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:** The Institute for Cancer Research

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 – 6/30/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Maria Minko Gill

4. **Grant Contact Person's Telephone Number:** 215-728-2659

5. **Grant SAP Number:** 4100054848

6. **Project Number and Title of Research Project:** 04-Targeting STAT3 in Ovarian Cancer

7. **Start and End Date of Research Project:** 1/1/2011 – 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Denise C. Connolly, 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:

   $457,376

   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
Connolly, Denise C	PI	10% year 1; 5% year 2	$10,771.22
O'Brien, Shane	Scientific Assistant	23% year 2; 15% year 3	$21,435.49
Gritsina, Galina	Graduate Student	54% year 1; 100% year 2; 50% year 3	$49,379.72
Neulight, Ellen B	Scientific Technician II	14% years 1-2	$11,195.78
Maglaty, Marisa	Technical Specialist	14% year 2; 8% year 3	$13,279.06
Litwin, Samuel	Associate Professor	5% years 1-2	$20,192.47

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
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___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 awarded:
STAT proteins and inflammation in ovarian cancer **	☐NIH ☐ Other federal (specify:_____) ☒ Nonfederal source (specify: Sandy Rollman Ovarian Cancer Foundation)	April 2011	$98,520	$98,520
R21 - Sensitizing Ovarian Carcinoma to Chemotherapy (resubmission planned for October 2014)	☒ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Feb 2014	$490,875	Not funded
P50 - FCCC-PENN SPORE in Ovarian Cancer Project 3: Targeting an HSP90 central hub for treatment of ovarian cancer	☒ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	May 2014	$2,078,875	Pending

**The grant submitted to the Sandy Rollman Ovarian Cancer Foundation stemmed from our initial hypothesis and early preliminary data from this Heath Research Grant Award. Shortly after the Heath Research Grant Award commenced, I met Dr. Ana Gamero from the Fels Institute for Cancer Research at Temple University. Dr. Gamero discovered an important role for STAT2 in cancer. Specifically, she showed that genetic loss of STAT2 inhibits tumor progression in mouse models of chemically-induced colorectal and skin cancer and that this inhibition is at least in part due to reduced activation of STAT3 and production of IL-6. Based on the established roles of STAT3 and IL-6 in EOC and Dr. Gamero's observation that STAT2 plays a critical role in cancer development by regulating STAT3 activation and IL-6 production, we initiated a collaborative project to investigate the relationship of STAT2 and STAT3 in the context of spontaneous ovarian tumor development and tumor associate inflammation.

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:

Pending the outcome of the resubmitted R21 grant and the FCCC-PENN SPORE in Ovarian Cancer we will submit proposals via other mechanisms (e.g., R01) or other agencies or Foundations (i.e., the Department of Defense, the Marsha Rivkin Center for Ovarian Cancer Research, or the Ovarian Cancer Research Fund) to test the hypothesis that STAT3 inhibition in ovarian cancer may target a key vulnerability in ovarian cancer stem cells.

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

The goal of this research project is to affirm the importance of STAT3 as a critical target for therapeutic intervention. Based on the work of this Heath Research Grant Award and work on an independent project that showed targeted inhibition of HSP90 results in inhibition of STAT3 activation in ovarian cancer, our goal continues to be development of preclinical data supporting the use of JAK2/STAT3 inhibitors as single agents or as combination therapy with conventional cytotoxic agents and/or HSP90 inhibitors to improve treatments available for ovarian cancer patients.

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	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
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.

This project involved collaboration between my group and those of Drs. Luis Sigal, Siddharth Balachandran and Glenn Rall, experts in immunology at the Fox Chase Cancer Center (FCCC). The project relied heavily on the FCCC Flow Cytometry Facility, directed by Dr. Richard Hardy. The project also relied on the FCCC Biological Imaging, Laboratory Animal, Biostatistics and Bioinformatic Facilities at FCCC.

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:

Dr. Ana Gamero, Fels Institute for Cancer Research at Temple University. We are working on collaborative project with Dr. Gamero to investigate the role of STAT2 in regulating STAT3 activation in ovarian cancer.

Dr. Nathalie Scholler, University of Pennsylvania (presently at SRI International). Based

on mutual interest in ovarian tumor-associated inflammation, we collaborated with Dr. Scholler to investigate the role of complement activation in ovarian cancer.

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.

Specific aims:
1) Evaluate the effects of a novel small molecule janus kinanase (JAK) 2 inhibitor on epithelial ovarian cancer cells *in vitro*.
2) Determine the therapeutic efficacy of STAT3 inhibition on ovarian carcinoma development and dissemination *in vivo*.
3) Test the hypothesis that targeting the STAT3 pathway will inhibit *in vivo* tumor development by direct effects on both tumor cells and on inflammatory cells present in the tumor microenvironment.

Progress:

In vitro effects of AZD1480 treatment on OC cell lines (Aim 1).

Constitutive activation of STAT3 is common in ovarian carcinoma cell lines and tumors. JAKs are key upstream mediators of STAT3 activation; therefore, we sought to determine the effects of a JAK inhibitor, AZD1480, on STAT3 activation, viability, apoptosis and proliferation in ovarian cancer (OC) cells. Exposure of human (A1847, OVCAR-5 and OVCAR-8) and murine (MOVCAR-5009 and MOVCAR-5447) OC cells to increasing concentrations of AZD1480 (0, 0.5, 0.1, 1, 5 and 10 µmol/L) for 24 hours resulted in dose dependent inhibition of STAT3 activation, measured by $pSTAT3^{Y705}$ levels. Substantial reductions were observed at 0.5 and 0.1 µmol/L, with little or no detectable $pSTAT3^{Y705}$ remaining in cells treated with 1 µmol/L. Consistent with previous studies on cell lines from several solid tumors (6, 16, 20), low concentrations (0, 0.5, 0.1 and 1 µmol/L) of AZD1480 had no effect on cell viability; inhibition of cell viability was only observed in the presence of high concentration (5 µmol/L) AZD1480. Significant accumulation of annexin V^+ apoptotic cells occurred in the presence of 5 µmol/L AZD1480, while cleaved poly ADP ribose polymerase (PARP) levels began to accumulate at lower concentrations (0.5-1.0 µmol/L) of drug. Similarly, inhibition of cell proliferation was observed in the presence of high concentration (5 µmol/L) AZD1480. It is possible that high concentrations of AZD1480 result in off-target effects; however, we were able to confirm that treatment of cells with 1.0 µmol/L AZD1480 resulted in abrogation of STAT3 DNA binding, confirming direct effects of AZD1480 on STAT3 activation. These results show that AZD1480-treatment has limited effects on cell viability, growth or survival of cultured OC cells and may reflect effects related to 2-D cell culture conditions or the additional requirement of the tumor microenvironment to observe full effect of the drug.

Dissemination of OC occurs primarily through local spread in the abdominal cavity, requiring the capacity of tumor cells to shed from the primary tumor, migrate and adhere to secondary sites in the peritoneum. Previous work showed that activated STAT3 is a key mediator of OC motility; therefore, the effects of AZD1480 on cell migration were evaluated using trans-well

chemotactic migration assays. Migration was significantly reduced in A1847, OVCAR-5 and OVCAR-8 cells treated with 1 µmol/L AZD1480. Similarly, pre-treatment of with 1 µmol/L AZD1480 inhibited the capacity of A1847, OVCAR-5 and OVCAR-8 cells to adhere to fibronectin or type I collagen. Taken together, these results show that AZD1480 inhibits STAT3 activation, migration and adhesion in OC cells, and suggest the potential for targeted inhibition of the JAK/STAT3 pathway to inhibit OC tumor growth and spread *in vivo*.

AZD1480 inhibits OC growth and ascites production in transgenic mice (Aim 2).

A transgenic mouse model of OC was used to evaluate the therapeutic potential of AZD1480 *in vivo*. Transgenic MISIIR-TAg mice develop spontaneous OC that resembles high grade serous OC with 100% penetrance. This model is particularly appropriate for analysis as full transformation by SV40 large TAg requires STAT3 and tumors and cell lines derived from tumor bearing mice exhibit high levels of constitutively activated STAT3. Moreover, the presence of a functional immune system enables evaluation the effects of JAK/STAT3 inhibition on the immune tumor microenvironment. To evaluate the effects of AZD1480 on tumor growth, mice were dosed twice daily with 30 mg/kg by oral gavage, based on previous studies and on pilot pharmacodynamic studies in tumor bearing MISIIR-TAg mice showed that treatment with 30 mg/kg AZD1480 resulted in dramatically reduced $pSTAT3^{Y705}$ levels at 2 and 6 hours post treatment, with recovery at 24 hours. The presence of tumors in mice was confirmed by magnetic resonance imaging (MRI); once ovary volume reached $50mm^3$ mice were randomized for treatment with AZD1480 or vehicle (n=17 mice/group) for up to 8 weeks and tumor growth was monitored and quantified by weekly MRI. Longitudinal growth data demonstrated a significant delay in tumor growth rate and smaller ovarian tumors in the AZD1480-treated mice compared to vehicle treated controls. Endpoint analyses confirmed the MRI data, showing significantly smaller tumors in AZD1480-treated mice. Moreover, at necropsy significantly fewer mice had malignant ascites; 2/17 (12%) AZD1480-treated mice compared to 12/17 (70%) vehicle-treated mice, p=0.00127.

To confirm AZD1480-mediated inhibition of STAT3 activation, snap frozen tumor tissue specimens were evaluated by immunoblot and ELISA. Immunoblot analysis showed significantly reduced $pSTAT3^{Y705}$ levels in AZD1480-treated tumors compared to vehicle-treated controls. An electrochemiluminescent ELISA assay was used to quantify $pSTAT3^{Y705}$ levels and further validated the significant effects of AZD1480 on STAT3 activation in tumors. Taken together, these results show that AZD1480 results in significant inhibition of tumor growth and progression and that these effects are at least partly due to reduction of STAT3 activation in primary tumors.

Expression of STAT3 target genes is altered in tumors from AZD1480-treated mice (Aim 2).

The primary functional consequences of STAT3 activation are related to its role as a transcription factor. To define the effects of AZD1480 treatment on STAT3-mediated transcription, RNA was isolated from ovarian tumors of mice that were treated with AZD1480 or vehicle (n=4/group) and global changes in gene expression were analyzed by genome wide microarray analysis. Unsupervised clustering analysis allowed separation into two groups; drug-treated tumors clustered separately from vehicle treated controls (data not shown). Using >2-fold

change and $p<0.01$ cutoff we identified a list of 10 upregulated and 87 downregulated genes in AZD1480 treated tumors. Notably, the list of differentially expressed genes included a large number of genes previously shown to be regulated by STAT3, including genes involved in cancer and wound healing (e.g., *Ccnd1*, *Ccl2*, *Cepd*, *Ifiti1*, *Mx1*, *Mx2*, *Oas1*, *Oas2*, *Usp18*). Gene ontology (GO) analysis identified genes enriched for ribonucleoside metabolic process and interferon and cytokine-mediated signaling pathways. A subset of genes was selected for individual validation by qRT-PCR. Differential expression was shown for *Ccdn1*, *Ccl12*, *Ifi204*, *Cd151*, *Igf1* and *Stat3*. These results confirm the direct inhibitory effects of AZD1480 on STAT3 transcriptional activity *in vivo*, and strongly suggest that the observed tumor growth inhibition result was due to inhibition of STAT3.

AZD1480 treatment inhibits tumor-associated integrin αvβ3 expression and MMP activity (Aim 2).

Expression of integrin αvβ3 and activation of tumor-associated matrix metalloproteinases (MMPs) in tumor and stromal cells is an essential component of OC growth and dissemination. As MMPs are transcriptional targets of STAT3, we hypothesized that the AZD1480-mediated tumor growth inhibitory effects were due in part to inhibition of tumor-associated integrin αvβ3 signaling and MMP activation. To investigate this directly, we used methods developed in our laboratory for combined anatomic and fluorescent molecular tomography (FMT) to detect and quantify *in vivo* integrin αvβ3 expression and MMP activation. Mice with ovarian tumors were treated with vehicle or AZD1480 (n=5/group) for four weeks and imaged weekly by MRI and FMT to determine tumor volume and to quantify integrin αvβ3 and MMP probe binding and activation. Once again, AZD1480 treated mice again exhibited significantly reduced tumor growth as evidenced by MRI. In addition, there was a significant reduction in integrin αvβ3 probe binding and in MMP probe activation. These results confirm the in vivo tumor growth inhibitory effects of AZD1480 treatment and suggest that the tumor inhibitory effects of this gene are mediated in part by decreased tumor-associated integrin αvβ3 expression and MMP activity.

Effects of JAK/STAT3 inhibition on immune cell populations (Aim 3).

The well-established feed-forward signaling between STAT3 in tumors and in the microenvironment and the lack of effect of AZD1480 treatment on cell growth, viability and survival in cultured OC cells, suggested that the tumor growth inhibitory effects of targeted disruption of the JAK/STAT3 pathway may not be confined to the activity of the drug only in tumor cells. Current thinking in OC research emphasizes the potential importance of targeting the pro-tumorigenic inflammatory response as an adjunct to conventional and tumor-targeted therapies. At the outset of this study, we hypothesized that targeted inhibition of JAK/STAT3 with AZD1480 would exert effects on both tumor cells and on immune cells in the tumor microenvironment, for example by inhibiting tumor associated macrophages, myeloid derived suppressor cells (MDSC) or regulatory T (T_{Reg}) cells. By employing an immunocompetent GEM model of OC, we were able to assess the effects of AZD1480 on immune cell populations in the spleen, tumors and in the peritoneal microenvironment, the principal site of OC dissemination. Immune cell populations were stained and analyzed by flow cytometry for detection of $CD45^+CD4^+$ T cells, $CD45^+F4/80^+CD11b^+$ macrophages, $CD45^+CD11b^+GR\text{-}1^+$ MDSCs and

$CD45^+CD4^+FoxP3^+$ Treg cells, in a subset of mice in the original cohort of 34 mice evaluated for drug efficacy. Analysis of tumors showed that the number of infiltrating leukocytes was small, and that the majority of these cells were $CD45^+F4/80^+CD11b^+$ macrophages. Notably, analysis of leukocyte populations present in the peritoneal cavity showed that while there were no significant differences in $CD45^+F4/80^+CD11b^+$ macrophages or $CD45^+CD11b^+GR\text{-}1^+$ MDSCs there was a significant reduction in both the number and percent of $CD45^+CD4^+$ T cells and $CD45^+CD4^+FoxP3^+$ T_{Reg} cells in AZD1480-treated mice compared to controls. No significant differences in $CD45^+CD4^+$ T cells or $CD45^+CD4^+FoxP3^+$ T_{Reg} cells were detected in the spleens of vehicle- and drug-treated mice. These observations suggest that the tumor growth inhibitory effects of AZD1480 may be due in part to reduction in the immunosuppressive $CD45^+CD4^+FoxP3^+$ T_{Reg} cell population in the tumor microenvironment. This observation is highly significant as the presence of T_{Reg} cells in patient tumors is significantly correlated with poor prognosis. Thus, the capacity of JAK2 inhibition to inhibit T_{Reg} cells may result in significant therapeutic benefit in patients.

In summary, in this study we showed that targeted inhibition of the JAK/STAT3 pathway inhibited *in vivo* ovarian carcinoma growth, ascites production and tumor-associated protease activity, as well as pro-tumorigenic cancer-associated inflammatory mediators. These observations support further investigation of targeting the JAK/STAT3 pathway as a potentially effective strategy for treatment of ovarian cancer.

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, 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. Targeted blockade of JAK/STAT3 signaling inhibits ovarian carcinoma growth	Galina Gritsina, Fang Xiao, Shane W. O'Brien, Rashid Gabbasov, Marisa A. Maglaty, Ren-Huan Xu, Roshan J. Thapa, Yan Zhou, Samuel Litwin, Siddharth Balachandran, Luis J. Sigal, Dennis Huszar and Denise C. Connolly	Molecular Cancer Therapy	Aug 2014	☒ Submitted ☐Accepted ☐Published

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

Yes_____ No____X_____

If yes, please describe your plans:

Based on the three aims proposed in this Heath Research Grant Award and the results obtained from the study, we felt that the results would best be reported as a single manuscript to improve the impact and convey the complete story. As we proceed with the project evaluating the role of STAT2 in regulating STAT3 activation in the same transgenic mouse model, we believe that gene expression analyses performed for both studies will lead to the generation of new hypotheses that may be tested in the context of the STAT2 project or beyond as individual projects. Thus, while there is no immediate plan to submit additional publications for the three described aims, it is likely that future publications would include data/findings derived from this study.

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.

Funds from this mechanism allowed for a thorough and detailed pre-clinical analysis of targeted inhibition of JAK2 and as a consequence, STAT3 activation in transgenic mouse model of ovarian carcinoma. The use of our immunocompetent transgenic mouse model enabled analysis of the response of immune cell subpopulations to targeted JAK2/STAT3 inhibition, leading to the discovery that a potently immunosuppressive subpopulation of T_{Reg} cells, associated with poor prognosis in patients, was inhibited by AZD1480 treatment. This analysis would have been impossible in conventional human cell line xenograft models.

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.

Targeted inhibition of JAK2 and/or STAT3 is a potentially effective strategy for treatment of ovarian cancer and merits additional clinical investigation on patients with ovarian carcinoma.

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	POSITION TITLE
Connolly, Denise C., Ph.D.	Associate Professor
eRA COMMONS USER NAME dcconnol	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Potsdam College (S.U.N.Y.), Potsdam, NY	B.A.	05/84	Biology
Miami University, Oxford, OH	M.S.	12/86	Microbiology
Miami University, Oxford, OH	Ph.D.	09/89	Microbiology

Positions and Honors:

Postdoctoral Fellow, Mayo Clinic/Foundation, Rochester, MN	1989-1995
Senior Research Fellow, Johns Hopkins School of Medicine, Baltimore, MD	1995-1998
Research Associate, University of Michigan, Ann Arbor, MI	1998-1999
Research Associate, Fox Chase Cancer Center, Philadelphia, PA	2000-2003
Assistant Member, Fox Chase Cancer Center, Philadelphia, PA	2003-2006
Co-Director, Mouse Engineering Core, FCCC – NIH Ovarian Cancer SPORE	2004-2009
Associate Member/Assistant Professor, Fox Chase Cancer Center, Philadelphia, PA	2006-2013
Associate Professor, Fox Chase Cancer Center, Philadelphia, PA	2013-
Director, Fox Chase Cancer Center Biosample Repsitory Facility	2011-
Co-Director, Pathology Core, FCCC–Univ of Penn NCI Ovarian Cancer SPORE	2011-
Society of Gynecological Oncologists' 2003 Basic Science Award	2003

Selected Peer-reviewed Publications (from a total of 46)

1. **Connolly, D.C.**, Bao, R., Nikitin, A.Y., Stephens, K.C., Poole, T., Hua, X., Harris, S.S., Vanderhyden, B.C., Hamilton, T.C. Female mice chimeric for the expression of the SV40 TAg under control of the MISIIR promoter develop epithelial ovarian cancer. *Cancer Res*. **63**:1389-1397, 2003.
2. Pieretti-Vanmarcke, R., Donahoe, P.K., Szotek, P., Manganaro, T., Lorenzen, M.K., Lorenzen, J., **Connolly, D.C.**, Halpern, E.F., MacLaughlin, D.T. Recombinant human Müllerian inhibiting substance inhibits long term growth of MIS type II receptor-directed transgenic mouse ovarian cancers *in vivo*. *Clin. Cancer Res*. **12**:1593-1598, 2006.
3. Pieretti-Vanmarcke, R., Donahoe, P.K., Pearsall, L., **Connolly, D.C.**, Dinulescu, D.M., Halpern, E.F., Seiden, M.V., McLaughlin, D.T. 2006. MIS Enhances Subclinical Doses of Chemotherapeutic Agents to Inhibit Human and Mouse Ovarian Cancer. *Proc. Natl. Acad. Sci. USA*, 103:17426-17431.
4. Szotek, P.P., Pieretti-Vanmarcke, R., Masiakos, P.T., Dinulescu, D.M., **Connolly, D.**, Foster, R., Dombkowski, D., Preffer, F., MacLaughlin, D.T., Donahoe, P.K. Ovarian cancer side population defines cells with stem-cell characteristics and Müllerian inhibiting substance responsiveness. *Proc. Natl. Acad. Sci. U.S.A.* **103**:11154-11159, 2006.
5. Daikoku, T., Tranguch, S., Trofimova, I.N., Dinulescu, D.M., Jacks, T., Nikitin, A.Yu., **Connolly, D.C.**, Dey, S.K. 2006. Cyclooxygenase-1 is overexpressed in multiple genetically engineered mouse models of epithelial ovarian cancer. *Cancer Res*., 66:2527-2531.
6. Hensley, H., Quinn, B.A., Wolf. R.L., Litwin, S., Mabuchi, S., Williams, S.J., Williams, C., Hamilton, T.C., **Connolly, D.C.** Magnetic resonance imaging for detection and

determination of tumor volume in a genetically engineered mouse model of ovarian cancer. *Cancer Biol. Ther.* **6**:1717-1725, 2007.

7.Mabuchi, S., Altomare, D.A., **Connolly, D.C.**, Klein Szanto, A., Litwin, S., Hoelzle, M.K., Hensley, H.H., Hamilton, T.C., Testa, J.R. RAD001 delays tumor onset and progression in a transgenic mouse model of ovarian cancer. *Cancer Res.* **67**:2408-2413, 2007.

8.Izumchenko, E., Singh, M.K., Plotnikova, O.V., Tikhmyanova, N., Little, J.L., Serebriiskii, I.G., Seo, S., Kurokawa, M., Egleston, B.L., Klein-Szanto, A., Pugacheva, E.N., Hardy, R.R., Wolfson, M., **Connolly, D.C.,** Golemis, E.A. NEDD9 promotes oncogenic signaling in mammary tumor development. *Cancer Res.* **69**:7198-7206, 2009.

9.Quinn, B.A., Brake, T., Hua, X., Baxter-Jones, K., Ellenson, L.H., Litwin, S., **Connolly, D.C.** Induction of ovarian leiomyosarcomas in mice by conditional inactivation of *Brca1* and *p53*. *PLoS One* **4**(12):e8404, 2009.

10. **Connolly, D.C.,** Hensley, H.H. Xenograft and transgenic mouse models of epithelial ovarian cancer and non-invasive imaging modalities to monitor ovarian tumor growth in situ: applications in evaluating novel therapeutic agents. *Curr. Protoc. Pharmacol.* **45**:14.12.1-14.12.36, 2009.

11. Deng, S., Yang, X., Lassus, H., Liang, S., Kaur, S., Ye, Q., Li, C., Wang, L.-P., Roby, K.F., Orsulic, S. **Connolly, D.C.**, Zhang, Y., Montone, K., Bützow, R., Coukos, G., Zhang, L. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. *PLoS One* **5**(4):e10277, 2010.

12. Quinn, B.A., Xiao, F, Bickel, L., Marin, L., Hua, X., Klein-Szanto, A., **Connolly, D.C.** Development of a syngeneic mouse model of epithelial ovarian cancer. *J. Ovarian Res.* **3**:24, 2010.

13. Ratushny, V., Pathak, H.B., Beharry, N. Tikhmyanova, N., Xiao, F., Li, T., Litwin, S. **Connolly, D.C.**, Yen, T.J. Weiner, L.M., Godwin, A.K., Golemis, E.A. Dual inhibition of SRC and Aurora kinases induces post-mitotic attachment defects and cell death. *Oncogene* 31:1217-1227, 2012.

14. Hensley, H.H., Roder, N.A., O'Brien, S.W., Bickel, L.E., Xiao, F., Litwin S., **Connolly, D.C.** Combined *in vivo* molecular and anatomic imaging for detection of ovarian carcinoma-associated protease activity and integrin expression in mice. *Neoplasia* 14:451-462, 2012.

15. Nunez-Cruz, S., Gimotty, P.A., Guerra, M.W., **Connolly, D.C.**, Wu, Y.Q., DeAngelis, R.A., Lambris, J.D., Coukos G., Scholler, N. 2012. Genetic and pharmacologic inhibition of complement impairs endothelial cell function and ablates ovarian cancer neovascularization. *Neoplasia*, 14:994-1004.

16. Do, T.-V., Xiao, F., Bickel, L.E., Klein-Szanto, A.J., Pathak, H.B., Hua, X., Howe, C., O'Brien, S. W., Maglaty, M., Ecsedy, J.A., Golemis, E.A., Schilder, R.J., Godwin, A.K., **Connolly, D.C**. Aurora kinase A mediates epithelial ovarian cancer migration and adhesion. *Oncogene* 33:539-549, 2014.

17. Liu, H., Xiao, F., Serebriiskii, I.G., O'Brien, S.W., Maglaty, M.A., Astsaturov, I., Martin, L.P., Litwin, S., Proia, D.A., Golemis, E.A., **Connolly, D.C.** Network analysis identifies an HSP90-central hub susceptible in ovarian cancer. *Clin. Cancer Res.*, 19:5053-67, 2013.

18. Shah, N.R., Tancioni, I., Ward, K.K., Lawson, C., Chen, X.L., Jean, C., Sulzmaier, F., Uryu, S., Miller, N.L., **Connolly, D.C.,** Schlaepfer, D.D. Analyses of merlin/NF2 connection to FAK inhibitor responsiveness in serous ovarian cancer. *Gynecol. Oncol.* 134:104-111, 2014.