doc Fellow	100% Yr 1,2,3	$94,307.41
Kovatich	Student	100% Yr 1,2,3	$12,730.00
DeCamp	Student	100% Yr 1	$3,500
Asihene	Research Tech A	100% Yr 1, 75% Yr 2	$26,953.65
Sato	Work Study Student	25% Yr 1, 2,3	$12,968.00

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
Slidebook automation module	Purchased to upgrade existing equipment so that higher resolution and better image	$4,850
Slidebook analysis software	capture would be possible which was critical	$4,950
Slidebook deconvolution	for project completion.	$4,700

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 ___X___ No_____

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:
Novel approaches to modeling of human estrogen receptor-positive breast cancer in vivo	☐NIH ☒ Other federal (specify Department of Defense (DOD)) ☐ Nonfederal source (specify:_)	12/2012	$581,250	$
Coordinated Loss of miR-195/497 in Breast Cancer	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	07/2011	$1,937,500	$
Molecular features of patient-derived luminal breast cancer xenotransplant models	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	03/2013	$581,000	$

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:

Two R01 grant proposals, each of $ 1,938,000 total budget, are in preparation for NIH/NCI that relate to progress on prolactin-regulation of BCL6 and PTHrP proteins in human breast cancer.

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

The project has supported our original hypothesis that prolactin maintains differentiation of breast cancer cells and several mechanisms by which this occurs have been identified in the course of this project that will be the subject of further research progress. Importantly, the observations have direct implications for development of better diagnostic tests and therapeutic strategies.

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	1
Female	1	1	2	2
Unknown				
Total	**1**	**1**	**3**	**3**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	1	1	3	3
Unknown				
Total	**1**	**1**	**3**	**3**

	Undergraduate	Masters	Pre-doc	Post-doc
White	1	1	2	1
Black				
Asian			1	2
Other				
Unknown				
Total	**1**	**1**	**3**	**3**

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.

The research strengthened collaborative interaction at the institution and brought new technologies and concepts to a broad group of colleagues and peers at the institution.

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:

Collaborative interactions included investigators at University of Pennsylvania, Philadelphia, PA (Serge Fuchs), Walter Reed and the Joyce Murtha National Military Cancer Center, Bethesda, MD (Craig Shriver, Jeffrey Hooke), University of Nebraska Omaha, NE (Kay-Uwe Wagner) and a company, MDR Global Incm, Windber, PA (Albert Kovatich).

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.

GOAL

Progression of breast cancer from early solitary lesions to advanced metastatic disease requires loss of cellular differentiation and epithelial-to-mesenchymal transition (EMT). While extensive efforts have been devoted to understanding the factors that allow this transition to occur in breast cancer, little work has focused on factors that prevent EMT, or on molecular strategies to reverse EMT for use in differentiation therapy. The long-range goal of this research is to identify molecular mechanisms that govern growth and cellular differentiation of human breast cancer to improve therapies, and to use this knowledge to improve drug responsiveness of individual tumors, so that more effective, personalized medical treatment can be realized for cancer patients.

The central hypothesis is that the prolactin receptor (PRLR)-Jak2-Stat5 pathway promotes cellular differentiation and suppresses epithelial-to-mesenchymal transition (EMT), loss of estrogen receptor (ER) expression, and drug resistance of luminal breast cancer.

SPECIFIC AIMS

Aim 1 Determine whether the PRLR-Jak2-Stat5 pathway in human breast cancer is inhibited by EMT-promoting factors in the tumor microenvironment.

Aim 2 Determine whether Stat5a activation is sufficient to overcome dedifferentiation, EMT, and loss of ERα in human breast cancer.

SUMMARY OF RESEARCH

Aim 1 Determine whether the PRLR-Jak2-Stat5 pathway in human breast cancer is inhibited by EMT-promoting factors in the tumor microenvironment.

1) BCL6 is a transcriptional repressor that recognizes DNA target sequences similar to those recognized by signal transducer and activator of transcriptions 5 (Stat5). BCL6 disrupts differentiation of breast epithelia, is downregulated during lactation, and is upregulated in poorly differentiated breast cancer. In contrast, Stat5a mediates prolactin-induced differentiation of mammary epithelia, and loss of Stat5 signaling in human breast cancer is associated with undifferentiated histology and poor prognosis. Here, we identify the mammary cell growth factor prolactin as a potent suppressor of BCL6 protein expression in human breast cancer through a mechanism that requires Stat5a, but not prolactin-activated Stat5b, MEK-ERK, or PI3K-AKT pathways.

Results: Prolactin rapidly suppressed BCL6 mRNA in T47D, MCF7, ZR75.1, and SKBr3 breast cancer cell lines, followed by prolonged reduction of BCL6 protein levels within 3 hours. Prolactin suppression of BCL6 was enhanced by overexpression of Stat5a but not Stat5b, was mimicked by constitutively active Stat5a, but did not require the transactivation domain of Stat5a. Stat5 chromatin immunoprecipitation demonstrated physical interaction with a BCL6 gene regulatory region, and BCL6 transcript repression required histone deacetylase activity based on sensitivity to trichostatin A. Functionally, BCL6 overexpression disrupted prolactin induction of Stat5 reporter genes. Prolactin suppression of BCL6 was extended to xenotransplant tumors in nude mice *in vivo* and to freshly isolated human breast cancer explants ex vivo. Quantitative immunohistochemistry revealed elevated BCL6 in high-grade and metastatic breast cancer compared with ductal carcinoma in situ and nonmalignant breast, and cellular BCL6 protein levels correlated negatively with nuclear Stat5a ($r = -0.52$; $P < 0.001$) but not with Stat5b. Loss of prolactin-Stat5a signaling and concomitant upregulation of BCL6 may represent a regulatory switch facilitating undifferentiated histology and poor prognosis of breast cancer.

2) Basal levels of nuclear localized, tyrosine phosphorylated Stat5 are present in healthy human breast epithelia. In contrast, Stat5 phosphorylation is frequently lost during breast cancer progression, a finding that correlates with loss of histological differentiation and poor patient prognosis. Identifying the mechanisms underlying loss of Stat5 phosphorylation could provide novel targets for breast cancer therapy.

Results: Pervanadate, a general tyrosine phosphatase inhibitor, revealed marked phosphatase regulation of Stat5 activity in breast cancer cells. Lentiviral-mediated shRNA allowed specific examination of the regulatory role of five tyrosine phosphatases (PTP1B, TC-PTP, SHP1, SHP2, and VHR), previously implicated in Stat5 regulation in various systems. Enhanced and sustained prolactin-induced Stat5 tyrosine phosphorylation was observed in T47D and MCF7 breast cancer cells selectively in response to PTP1B depletion. Conversely, PTP1B overexpression suppressed prolactin-induced Stat5 tyrosine phosphorylation. Furthermore, PTP1B knockdown increased Stat5 reporter gene activity. Mechanistically, PTP1B suppression of Stat5 phosphorylation was mediated, at least in part, through inhibitory dephosphorylation of the Stat5 tyrosine kinase, Jak2. PTP1B knockdown enhanced sensitivity of T47D cells to prolactin phosphorylation of Stat5 by reducing the EC50 from 7.2 nmol/L to 2.5 nmol/L. Immunohistochemical analyses of two independent clinical breast cancer materials revealed significant negative correlations between levels of active Stat5 and PTP1B, but not TC-PTP. Collectively, our data implicate PTP1B as an important negative regulator of Stat5 phosphorylation in invasive breast cancer.

3) Extracellular acidosis in malignant tumors favors evolution of more aggressive and therapy-resistant cancer. Molecular alterations induced by acidosis remain poorly defined and may vary between cancer types. Prolactin maintains nuclear tyrosine phosphorylated Stat5 (Nuc-pYStat5) and serves as a prodifferentiation and survival factor for luminal breast epithelial cells. Loss of Nuc-pYStat5 in breast cancer correlates with poor prognosis and anti-estrogen resistance. Experimental activation of Stat5 inhibits invasive characteristics of breast cancer cells. Acidic pH 6.0 associated with early endosomes dissociates prolactin from receptor, but to what extent modest extracellular acidosis of pH 6.5-6.9 in breast cancer blocks prolactin signaling remains unknown. Here we show consistent disruption at pH 6.8 of signaling by prolactin receptors but not receptors for EGF, OSM, or growth hormone.

Results: Quantitative immunofluorescence analysis of human breast cancer specimens established mutually exclusive expression of Nuc-pYStat5 and GLUT1, a glucose transporter upregulated in glycolysis-dependent carcinoma cells and an indirect marker of lactacidosis. Mutually exclusive expression of GLUT1 and Nuc-pYStat5 occurred globally or regionally within tumors, consistent with global or regional acidosis. All prolactin-induced signals and transcripts were suppressed by acidosis, and the pH effect was rapid and immediately reversible, supporting a mechanism of acidosis-disruption of prolactin binding to receptor. T47D breast cancer xenotransplants in mice displayed variable acidosis (pH 6.5-6.9) and tumor regions with elevated GLUT1 displayed resistance to exogenous prolactin despite unaltered levels of prolactin receptors and Stat5. We propose that acidosis-induced prolactin resistance is a previously unrecognized mechanism by which breast cancer cells elude normal homeostatic control.

4) Signal transducer and activator of transcription-3 (Stat3) is frequently activated in breast cancer and multiple lines of evidence suggest that Stat3 promotes tumor progression. However, the prognostic value of Stat3 in human breast cancer remains controversial and associations range from favorable to unfavorable based on four outcome studies of 62, 102, 255 and 517 patients. Cellular Stat3 protein expression was measured in three studies whereas nuclear localized, tyrosine phosphorylated Stat3 (Nuc-pYStat3) was used as the

readout in only one study. We therefore retrospectively analyzed the prognostic value of Nuc-pYStat3 in a larger material of 721 breast cancer specimens. Overall, patients whose tumors were positive for Nuc-pYStat3 tended to have improved survival, but the trend did not reach statistical significance (P=0.08).

Results: When specimens were stratified by tumor grade, patients with low grade but not high grade tumors that were positive for Nuc-pYStat3 had significantly prolonged overall survival in univariate analysis (P=0.014) but not in multivariate analyses. Unexpectedly, quantitative immunofluoresence detection revealed highest levels of Nuc-pYStat3 in normal breast epithelia and gradual loss of Nuc-pYStat3 during progression from DCIS, invasive ductal carcinoma, and lymph node metastases. Levels of Nuc-pYStat3 correlated positively with levels of Nuc-pYStat5, a favorable prognostic marker, in invasive ductal carcinomas. Furthermore, Nuc-pYStat3 levels correlated strongly with protein levels of nuclear localized Stat5a (r=0.633, P<0.001) but notStat5b. Our data does not support the notion that Nuc-pYStat3 is an independent marker of prognosis in breast cancer, although future studies may reveal prognostic utility within molecularly characterized subtypes of breast cancer.

Aim 2 Determine whether Stat5a activation is sufficient to overcome dedifferentiation, EMT, and loss of ERα in human breast cancer.

1) To investigate nuclear localized and tyrosine phosphorylated Stat5 (Nuc-pYStat5) as a marker of prognosis in node-negative breast cancer and as a predictor of response to antiestrogen therapy. Levels of Nuc-pYStat5 were analyzed in five archival cohorts of breast cancer by traditional diaminobenzidine-chromogen immunostaining and pathologist scoring of whole tissue sections or by immunofluorescence and automated quantitative analysis (AQUA) of tissue microarrays.

Results: Nuc-pYStat5 was an independent prognostic marker as measured by cancer-specific survival (CSS) in patients with node-negative breast cancer who did not receive systemic adjuvant therapy, when adjusted for common pathology parameters in multivariate analyses both by standard chromogen detection with pathologist scoring of whole tissue sections (cohort I; n=233) and quantitative immunofluorescence of a tissue microarray (cohort II; n=291). Two distinct monoclonal antibodies gave concordant results. A progression array (cohort III; n=180) revealed frequent loss of Nuc-pYStat5 in invasive carcinoma compared to normal breast epithelia or ductal carcinoma in situ, and general loss of Nuc-pYStat5 in lymph node metastases. In cohort IV (n=221), loss of Nuc-pYStat5 was associated with increased risk of antiestrogen therapy failure as measured by univariate CSS and time to recurrence (TTR). More sensitive AQUA quantification of Nuc-pYStat5 in antiestrogen-treated patients (cohort V; n=97) identified by multivariate analysis patients with low Nuc-pYStat5 at elevated risk for therapy failure (CSS hazard ratio [HR], 21.55; 95% CI, 5.61 to 82.77; P=0.001; TTR HR, 7.30; 95% CI, 2.34 to 22.78; P=0.001). Conclusion: Nuc-pYStat5 is an independent prognostic marker in node-negative breast cancer. If confirmed in prospective studies, Nuc-pYStat5 may become a useful predictive marker of response to adjuvant hormone therapy.

2) Signal transducer and activator of transcripton-5a (Stat5a) and its close homologue, Stat5b, mediate key physiological effects of prolactin and growth hormone in mammary glands. In

breast cancer, loss of nuclear localized and tyrosine phosphorylated Stat5a/b is associated with poor prognosis and increased risk of antiestrogen therapy failure. Here we quantify for the first time levels of Stat5a and Stat5b over breast cancer progression, and explore their potential association with clinical outcome. METHODS: Stat5a and Stat5b protein levels were quantified in situ in breast-cancer progression material. Stat5a and Stat5b transcript levels in breast cancer were correlated with clinical outcome in 936 patients. Stat5a protein was further quantified in four archival cohorts totaling 686 patients with clinical outcome data by using multivariate models.

Results: Protein levels of Stat5a but not Stat5b were reduced in primary breast cancer and lymph node metastases compared with normal epithelia. Low tumor levels of Stat5a but not Stat5b mRNA were associated with poor prognosis. Experimentally, only limited overlap between Stat5a- and Stat5b-modulated genes was found. In two cohorts of therapy-naïve, node-negative breast cancer patients, low nuclear Stat5a protein levels were an independent marker of poor prognosis. Multivariate analysis of two cohorts treated with antiestrogen monotherapy revealed that low nuclear Stat5a levels were associated with a more than fourfold risk of unfavorable outcome. Loss of Stat5a represents a new independent marker of poor prognosis in node-negative breast cancer and may be a predictor of response to antiestrogen therapy if validated in randomized clinical trials.

3) Prolactin controls the development and function of milk-producing breast epithelia but also supports growth and differentiation of breast cancer, especially luminal subtypes. A principal signaling mediator of prolactin, Stat5, promotes cellular differentiation of breast cancer cells *in vitro*, and loss of active Stat5 in tumors is associated with tamoxifen therapy failure in patients. In luminal breast cancer progesterone induces a cytokeratin-5 (CK5)-positive basal cell-like population. This population possesses characteristics of tumor stem cells including quiescence, therapy-resistance, and tumor-initiating capacity. Here we report that prolactin counteracts induction of the CK5-positive population by the synthetic progestin R5020 (Pg) in luminal breast cancer cells both *in vitro* and *in vivo*. CK5-positive cells were chemoresistant as determined by four-fold reduced rate of apoptosis following docetaxel exposure. Pg-induction of CK5 was preceded by marked up-regulation of BCL6, an oncogene and transcriptional repressor critical for the maintenance of leukemia-initiating cells. Knockdown of BCL6 prevented induction of CK5-positive cell population by Pg. Prolactin suppressed Pg-induced BCL6 through Stat5 but not Erk- or Akt-dependent pathways.

Results: Clinical relevance of these findings was reflected in a positive correlation between tumor levels of BCL6 and CK5 protein in progesterone receptor-positive breast cancer among premenopausal but not postmenopausal patients. Suppression of Pg-induced CK5-positive luminobasal cells represents a novel pro-differentiation effect of prolactin in breast cancer. These insights may have direct implications for breast cancer progression and therapy since loss of prolactin receptor signaling is frequent and BCL6 inhibitors are emerging for lymphoma treatment.

Key Research Accomplishments

Prodifferentiation and progression-suppressive effects of Prolactin-Stat5 in human breast cancer:

- Prolactin-Jak2-Stat5 signaling effectively suppresses expression of the BCL6 oncogene in human breast cancer.
- Prolactin blocks progesterone-induction of CK5-positive drug resistant cells through a mechanism that requires progestin-induction of BCL6.
- Loss of prolactin-Stat5a signaling and concomitant upregulation of BCL6 may represent a regulatory switch facilitating undifferentiated histology and poor prognosis of breast cancer.
- Collectively these novel discoveries implicate prolactin-Stat5 in preventing progestin-induced dedifferentiation and drug resistance in luminal breast cancer. New therapeutic approaches to breast cancer that involve prolactin receptor activation and BCL6 inhibitors may be a result of these observations.

Consequences and causes of loss of Stat5 activation in progressing human breast cancer:

- Loss of Stat5 activation in breast cancer is associated with poor clinical outcome and increased risk of anti-estrogen therapy response failure.
- Loss of Stat5a may be that key contributor to loss of differentiation.
- Our data does not support the notion that Nuc-pYStat3 is an independent marker of prognosis in breast cancer, although future studies may reveal prognostic utility within molecularly characterized subtypes of breast cancer.
- Mechanisms for loss of Stat5 activation in human breast cancer include increased PTP1B activity, a known mediator of Her2/EGFR effects, and acidosis in the tumor microenvironment due to glycolysis and lactacidosis.
- Collectively these novel discoveries support the utility of Stat5 pathway markers in diagnosis and therapy management of human breast cancer, and identify at least two distinct mechanism whereby Stat5 inactivation in breast cancer occurs.

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. Prolactin Inhibits Expression of the Proto-oncogene BCL6 in Breast Cancer through a Stat5 Dependent Mechanism.	Tran TH, Utama FE, Lin J, Yang N, Sjolund AB, Ryder A, Johnson KJ, Neilson LM, Liu C, Brill KL, Rosenberg AL, Witkiewicz AK, Rui H.	Cancer Research, 70, 1711–21, 2010.	June 2009	☐Submitted ☐Accepted ☒Published
2. PTP1B suppresses prolactin activation of Stat5 in breast cancer cells.	Johnson KJ, Peck AR, Liu C, Tran TH, Utama FE, Sjolund AB, Schaber JD, Witkiewicz AK, Rui H.	Am J Pathol, 177, 2971–2983, 2010.	Aug 2010	☐Submitted ☐Accepted ☒Published
3.Signal transducer and activator of transcription-3 and breast cancer prognosis.	Sato T, Neilson LM, Peck AR, Liu C, Tran TH, Witkiewicz A, Hyslop T, Nevalainen MT, Sauter G, and Rui H.	American Journal of Cancer Research 1:347-355, 2011.	Dec 2010	☐Submitted ☐Accepted ☒Published
4. Loss of Nuclear Localized and Tyrosine Phosphorylated Stat5 in Breast Cancer Predicts Poor Clinical Outcome and Increased Risk of Anti-Estrogen Therapy Failure. Subject of Editorial: Tweardy D, Chang JC. Stat5: from breast development to cancer prognosis, prediction, and progression. J Clin Oncol. 29, 2443-4, 2011.	Peck AR, Witkiewicz AK, Liu C, Stringer GA, Klimowicz AC, Pequignot E, Freydin B, Tran TH, Yang N, Rosenberg AL, Hooke JA, Kovatich AJ, Nevalainen MT, Shriver CD, Hyslop T, Sauter G, Rimm DL, Magliocco AM, Rui H.	J Clin Oncol, 18, 2448-53, 2011.	May 2010	☐Submitted ☐Accepted ☒Published
5. Low levels of Stat5a protein in breast cancer are associated with tumor progression	Peck AR, Witkiewicz AK, Liu C, Klimowicz AC, Stringer GA, Pequignot E, Freydin B, Yang N, Ertel A, Tran	Breast Cancer Research, 14:R130, 2012 (16	April 2012	☐Submitted ☐Accepted ☒Published

and unfavorable clinical outcomes.	TH, Girondo MA, Rosenberg AL, Hooke JA, Kovatich AJ, Shriver CD, Rimm DL, Magliocco AM, Hyslop T and Rui H.	pages).		

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:

This research is ongoing and we will continue to submit manuscripts to the journals above.

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.

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Rui, Hallgeir, M.D., Ph.D.	Professor of Cancer Biology, Medical Oncology, and Pathology
eRA COMMONS USER NAME hallgeir	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Oslo, Faculty of Medicine, Norway	M.D	1987	Medicine
University of Oslo, Institute of Pathology, Norway	Ph.D.	1988	Pathology

Positions and Honors.

1987-88 Clinical Residency, Notodden Hospital, Notodden, Norway (internal medicine/surgery).

1988 Clinical Residency, Notodden/Hjartdal Community Health Center, Norway (community med.)

1989 Research fellow (Norw. Cancer Society), Inst. of Pathology, Natl. Hosp., Oslo, Norway.

1989 Post-doc fellow (Fulbright), Dr. Diane H. Russell (died), University of South Florida, Tampa, FL.

1989-91	Post-doc Fellow (Fogarty), Dr. Julie Y. Djeu, Moffitt Cancer Center, USF, Tampa, FL.
1991-95	Visiting Research Fellow (Fogarty), Section of Dr. W.L. Farrar, NCI/NIH, Frederick, MD.
1995-01	Assistant Professor, Dept of Pathology, Uniformed Services University, Bethesda, MD.
2001-02	Associate Professor w/tenure, Dept of Pathology, Uniformed Services University, Bethesda, MD
2002-06	Associate Professor, Dept. of Oncology, Georgetown U., Lombardi Comprehensive Cancer Center, Washington DC.
2006-	Professor w/tenure, Dept of Cancer Biology, Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA.

Honors

Fulbright fellowship from the Fulbright Association, 1989.
Fogarty fellowship (NIH) 1989-1991.
"Lillemor Grobstok's Scholarship for Studies of Cancer Abroad", 1989, 1992-95. (Norwegian Cancer Society)
Invited speaker at The Gordon Research Conference on Prolactin. Ventura, CA, 02/4-9/96.
Special Guest Lecturer, 9th Ann. Meeting, American Academy of Allergy, Asthma & Immunology, 02/24/97
Chair, Signal Transduction Session at Gordon Research Conference on Prolactin. Ventura,CA 1998/2004.
Symposium speaker, Endocrine Society Meetings 2003 (Philadelphia, PA) and 2004 (New Orleans, LA)
Co-chair of Gordon Research Conference on Prolactin 2006, Ventura, CA.
Elected Chair of Gordon Conference on Prolactin and Growth Hormone 2008, Ventura, CA.
Recipient of Komen for the Cure Promise Award, $ 6.7 million for translational breast cancer research, 2009.

Scientific Societies

Fulbright Alumni Association, American Society for the Advancement of Science, International Cytokine Society, Endocrine Society/FASEB, American Association for Cancer Research.

Federal Government committees

Grant review committees since 1999: Fourteen NIH panels, ten DOD panels, one VA panel, one NSF panel.