

Response Form for the Final Performance Summary Report*

1. Name of Grantee: Thomas Jefferson University
2. Year of Grant: 2008 Formula Grant

A. For the overall grant, briefly describe your grant oversight process. How will you ensure that future health research grants and projects are completed and required reports (Annual Reports, Final Progress Reports, Audit Reports, etc.) are submitted to the Department in accordance with Grant Agreements? If any of the research projects contained in the grant received an “unfavorable” rating, please describe how you will ensure the Principal Investigator is more closely monitored (or not funded) when conducting future formula funded health research.

Thomas Jefferson University instituted a grant oversight process when the CURE grants were originally awarded in 2002. A grant oversight staff/office under the direction of the Vice President for Research (VPR) was established in 2002. There are currently two staff positions in this office which assist the VPR in the monitoring and administration of these grants. This staff ensures that all reporting requirements are met in a timely fashion including annual and final reports. The reports are edited and proofread and returned to the investigator if any reporting requirements are not met prior to submission. This process ensures that investigators are performing the research requirements as stated in their research plan. Principal investigators who receive a final performance review rating of unfavorable are not eligible for subsequent Formula Fund awards except in exceptional instances reviewed and approved by the Vice President for Research or his/her designee.

* Please note that grantees' Final Performance Summary Reports, Response Forms, and Final Progress Reports *will be made publicly available on the CURE Program's Web site.*

Project Number: 0865201

Project Title: Role and Regulation of Focal Adhesion Kinase in Melanoma

Investigator: Aplin, Andrew

B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. While the investigators did secure a small extramural grant, it does not appear that they made a larger effort to secure larger extramural grants (such as the NIH R-series) except in passing mention in the final progress report. The final progress report was designed to allow reporting of grants that were submitted and not funded, and if the investigators did indeed apply for more extramural funding (even if unsuccessful); these grants should be listed in the final progress report. Furthermore, the mention of an NIH grant submitted in February 2013 does not include relevant information including grant title, RFA, NIH institute where grant will be reviewed, and grant number if assigned.

Future recommendations: I would recommend more aggressive efforts to leverage funding, and inclusion of relevant grant data for submitted applications regardless of funding outcome.

Response: Within the funding period I have received 3 R01s, 2 DoD grants, 3 foundation grants and 1 industrial award. This seems to have been an administrative omission.

2. For a grant whose goal was to elucidate biomarkers that might have prognostic value, there was no clinical collaboration or use of in vivo models (either at TJU or through collaborative efforts). For future grants with this objective, I would recommend that the investigators seek collaborators who can work with them to either secure patient samples for biomarker testing or to confirm their findings in mouse models (either transgenic or xenograft-based).

Future recommendations: I would recommend establishing collaborations to increase the chances of obtaining more pre-clinical data if the objective is indeed the establishment of clinical biomarkers.

Response: I am currently working within a collaborative group funded that includes both basic/translational researchers and clinicians.

3. For many of these experiments, a limited number of cell lines were used (likely due to limited resources). Given the limitations of laboratory melanoma models in replicating the human disease, future studies should consider use of a broader set of lines (genetically characterized to some extent, i.e., BRAF/NRAS mutation status, PTEN status, CDKN2A status, etc.).

I would recommend using a broader panel of genetically characterized cell lines for all experiments.

Response: I agree. We analyze our findings as broad a panel as possible, as well as in fresh tumor samples.

4. While six publications were associated with the final progress report, only three represent actual original research that is directly relevant to this award. The other three reviews, while interesting and also related in part to the research, are not publications I would link directly with this award.

I would (as a reviewer) prefer focusing primarily on publications that report original research funded through this grant proposal.

Response: Next time, I will highlight references primarily linked to the funded research.

Reviewer 2:

None.

Reviewer 3:

It would improve the beneficial impact of this study, if either antibody directed against p-FAK ser-910 or small molecule inhibitor specific to p-FAK ser-910 are developed and tested in animal models.

Response: A antibody against pS910 was used in the studies. A small molecule inhibitor that blocks the kinase that phosphorylates S910 is in current use in the lab

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: N/A

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: N/A

E. Generic Recommendations for Thomas Jefferson University

Reviewer 1:

I am unaware if this grant was made with matching funds from the institution, but given the rather ambitious scope of the application, similar future applications could benefit significantly from some other type of either linked supplement or matching funds to help finance the research.

Response: Dr. Aplin meets regularly with his Chair to review his research portfolio, his level of grant funding and needs he has to support his work. The Chair meets regularly with the Dean of the Medical School and has the opportunity to discuss their faculty and request additional research support. The Institution does not provide formal matching funds.

Project Number: 0865202
Project Title: Prolactin and Growth Factor Signaling in Breast Cancer
Investigator: Rui, Hallgeir

B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

None.

Reviewer 2:

None.

Reviewer 3:

Weaknesses: The major criticism is the lack of any progress on the stated objectives in the project, particularly with regard to the potential interface between the EGFR/Her2 axis and the prolactin/Stat5a signaling cascade. Nevertheless, the applicant did succeed in identifying BCL6 as a potentially important target of prolactin signaling as well as identifying PTP1B as a potential negative modifier of Stat5a signaling. The clinical studies identifying Stat5a as a predictive factor are also interesting, but not related to the original objectives. In the absence of information regarding the stated goals of the other funding sources listed on each of the published papers, it is not easy to identify how funds from the Pennsylvania Department of Health expedited progress on these projects.

Recommendations:

1. New studies would be needed to identify and characterize interactions between EGFR/Her2 signaling and the prolactin/prolactin receptor/Stat5a axis.

Response: We thank the Reviewers for their thoughtful comments and favorable rating. Reviewer 3 mentions two publications resulting from this three-year project. We would like to highlight that in total seven high impact publications (listed below) resulted from this generous support from PA Department of Health. These were listed in our final progress report, although the last two manuscript were still listed as "in press" at the time so full published references are now included. We would like to highlight that several of

the points brought up by Reviewer 3 are addressed by some of these additional publications.

1. 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**. Prolactin Inhibits Expression of the Proto-oncogene BCL6 in Breast Cancer through a Stat5 Dependent Mechanism. *Cancer Research*, 70, 1711–21, 2010.
2. Johnson KJ, Peck AR, Liu C, Tran TH, Utama FE, Sjolund AB, Schaber JD, Witkiewicz AK, **Rui H**. PTP1B suppresses prolactin activation of Stat5 in breast cancer cells. *Am J Pathol*, 177, 2971–2983, 2010.
3. Sato T, Neilson LM, Peck AR, Liu C, Tran TH, Witkiewicz A, Hyslop T, Nevalainen MT, Sauter G, and **Rui H**. Signal transducer and activator of transcription-3 and breast cancer prognosis. *American Journal of Cancer Research* 1:347-355, 2011.
4. 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**. Loss of Nuclear Localized and Tyrosine Phosphorylated Stat5 in Breast Cancer Predicts Poor Clinical Outcome and Increased Risk of Anti-Estrogen Therapy Failure. *J Clin Oncol*, 18, 2448-53, 2011.
Subject of Editorial: Tweardy D, Chang JC. Stat5: from breast development to cancer prognosis, prediction, and progression. *J Clin Oncol*. 29, 2443-4, 2011.
5. Peck AR, Witkiewicz AK, Liu C, Klimowicz AC, Stringer GA, Pequignot E, Freydin B, Yang N, Ertel A, Tran TH, Gironde MA, Rosenberg AL, Hooke JA, Kovatich AJ, Shriver CD, Rimm DL, Magliocco AM, Hyslop T and **Rui H**. Low levels of Stat5a protein in breast cancer are associated with tumor progression and unfavorable clinical outcomes. *Breast Cancer Research*, 14:R130, 2012 (16 pages).
6. Yang N, Liu C, Peck AR, Gironde MA, Yanac AF, Tran TH, Utama FE, Tanaka T, Freydin B, Chervoneva I, Hyslop T, Kovatich AJ, Hooke JA, Shriver CD, and **Rui H**. Prolactin-Stat5 signaling in breast cancer is potently disrupted by acidosis within the tumor microenvironment. *Breast Cancer Research*, 15, R73, 2013.
7. Sato T, Tran TH, Peck AR, Gironde MA, Liu C, Goodman CR, Neilson LM, Freydin B, Chervoneva I, Hyslop T, Kovatich AJ, Hooke JA, Shriver CD, Fuchs SY and **Rui H**. Prolactin suppresses a progesterin-induced CK5-positive cell population in luminal breast cancer through inhibition of progesterin-driven BCL6 expression, *Oncogene*, 33, 2215-24, 2014 May 27. doi: 10.1038/onc.2013.172.

Regarding the first specific comment, it turned out that the interphase between EGFR/Her2 and the prolactin/Stat5a signaling cascade was much more complex than originally anticipated based on our preliminary data at the time. An eighth manuscript is in preparation documenting interaction between prolactin receptors and Her2, in which we propose that Her2 is “hijacking” ligand-free prolactin receptors and rewiring signaling away from prolactin-driven differentiation toward Her2-driven proliferation. However work is still ongoing to fully test this hypothesis and complete the study. In addition, an unforeseen complication for this project was that the postdoc who made the original observation that EGF treatment directly inhibited prolactin signaling, erroneously

dissolved EGF stock solution in high concentration of acetic acid instead of the recommended 10 mM acetic acid (which was used for vehicle control). After her departure from the lab we discovered that the initial inhibitory effects with the highly acidic stock EGF were in fact due to protons (acidosis) rather than EGF. Another trainee took this serendipitous finding and completed a publication showing that tumor acidosis, a frequent occurrence in solid tumors driven by growth factors, potentially blocks prolactin receptor activation by disrupting prolactin binding. We published this study last year (Reference 6; Yang et al, Breast Cancer Research, 2013). We have acknowledged funding from PA Department of Health for this project. Because growth factor signaling in solid tumors promotes acidosis, EMT, and loss of differentiation these additional studies do in fact supported the broader aspects of the original objective.

2. Characterize whether EGFR signaling and ERK1/2, Jnk or Akt kinase hyper-activation correlate with Stat5a inactivation.

Response: We have assembled a bank of more than 2,000 breast cancer specimens, generated tissue arrays and are continuing these studies.

3. Characterize the role of Stat5a activation in restoring ER α expression in ER α -negative/Her2-positive tumors in vitro and in vivo.

Response: This original hypothesis was not supported by our experimental data. Reversing loss of ER α expression through Stat5 activation was not readily achievable. However, two of our publications (Refs 1 and 7; Tran et al, Oncogene 2010, Sato et al, Oncogene, 2013) demonstrated that prolactin-Stat5a signaling blocks BCL6 expression and prevents emergence of a therapy-resistant and ER α -negative cell population that expresses the basal marker, cytokeratin-5. Thus it remains a distinct possibility that prolactin-Stat5a signaling inhibits loss of ER α expression in ER α -positive tumors. We have acknowledged funding from PA Department of Health in these published projects.

4. Characterize the role of IGF-1 in regulating the PRLR-Stat5 axis, examine Her2, Her3, Her4 expression in human breast cancer patients with inactive Stat5.

Response: This work is still ongoing. We have just completed staining of IGF1R, Her1, Her2, Her3 and Her4 in 2000 breast cancer specimens, and are performing image analyses of immunofluorescence scans of these specimens. A publication is expected in 2015.

5. Characterize the role of Stat5 activation in regulating breast cancer function in 3D culture systems.

Response: We used 3D cultures to show that acidosis blocks prolactin-induced Stat5 activation in the recently published paper (Ref 6; Yang et al, Breast Cancer Research, 2013) that was derived from the work on EGF as originally proposed. We have acknowledged funding from PA Department of Health for this derivative project. We

have focused our efforts on in vivo studies of 3D tissue responses in tumor xenografts and novel models of patient-derived breast cancer.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: N/A

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response: We thank the reviewers for their conscientious effort and for the favorable reviews.

Project Number: 0865203
Project Title: Stat5 and ErbB2 in Prostate Cancer
Investigator: Nevalainen, Marja

B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.

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SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. The main weakness regarding progress is that work did not progress as far as it could have, given the resources and time provided. The researchers did address 3 of their 5 stated milestone goals, and so did examine the questions they set out to answer. However, they were not able to make sufficient progress to publish a paper and to get a grant. It is unclear from the progress report whether they encountered technical difficulties or had personnel issues or what the reasons were.

Recommendation: Require the PI to explicitly address any problems encountered in the pursuit of their work to allow more comprehensive assessment of progress.

Response:

- 1) The studies proposed in the original Aim #2 to determine if Stat5 is the mediator of synergetic interaction between ErbB2 and androgen receptor (AR) encountered technical difficulties. These difficulties were related to the findings emerging during the project indicating that inhibition of Stat5a/b, by genetic knockdown or pharmacologically, induce extensive apoptotic death of prostate cancer cells (1, 2). In other words, since inhibition of Stat5 kills prostate cancers cells, mechanistic studies evaluating Stat5 as a mediator of ErbB2 induction of transcriptional activity of AR are extremely challenging due to low or no surviving prostate cancer cells after Stat5a/b knockdown.
- 2) The post-doctoral fellow working on this project moved to a junior faculty position in another institution. Nonetheless, this project is currently being finished for a manuscript submission.
- 3) The results of the proposed studies served as critical preliminary data for a new RO1 grant submission in 2010. However, the grant did not receive high enough priority score for funding.

2. It appears that this project was closely related to an existing (since 2005) R01 that the PI had. I don't know whether overlap was considered during the initial award of the funds, but I would recommend a close look at how this is addressed. The abstract from 2006 in the NIH database includes the following text, which suggests some overlap at least: "...three specific aims: 1: Determine upstream mechanisms of constitutive Stat5a and/or 5b activation in human prostate cancer. 2: Determine whether active Stat5a and/or 5b inhibits homotypic adhesion of human prostate cancer cells and stimulates heterotypic adhesion, motility and invasion of prostate cancer cells, in vitro and in vivo. 3: Determine the prognostic values of Stat5a and Stat5b in human prostate cancer with disease recurrence as endpoint. "http://projectreporter.nih.gov/project_info_description.cfm?aid=7100589&icde=18066748

Recommendation: Request a statement from the PI about overlap of funding at the time of application.

Response: There was no overlap with this project and the funded RO1 grant. The specific aims of the funded RO1 grant were the following:

Aim #1: Determine upstream mechanisms of constitutive Stat5a/b activation in human prostate cancer. Stat5ab is constitutively activated in primary prostate cancer with strong association with high histological grade of the cancer(3, 4), in hormone-refractory recurrent prostate cancer(5) and in prostate cancer metastasis. ***We propose*** that activation of Stat5a/b in prostate cancer is due to elevated activity of the components of Prl-Jak2-Stat5a/b signaling pathway in prostate cancer. Specifically, ***we hypothesize*** that increased activation of Stat5a/b is due to: a) amplification of Stat5a/b genes in prostate cancer, b) activating mutation of Jak2(6-8) in prostate cancer, and/or c) autocrine Prl-production in prostate cancer. We will test our hypothesis in prostate cancer specimens on tissue microarrays (array n=5, sample n=1724) with clinical follow-up data. Moreover, we have 64 fresh frozen human prostate specimens available for studies b and c.

Specific Aim #2: Establish whether Stat5a/b suppresses homotypic adhesion and stimulates motility and invasion of prostate cancer cells in vitro and in vivo. Our ***preliminary data*** indicate that Stat5ab is highly activated in both lymph node and bone metastases of human prostate cancer, and that Stat5a/b regulates cell clustering and E-Cadherin expression in prostate cancer cells. ***We hypothesize*** that Stat5a/b stimulates prostate cancer cell invasion by decreasing homotypic adhesion through down-regulation of E-Cadherin/ β -catenin complexes, by stimulation of motility through RhoGTPases and stimulation of heterotypic adhesion

Aim #3: Determine whether activation of Stat5a and/or Stat5b in human prostate cancer predicts clinical outcome of the disease.

3a. Determine the independent prognostic values of active Stat5a and/or Stat5b in clinical outcome of prostate cancer with disease recurrence as endpoint.

3b. Determine whether activation of Stat5a and/or Stat5b in prostate cancer cells predicts early disease recurrence in patients with intermediate risk clinical features (Gleason grade 3 and 4).

3. The scope of the work proposed and performed is not very exploratory. In essence the PI proposed to examine specific signaling pathways, rather narrowly, confirming what is known elsewhere. The opportunity for new insights and the creation of new areas of investigation is therefore quite limited. This also negatively impacts leveraging opportunities.

Recommendation: Allow/encourage hypothesis generating research, including -omics investigations, which can lead to many new insights and hypothesis-driven investigations, and provide opportunities for leverage.

Response:

Often hypothesis-generating proposals are criticized by the reviewers for being fishing expeditions and lacking hypothesis-driven studies.

Reviewer 2:

1. It would seem important to develop a pre-clinical model of Erb2 dependent prostate tumorigenesis to obtain proof of principle that anti-Erb2 therapy can show efficacy in vivo in prostate cancer driven by the Erb-Stat5 axis.

Response:

The PI's laboratory does not generate mouse models.

2. Developing a set of validated antibodies to monitor Erb2 and Stat5b expression and activation state would be required to translate this work into the clinic. As it turns out, the approaches for validating these antibodies were not well articulated. I would suggest going to the human protein atlas web site and looking over their stringent criteria for antibody validation and selection in future applications. Overall, the strengths outweigh the weaknesses.

Response:

Antibodies for the detection of ErbB2 are routinely in use in the clinic all over the world for breast cancer diagnostics. Antibodies for Stat5a/b are available and have been well validated as documented (4, 9-13).

Reviewer 3:

None.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: N/A

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response:

1. Gu L, Dagvadorj A, Lutz J, Leiby B, Bonuccelli G, Lisanti MP, et al. Transcription factor Stat3 stimulates metastatic behavior of human prostate cancer cells in vivo, whereas Stat5b has a preferential role in the promotion of prostate cancer cell viability and tumor growth. *Am J Pathol.* 2010;176:1959-72.
2. Gu L, Liao Z, Hoang DT, Dagvadorj A, Gupta S, Blackmon S, et al. Pharmacologic Inhibition of Jak2-Stat5 Signaling By Jak2 Inhibitor AZD1480 Potently Suppresses Growth of Both Primary and Castrate-Resistant Prostate Cancer. *Clin Cancer Res.* 2013;19:5658-74.
3. Li HZ, Zhang, Y., Glass, A., Zellweger, T., Gehan, E., Bubendorf, L., Gelmann, E.P., Nevalainen, M.T. Activation of Transcription Factor Stat5 (Stat5) in Prostate Cancer Predicts Early Recurrence. *Clinical Cancer Res.* 2005, in press.
4. Li H, Ahonen TJ, Alanen K, Xie J, LeBaron MJ, Pretlow TG, et al. Activation of signal transducer and activator of transcription 5 in human prostate cancer is associated with high histological grade. *Cancer Res.* 2004;64:4774-82.
5. Dagvadorj A, Li H, Tan S, King RL, Zhang Y, Culig Z, et al. Transcription factor Stat5 provides a survival signal for prostate cancer cells during androgen deprivation. In preparation. 2005.
6. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet.* 2005;365:1054-61.
7. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature.* 2005;434:1144-8.
8. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell.* 2005;7:387-97.
9. Li H, Zhang Y, Glass A, Zellweger T, Gehan E, Bubendorf L, et al. Activation of signal transducer and activator of transcription-5 in prostate cancer predicts early recurrence. *Clin Cancer Res.* 2005;11:5863-8.
10. Mirtti T, Leiby BE, Abdulghani J, Aaltonen E, Pavela M, Mamtani A, et al. Nuclear Stat5a/b predicts early recurrence and prostate cancer-specific death in patients treated by radical prostatectomy. *Hum Pathol.* 2012;In Press
11. Nevalainen MT, Xie J, Bubendorf L, Wagner KU, Rui H. Basal activation of transcription factor signal transducer and activator of transcription (Stat5) in nonpregnant mouse and human breast epithelium. *Mol Endocrinol.* 2002;16:1108-24.
12. Nevalainen MT, Xie J, Torhorst J, Bubendorf L, Haas P, Kononen J, et al. Signal transducer and activator of transcription-5 activation and breast cancer prognosis. *J Clin Oncol.* 2004;22:2053-60.

13. Peck AR, Witkiewicz AK, Liu C, Stringer GA, Klimowicz AC, Pequignot E, et al. Loss of nuclear localized and tyrosine phosphorylated Stat5 in breast cancer predicts poor clinical outcome and increased risk of antiestrogen therapy failure. *J Clin Oncol.* 2011;29:2448-58.

Project Number: 0865204
Project Title: Targeting the IGF-1 Receptor in Cancer
Investigator: Baserga, Renato

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SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

1. This project's accomplishments are marginally favorable. While the intended area of investigation was apparently well-explored, the specific milestones put forward were incompletely explored. Significantly, no publications were cited in the final report and plans for transfer of the work to another PI and the seeking of further funding are not evident. Therefore, the goals of the investment, to enhance research and support the creation of future projects, were not fulfilled.

Recommendation: Specify whether the work of the retiring PI will be carried on by someone else.

Response: We did find acetylation of IRS-1 with commercial antibodies and we repeated the observation at least 3 times. But when we looked for acetylated residues of IRS-1, we could not find them, despite several attempts. We then went to a well-known University that does this type of analysis on a fee basis, we sent them IRS-1 (stimulated or not), and the answer was they could not find acetylated residues in IRS-1. While the results provided a dead end, that line of inquiry was sound and the results obtained were useful. It is difficult to publish negative results.

2. It was difficult to assess the progress made, as the progress report consisted of very general statements of findings made, and insights gathered. There was little cohesion or connection between different parts of the final report, which was created by combining elements from prior reports. No data were shown, or experiments described.

Recommendation: The PI should more concretely describe the experiments/studies performed and the findings obtained, since this cannot be seen in a published paper or even a submitted manuscript.

Response: I have written many papers on the IGF-I receptor and IRS-1 since 1989, and most of them were valid and have been cited by other investigators. Before retiring, I wrote a last paper in the Journal of Cellular Physiology, entitled “The Decline and Fall of the IGF-1 Receptor”. In it, I pointed out the meager results obtained with antibodies to the receptor (I participated in one of them with Amgen, and my name is on one of the papers on that antibody). The problem with targeting the IGF-I receptor or IRS-1 is tumor consists usually of several subclones, and when you target one of these subclones, the tumor promptly switches to another receptor or another pathway. I have given the references on subclones in many tumors in the JCP paper, and had I not retired, that is where I would have gone to work next. The fact is that most tumors consist of subclones and that this probably is the reason why anticancer therapies are so unsuccessful in the last stages of the disease.

Reviewer 2:

1. No actual data were presented in the report making it difficult to evaluate how the results obtained help in reaching the original objectives. Presentation of tables and/or figures summarizing findings would strengthen the report.

Response: We have done a good job with the IGF-1 receptor. Yes, there was a difference between normal cells and tumor cells in cultures (confirmed by other investigators), even though our first experiments were with antisense strategies (I have NEVER seen an antisense to work in human cancer, although residual workers always try to resurrect it), but then we confirmed it using antibodies and especially the role of IRS-1, whose absence caused cells with the receptor to differentiate instead of stimulating cell proliferation. But again, subclones will avoid targeting of IRS-1, and we go back to the usual problem.

2. No publications were listed. Some were to be submitted and more information on this would be helpful. I did find publications mentioned in one of the earlier annual reports, however. If results are not published then it will clearly not have any impact on future clinical applications of the alleged findings.

Response: There were no publications from this project.

3. In the final report, there is no indication of how results obtained relate to previous work by the investigators and others. No citations are included.

Response: I have written many papers on the IGF-I receptor and IRS-1 since 1989, and most of them were valid and have been cited by other investigators. Before retiring, I wrote a last paper in the Journal of Cellular Physiology, entitled “The Decline and Fall of the IGF-1 Receptor”. In it, I pointed out the meager results obtained with antibodies to the receptor (I participated in one of them with Amgen, and my name is on one of the papers on that antibody). The problem with targeting the IGF-I receptor or IRS-1 is tumor consists usually of several subclones, and when you target one of these subclones, the tumor promptly switches to another receptor or another pathway. I have given the

references on subclones in many tumors in the JCP paper, and had I not retired, that is where I would have gone to work next. The fact is that most tumors consist of subclones and that this probably is the reason why anticancer therapies are so unsuccessful in the last stages of the disease.

Reviewer 3:

1. The determination of specific sites of acetylation should be discontinued. These are difficult analyses and require a mass spectrometry expert spending full time on the project. It might be useful in the future to combine this analysis with determination of additional post-translational modifications, including ubiquitination, phosphorylation (Ser/Thr/Tyr) and O-GlcNacylation. The miR-145 studies represent an area for future investigations in order to determine which targets are being affected besides IGF-1R and IRS-1. The studies on DACH1, which were not part of the proposed studies, may also be an area worthy of future examination.

Response: Agreed.

2. As indicated above, although the overall progress on this project was modest, some key areas of future studies have been developed. These could be developed by one of the co-investigators on the project.

Response: The project will not be continued as I have retired

3. Because there were no additional sources of funding reported or plans for future funding of this project, it is difficult to make a recommendation. Overall, the project objective is of significant interest, such that one of the co-investigators may be interested in pursuing this line of research.

Response: Agreed

4. In the final report, there were no plans for publishing any of the findings from this study in the future. If there are still findings that have not been published, I strongly encourage the PI/co-investigators to submit these data for publication.

Response: The project will not be continued as I have retired

5. Given that this project appears to have been terminated, there are no recommendations. If one of the co-investigators is still at the grantee institution, they would be the likely individual to continue this work in the future.

Response: This project has ended.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: N/A

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response:

E. Generic Recommendations for Thomas Jefferson University

Reviewer 3:

It would appear from the progress reports submitted over the years that progress was slow on this project. It may be useful to intercede at an earlier point to determine whether there are problems with personnel, etc., in order to determine whether funding should be terminated prior to the end of the cycle.

Response: In this particular case, and given Dr. Baserga’s international reputation and substantial contributions in the IGF-1 field, it was reasonable to assume that he would be productive. We agree that future reviews of progress should include a recommendation if a project should be halted or reassigned.

Project Number: 0865205
Project Title: The Role of MicroRNA (miRNA) Gene Expression in
Therapy Resistance of Human Breast Cancer
Investigator: Pestell, Richard

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SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

Publication record is low. Recommendation: Publish.

Response: We thank the reviewer for the comment. We have included additional updated publications since the time of the original report to now include

miR-221/222 promotes S-phase entry and cellular migration in control of basal-like breast cancer. Li Y, Liang C, Ma H, Zhao Q, Lu Y, Xiang Z, Li L, Qin J, Chen Y, Cho WC, **Pestell RG**, Liang L, Yu Z. *Molecules*. 2014 May 30;19(6):7122-37. doi: 10.3390/molecules19067122.

The metastatic potential of triple-negative breast cancer is decreased via caloric restriction-mediated reduction of the miR-17~92 cluster. Jin L, Lim M, Zhao S, Sano Y, Simone BA, Savage JE, Wickstrom E, Camphausen K, **Pestell RG**, Simone NL. *Breast Cancer Res Treat*. 2014 Jul;146(1):41-50. doi: 10.1007/s10549-014-2978-7. Epub 2014 May 27.

MicroRNA-Mediated Cancer Metastasis Regulation via Heterotypic Signals in the Microenvironment. Haizhong M, Liang C, Wang G, Jia S, Zhao Q, Xiang Z, Yuan L, Cho WC, **Pestell RG**, Liang L, Zuoren YU. *Curr Pharm Biotechnol*. 2014 May 16.

miR-17/20 sensitization of breast cancer cells to chemotherapy-induced apoptosis requires Akt1. Yu Z, Xu Z, Disante G, Wright J, Wang M, Li Y, Zhao Q, Ren T, Ju X, Gutman E, Wang G, Addya S, Li T, Xiang Z, Wang C, Yang X, Yang X, **Pestell R**. *Oncotarget*. 2014 Feb 28;5(4):1083-90.

Cyclin D1 induction of Dicer governs microRNA processing and expression in breast cancer.

Yu Z, Wang L, Wang C, Ju X, Wang M, Chen K, Loro E, Li Z, Zhang Y, Wu K, Casimiro MC, Gormley M, Ertel A, Fortina P, Chen Y, Tozeren A, Liu Z, **Pestell RG**. Nat Commun. 2013;4:2812. doi: 10.1038/ncomms3812.

MicroRNAs and cancer stem cells: the sword and the shield. Sun X, Jiao X, Pestell TG, Fan C, Qin S, Mirabelli E, Ren H, **Pestell RG**. Oncogene. 2013 Nov 18. doi: 10.1038/onc.2013.492. [Epub ahead of print]

Reviewer 2:

None.

Reviewer 3:

None.

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: N/A

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response:

Project Number: 0865206
Project Title: Mechanisms for Metastasis Suppression through
Kisspeptin Regulation of the Microenvironment
Investigator: Peiper, Stephen

B. To ensure that feedback provided in the Final Performance Summary Report is utilized to improve ongoing and future research efforts, briefly describe your plans to address each specific weakness and recommendation as noted in Section B of the Final Performance Summary Report and listed below. If no weaknesses are listed below, no response is required.

(As you prepare your response please be aware that the Final Performance Summary Report, this Response Form, and the Final Progress Report will be made publicly available on the CURE Program's Web site.)

SPECIFIC WEAKNESSES AND RECOMMENDATIONS

Reviewer 1:

There was low productivity on the ovarian model and no test of any of the central hypotheses in this model. It was clear after year 1 that the central hypothesis was in jeopardy of being disproven. The PI should have refocused sooner.

Response: Dr. Navenot played a major role in the development of the proposal. Dr. Navenot unexpectedly left Jefferson early in the project. As a result, the work was re-focused. This has resulted in a successful application for a federal grant (R21, multi-PI with Dr. Bing-Hua Jiang). I believe that this fulfills the goal of the program.

Reviewer 2:

1. The hypothesis for this proposal was based on weak preliminary data.

Response: N/A

2. The PI performed a number of experiments which ultimately demonstrated that the hypothesis was incorrect and that the experimental models were unreliable.

Response: N/A

3. The methods that were proposed were unlikely to have succeeded given the complexity of the experimental models.

Response: N/A

4. The experiments that were performed to study another problem were not convincing and perhaps also unjustified.

Response: N/A

Reviewer 3:

1. No long-term funding achieved. The PI should submit more external long-term grants to the NIIH, Department of Defense, etc., based on preliminary findings.

Response: N/A

2. No pre-doctoral or post-doctoral students were trained. The training of students should be a priority for this research and funding mechanism.

Response: N/A

3. There was only a single publication peer reviewed over three years ago in a mid-tier specialized journal. These data presented largely negative findings regarding the role of KISS1 as a metastatic suppressor. The ovarian data should be published to strengthen the argument that KISS1 has a functional role in this process.

Response: N/A

4. Many figures completely lack a legend other than the title and many are missing statistics. These must be included in any future write-ups.

Response: N/A

C. If the research project received an “unfavorable” rating, please indicate the steps that you intend to take to address the criteria that the project failed to meet and to modify research project oversight so that future projects will not receive “unfavorable” ratings.

Response: Dr. Navenot played a major role in the development of the proposal. Dr. Navenot unexpectedly left Jefferson early in the project. As a result, the work was re-focused. This has resulted in a successful application for a federal grant (R21, multi-PI with Dr. Bing-Hua Jiang). I believe that this fulfills the goal of the program.

D. Additional comments in response to the Final Performance Review Report (OPTIONAL):

Response:

E. Generic Recommendations for Thomas Jefferson University

Reviewer 2:

The decision to include this project in funding was questionable even before the experiments were performed. This grant was based on weak evidence and proposed an ambitious set of experiments using complicated techniques. Although the PI showed that the basic premise was flawed, the number and types of experiments did not merit this level of funding.

Response: The project was deemed high risk/high reward. A longtime member of Dr. Peiper's lab, Dr. Navenot, played a major role in the development of the proposal. Dr. Navenot unexpectedly left Jefferson early in the project. As a result, the work was re-focused by Dr. Peiper. This has resulted in a successful application for a federal grant (R21, multi-PI with Dr. Bing-Hua Jiang), which will be awarded shortly. In the future, consideration will be given to less risky projects with a more robust set of preliminary experiments.