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):** 1/1/2012 – 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:** 4100057660

6. **Project Number and Title of Research Project:** 06-Chemosensitization of Cancer Cells by Inhibition of a Transcription Network

7. **Start and End Date of Research Project:** 1/1/2012 – 6/30/2014

8. **Name of Principal Investigator for the Research Project:** Timothy J. Yen, 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:

   $300,824

   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
Yen, Timothy	PI	5% years 1-3	$45,601.67
Stobbe, Corinne	Scientific Assistant	10% year 1	$8,408.62
Beeharry, Neil	Research Associate	100% year 1	$60,545.50
Hittle, James	Technical Specialist	70% year 3	$8,216.72
Bhattacharjee, Vikram	Postdoctoral Associate	65% year 2	$32,462.07

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:
R21 - TDG as a Novel Target to Enhance Gemcitabine Killing of Pancreatic Cancer Cells	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	February 2014	$490,875	Not funded
R21 - Chemosensitization of Pancreatic Cancer Cells by Curcumin and Vitamin D Receptor	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	July 2012	$490,875	$420,074
R21 - Characterization of Drug Survival by Pancreatic Cancer Cells In Vitro and In Vivo	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	November 2013	$490,875	$427,062
R21 - Germline Variants in Mitotic Genes that may Predispose to Familial Colon Cancer	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	July 2014	$490,875	Pending
Loss-of-Function Analysis in Human TSC Mutant Cells: Identification of Genes that Play a Role in Survival and Rapamycin Resistance	☐NIH ☒ Other federal (specify: DOD) ☐ Nonfederal source (specify: _____)	July 2014	$908,624	Pending
Chemosensitization of ovarian cancer cells with clinically relevant kinase inhibitors that exhibit novel checkpoint override activities	☐NIH ☒ Other federal (specify: DOD) ☐ Nonfederal source (specify: _____)	August 2014	$401,625	Pending
Loss-of-Function Analysis in Human Pancreatic	☐NIH ☐ Other federal	August 2014	$50,000	Pending

Cancer Cells: Identification of Genes that Play a Role in Gemcitabine Survival and Resistance	(specify:_____) ☒ Nonfederal source (specify: Hirshberg Foundation)			
CRISPR/Cas9 Loss of Function Screen to Identify Genes that Specify Survival of Ovarian Cancer Cells to Cisplatin	☐NIH ☐ Other federal (specify:_____) ☒ Nonfederal source (specify: Sandy Rollman Ovarian Cancer Foundation)	August 2014	$50,000	Pending

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:

I plan to submit an NIH proposal to characterize the role of VDR in pancreatic cancer progression. This proposal will be based on work supported by CURE. Our studies of the importance of VDR revealed an additional role as a repressor of EMT.

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

We plan to focus on characterizing VDR that we identified in our siRNA screen enhances gemcitabine sensitivity. We now know that loss of VDR in pancreatic cancer may be lethal for some cell lines and this is something we want to verify with a new gene knockout technology called CRISPR/Cas9. Since VDR is not an essential gene in normal development, our finding suggests that in PCa, the tumorogenesis program has recruited VDR to perform functions that are essential for their viability. One possibility that is linked to our gemcitabine sensitization studies is that VDR is required to facilitate the repair of damaged DNA that arise from elevated levels of genotoxic stress. Our longterm goal is therefore to understand how the tumorogenesis program initiated in PCa recruits VDR to perform functions that become essential for viability. VDR may be viewed as the Achilles heel of PCa.

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				2
Female				
Unknown				
Total				**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				2
Unknown				
Total				**2**

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

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.

    Our discovery of Bosutinib as a chemosensitizer has stimulated efforts by medical oncologists at FCCC to conduct a clinical trial on its use to improve outcomes for pancreatic cancer patients. Our discovery has also expanded my interactions with clinician researchers in the area of ovarian and esophageal cancers as they are interested in testing if our drug will sensitize killing of these cancers with standard chemo or radio therapies.

Our discovery of VDR as a target for gemcitabine sensitization has provided me opportunities to collaborate with FCCC investigators whose research is in the area of cholesterol metabolism.

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. Jonathan Brody at Thomas Jefferson University in Philadelphia. We co-authored a paper on a novel mechanism that regulates Wee1 kinase mRNA. The Brody lab characterized the mechanism of mRNA stability, we characterized the cell biological aspects of the study.

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

Yes____X_____ No_____

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

We did file 2 patents on our discovery of VDR as a chemosensitization target, and the novel use of Bosutinib and related drugs in overriding DNA damage checkpoints.

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

Yes____X_____ No_____

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

Members of the lab and I participate in the annual fundraiser for Pancreatic Cancer Network (PanCaN).

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

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period

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

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

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

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

Project Goals and Objectives.

The broad objectives of this research are to identify mechanisms that allow cancer cells to adapt and survive chemo and radiotherapies. This work is of significance because tumor recurrence after drug treatment remains a major obstacle to the long-term survival of cancer patients. Studying the mechanisms that cancer cells use to survive exposure to cytotoxic agents is critical to the development of strategies to enhance chemo-sensitization, and reduce the incidences of tumor recurrence. Our approach was to conduct a synthetic lethal screen using a genome-wide siRNA library. We chose pancreatic cancer for our studies because it is the 4th deadliest of all cancers in the US. Although gemcitabine is the drug of choice for pancreatic cancer patients, this drug only extends survival by a few months relative to other chemotherapies.

Our screen was conducted in the Panc 1 cell line, and candidate genes from our screen were validated in secondary screens using an independent set of si- or shRNA, as well as an additional cell line, BXPC3. One of the genes encoded the Vitamin D Receptor (VDR), a transcription

factor essential for calcium homeostasis and bone mineralization. In previous reports, I did not reveal the identity of VDR and simply referred to it as a transcription factor. The work described in this report has been submitted for publication and to the Patent Office. While VDR is not an essential gene, it regulates different sets of genes in many different tissues beyond bone tissue. We pursued VDR because it has not previously been reported to be involved in drug sensitization. Another two genes encode DNA damage response proteins, Chk1 (Checkpoint kinase 1) and 53BP1 (p53 tumor suppressor binding protein 1) that may be targets of these transcription factors. As the two transcription factors VDR and RXR can form heterodimers, we hypothesize that they regulate a network of survival genes that cancer cells use to overcome the effects of DNA damage induced by drugs such as gemcitabine. We propose the following aims to test our hypothesis:

1) Test drug sensitivities of a panel of cell lines derived from different origins of cancer to knockdown of the RUNX2 and VDR transcriptional network.
2) Test if sensitization is achieved with other genotoxic agents besides gemcitabine.
3) Compare and identify critical downstream target genes to characterize the mechanism of drug sensitization.

Outcomes of Aim 1. We proposed to test if ovarian, lung and colorectal cancer cell lines rely on this transcriptional network for survival to chemotherapeutic agents. Following the identical protocols used for pancreatic cancer cells, we focused on ovarian cancer cells first as they were readily available to us. We were unable to show that this specific transcription factor altered gemcitabine sensitivity amongst ovarian cancer cells OVCAR 3, 5, 10. The transcription factor was present in these cells as determined by western blot (data not shown). We attempted numerous different drug concentrations but no significant sensitization was observed. The amount of time devoted to testing ovarian cancer cells precluded efforts to test lung and colorectal cancer cells. This was in part due to developments in other fronts that became a priority (see below).

Outcomes Aim 2. We tested pancreatic cancer cells for response to cisplatin and doxorubicin, two drugs with different mechanisms of action than gemcitabine. siRNA knockdown of VDR sensitized Panc1 cells to gemcitabine as well as doxorubicin and cisplatin. VDR knockdown in BXPC3 cells only sensitized them to gemcitabine and doxorubicin. The lack of sensitization with cisplatin may be due to the possibility that the concentration we used was insufficient to damage DNA. Nevertheless, we demonstrated that VDR also dictated response of cells to doxorubicin and cisplatin.

Outcomes Aim 3. We have determined that the mechanism by which VDR sensitizes cells to killing by gemcitabine is at the level of DNA repair. Gemcitabine blocks DNA replication and causes replication forks to stall at sites where gemcitabine has been incorporated into DNA. The major mechanism to repair stalled forks is by homologous recombination (HR), whereby the damaged DNA uses its undamaged sister chromatid as a template to copy the genetic information. A key protein in HR is Rad51, an ortholog of the prokaryotic RecA protein that is also essential for stimulating strand invasion and pairing for HR. In PCa cells where we have blocked expression of VDR, Rad51 fail to be recruited to sites of damage. Consequently, the stalled fork eventually collapses and forms double stranded breaks that kill the cells. The

relationship between VDR and Rad51 is not clear, but we do know that it is not at the level of transcriptional regulation. Levels of Rad51 mRNA and protein are unaltered in control and cells depleted of VDR. Interestingly, we can restore Rad51 recruitment to sites of damage with an inhibitor HDAC (histone deacetylase). This suggests that VDR may rely on its interactions with chromatin modifying factors to dictate the recruitment of Rad51 to stalled replication forks. It is well established that Rad51 requires histone acetylation at sites of damage for its recruitment there. However, the importance of VDR in regulating Rad51 through histone acetylation is a novel discovery.

From a translational standpoint, the importance of VDR to HR suggested that it may expand the use of PARP inhibitors (PARPi) for PCa. PARPi selectively kill cancer cells with mutations in BRCA1 and 2, which are essential for HR. We found that if PCa cells are depleted of VDR, their failure to activate Rad51 for HR, increased their sensitivity to PARPi.

The VDR studies described above have been submitted for publication (August 24, 2014).

In addition to our efforts to characterize VDR as a novel sensitizer of gemcitabine, we had previously reported our efforts to identify clinically relevant kinase inhibitors that could be used to enhance gemcitabine activity.

We completed our studies of our screen to re-purpose clinically relevant kinase inhibitors as novel chemosensitizers. We determined that Bosutinib and its isomer (Bos-I) enhanced gemcitabine killing of pancreatic cancer cell lines in vitro and determined the concentration of drug that sensitized cells to killing at a comparable level as known inhibitors of Chk1, such as UCN-01. We submitted this as part of a manuscript, which required additional experiments as suggested by reviewers. These experiments included demonstrating that sensitization by Bos-I was also observed with other genotoxic drugs. Consistent with the observation for gemcitabine, Bos-I enhanced the cytotoxic effects of both cisplatin and doxorubicin. Sensitization was specific for DNA damaging drugs as no sensitization was observed with paclitaxel, which targets microtubules. We also tested the response of other cell lines such as MiaPaCa2 pancreatic adenocarcinoma cells and untransformed RPE cells. MiaPaCa2 cells treated with either gemcitabine or doxorubicin were more sensitive in the presence of Bos-I as compared to vehicle. Interestingly, normal RPE1 cells were not killed by these treatments suggesting that chemosensitization maybe preferentially achieved in tumor cells.

The characterization of the mechanism by which Bos-I enhance gemcitabine killing was completed. We showed by time-lapse, flow cytometry and immunofluorescence staining that Bosutinib isomer abrogated the DNA damage checkpoint and forced cells with incompletely replicated DNA into a catastrophic mitosis. In response to reviewers' comments, we next directly confirmed that Bosutinib and Bos-I dually inhibited Chk1 and Wee1, two kinases critical for the DNA damage checkpoint pathway. Furthermore, we showed by using phosphoH2AX as a pharmacodynamic marker that these drugs targeted these kinases in cells. When cells are treated with gemcitabine, phosphoH2AX staining increases. However, staining intensity further increased by 3-fold if the cells are also depleted of Wee1 (Fig.1). Treatment with Bos-I further increased staining. The Bos-I induced phosphoH2AX staining can be suppressed by overexpressing Wee1-wild type (Fig.1).

To understand the molecular basis for the differences in the specificity of bosutinib and Bos-I for Chk1 and Wee1, molecular models were constructed to visualize the interactions between the inhibitors and the ATP binding pocket. Structurally, the 2 compounds only differ in the positioning of the Cl and methoxy atoms on the aniline ring (Fig. 2), suggesting that the spatial position of these groups dictate their interaction within the ATP binding pocket. It is well established that the gatekeeper residue of kinases can dictate the ability for inhibitors to bind. Our molecular models showed that the methoxy group of authentic bosutinib (position 5) may sterically clash with gatekeeper residue in Wee1 (Asparagine, N376) and possibly reduce its efficacy. By contrast, the methoxy group in Bos-I (position 3) is positioned away from the gatekeeper (Fig. 3). This suggests that the spatial relationship between the gatekeeper residue and the methoxy group in bosutinib and Bos-I may dictate the ability of the inhibitors to bind and thereby inhibit. These observations were supported by relative binding energy calculations derived from our models of bosutinib and Bos-I bound to Chk1 and Wee1. Consistent with cellular and biochemical data presented above, the calculations indicated that Bos-I had a stronger preference for both Wee1 and Chk1 compared with bosutinib.

To explore the importance of the gatekeeper residue in determining the sensitivity to bosutinib or Bos-I binding, we used the aforementioned kinase inhibitor database to identify kinases that are preferentially inhibited (>50%) by Bos-I or bosutinib and then compared the gatekeeper residues. For Bos-I, the gatekeeper residues were predominantly methionine, or valine, while similar analysis of bosutinib sensitive kinases identified predominantly methionine, threonine or phenylalanine as gatekeeper residues. This analysis showed that the gatekeeper residues of bosutinib-sensitive kinases were enriched for threonine or phenylalanine and provided us with an opportunity to test whether these gatekeeper residues might dictate selectivity for bosutinib over Bos-I. We performed in vitro kinase assays using recombinant Wee1 wild-type (WT) as well as gatekeeper mutants N376F and N376T and tested the inhibitory activity of bosutinib and Bos-I using phosphorylation of Cdc2, its physiological substrate, as a read out. We observed that phosphorylation of $Cdc2^{Y15}$ by Wee1 WT was potently inhibited by Bos-I, while approximately 4-fold more bosutinib was needed to achieve similar inhibition (IC_{50} values: Bosutinib 6.9 µM vs. Bos-I 1.8 µM). Importantly, both the N376T and the N376F mutant exhibited increased sensitivity to bosutinib as its IC_{50} was reduced to 1.9 µM and 0.9 µM, respectively. The sensitivity of the N376T mutant to Bos-I was unchanged (Bos-I Wee1 WT 1.8 µM vs. N376T 1.6 µM), while the N376F mutant was now less sensitive to Bos-I (IC_{50}: WT 1.8 µM vs. N376F 8.3 µM). These findings demonstrate that while the N376T mutant improves sensitivity to bosutinib, the sensitivity to the N376F mutant toward the inhibitors is flipped, compared with the Wee1 WT. We next generated molecular models of both inhibitors bound to the mutants. For the N376F mutant, the orientation of the benzyl group of the phenylalanine differed depending on which inhibitor was bound. When bosutinib was bound, the benzyl group was rotated away from the 5-methoxy group leading to reduced steric hindrance. In contrast, when Bos-I was bound, the benzyl group of N376F was orientated such that it was proximal to the 4-methoxy group. This orientation would generate a steric clash between the benzyl group and the 4-methoxy group that could explain the resultant decrease in ability to inhibit Wee1. In performing similar analysis of the 376T mutant (Fig. 3), we were unable to identify structural differences that may account for why Bos-I is slightly more effective compared with the WT Wee1, nor for the dramatic increase in effectiveness of bosutinib as compared with WT Wee1. Using a combination of in vitro kinase

reactions and modeling studies, we provide evidence that both inhibitors can bind Wee1, albeit to slightly different abilities. Furthermore, the gatekeeper residue appears to be an important, but unlikely to be the sole, determinant for sensitivity.

We completed our xenograft studies that used patient derived pancreatic tumor cells. The xenograft data showed that tumors responded to either gemcitabine or Bos-I alone for 17 days. However, tumors resumed growth after 17d and no longer responded to single drug treatment. By contrast, tumors treated with gemcitabine + Bos-I failed to grow up until the third week when the experiment was stopped. In addition, we conducted immunohistochemical studies to demonstrate efficacy of the drug combinations.

To explore whether the mechanism of action for sensitization in vivo was similar to that in vitro, we used the phamacodynamic assay as employed above to measure γH2AX. In animals treated with gemcitabine alone there was an increase in γH2AX in the tumor cells compared with tumor cells from vehicle treated animals ($P < 0.01$). Moreover, γH2AX levels were highest in the tumor cells from animals treated with the combination of gemcitabine and Bos-I ($P < 0.0001$). We also observed that the mitotic index was reduced in tumors from animals treated with gemcitabine as compared with vehicle treated animals (vehicle 2.2 ± 0.7% of mitotic cells vs. gem-treated 0.4 ± 0.4% of mitotic cells; $P = 0.03$). However, in tumors from animals treated with the combination of gemcitabine and Bos-I, the mitotic index was increased compared with animals treated with gemcitabine alone (gem-treated 0.4 ± 0.4% of mitotic cells vs. gem+Bos-I treated 2.0 ± 0.6% of mitotic cells; $P = 0.05$), consistent with the in vitro data. Furthermore, the extent of DNA damage as assessed by γ-H2AX was approximately 2-fold higher in mitotic tumor cells in animals treated with gemcitabine and Bos-I compared with animals treated with vehicle alone. These findings show that Bos-I can enhance inhibition of tumor growth by gemcitabine in vivo.

Finally, we tested Bosutinib (at the same concentration as Bos-I) and found that it suppressed tumor growth as a single agent but tumor growth resumed after 17d. However, the combination of Bosutinib and gemcitabine did not suppress tumor growth as effectively as Bos-I+ gemcitabine, consistent with the weaker chemosensitization activity of Bosutinib.

Our studies of repurposing kinase inhibitors are not published.

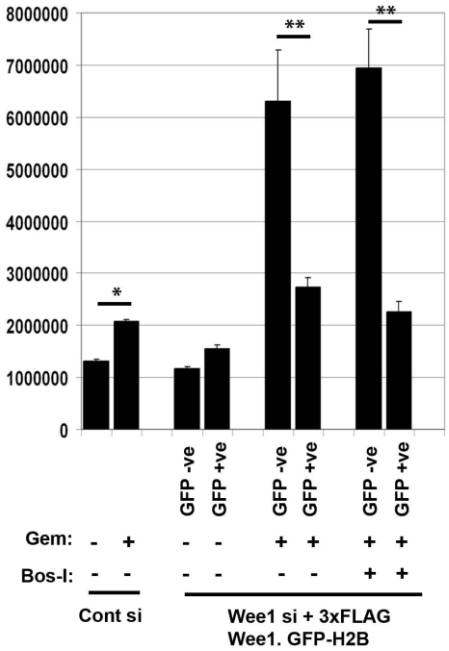

Fig.1. Wee1 is a target of Bos-I in cells. Panc1 cells were transfected with the indicated constructs, treated with Bos-I +/- gemcitabine and fixed and stained for pH2AX foci. The Wee1 expression construct also contained a gfpH2B that allowed identification of positively tranfected cells. Comparison of foci (Y axis) show that cells transfected with Wee1 (and thus gfpH2B positive) suppressed pH2AX foci that are induced by Gem +/- Bos-I.

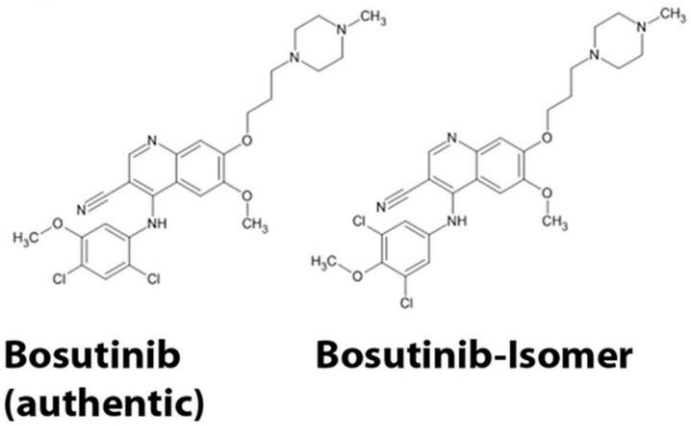

Fig.2. Comparison of the structures of Bosutinib and Bosutinib isomer. The aniline ring is at the bottom left of each structure. Note the differences in the positions of the –OCH3 methoxy and Cl between the structures.

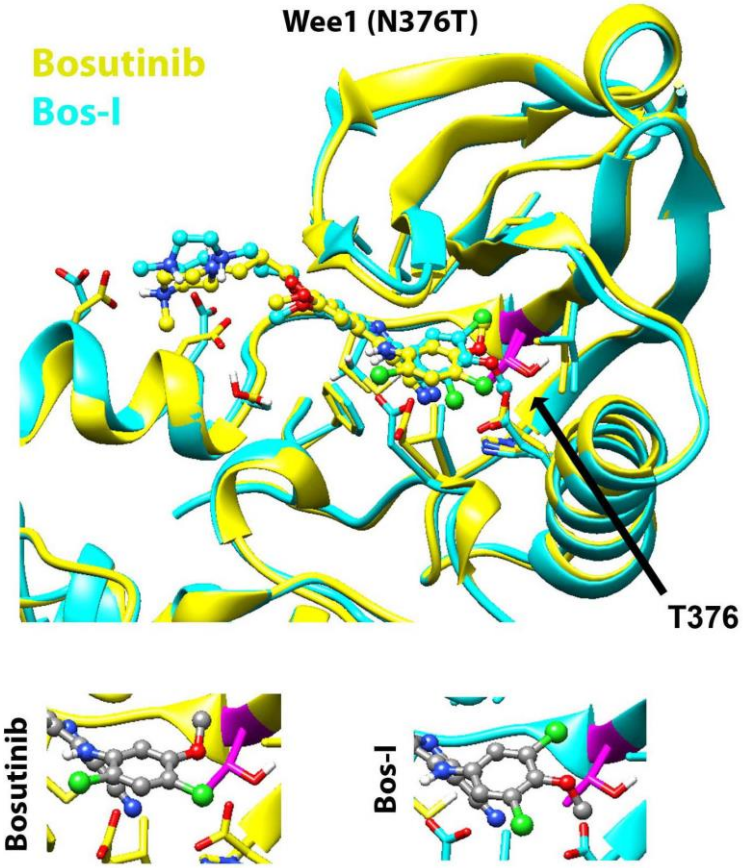

Fig.3. The gatekeeper residue in Wee1 contributes to inhibitor specificity. (**A**) Ribbon diagrams of published structures of Wee1 (PDB code 3BI6) bound with the inhibitors bosutinib or Bos-I which are superimposed (main). Insets show enlarged view of the pocket bound to their inhibitors (gray backbone). The gatekeeper residue T376 is shown in pink (black arrow). Note the steric clash of the Cl (Green) in Bosutinib with the gatekeeper.

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. Centromere fragmentation is a common mitotic defect of S and G2 checkpoint override	Beeharry, N., Rattner, J.B., Caviston, J.P., **Yen, T.**	*Cell Cycle* **12**:1588-1597, 2013. PMC3680538	Feb 2013	☐Submitted ☐Accepted ☒Published
2. HuR post-transcriptionally regulates WEE1: implications for the DNA damage response in pancreatic cancer cells.	Lal, S., Burkhart, R.A., Bhattacharjee, V., Beeharry, N., Londin, E.R., Cozzitorto, J.A., Kimbo, M., Norris, Z.A., Yeo, C.J., Sawicki, J.A., Rigoutsos, I., **Yen, T.J**., Brody, J.R.	*Cancer Res.* **74**:1128-1140. 2014. (doi: 10.1158/0008-5472.CAN-13-1915	July 2013	☐Submitted ☐Accepted ☒Published
3. Re-purposing clinical kinase inhibitors to enhance chemosensitivity by overriding checkpoints.	Beeharry, N., Banina, E., Hittle, J., Skobeleva, N., Khazak, V., Deacon, S., Andrake, M., Egleston, B.L., Peterson, J.R., Astsaturov, I., **Yen, T.J.**	*Cell Cycle* 13:2172-91, 2014. (doi: 10.4161/cc.29214. PMCID: PMC Journal – In Process)	April 2014	☐Submitted ☐Accepted ☒Published
4. Leukemia-associated RhoGEF (LARG) is a novel RhoGEF in cytokinesis and required for the proper completion of abscission	Martz MK, Grabocka E, Beeharry N, **Yen TJ**, Wedegaertner PB.	*Mol Biol Cell.* 2013 Sep;24(18):2785-94.	July 2012	☐Submitted ☐Accepted ☒Published

5. A synthetic lethal screen identifies the Vitamin D receptor as a novel gemcitabine sensitizer in pancreatic cancer cells.	Bhattacgarjee, V, Zhou, Y, **Yen, TJ**	Cell Cycle	August 2014	☒Submitted ☐Accepted ☐Published

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:

I have ongoing work on further characterization of VDR that I plan to submit. However, without funding, I cannot say when I can complete the work.

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.

Two major discoveries were made during the funding period.

1. The discovery of a clinically relevant kinase inhibitor, Bosutinib and Bosutinib-isomer, that can be repurposed to override DNA damage checkpoints and thus enhance gemcitabine sensitivity of pancreatic cancer cells in vitro and in vivo. Checkpoint inhibitors are known to effectively enhance killing by standard chemotherapies. Unfortunately, these highly potent inhibitors are not tolerated by patients and thus the

concept of overriding DNA damage checkpoints to improve treatment outcomes in patients has never been fully tested. We took advantage of the inherent promiscuity of kinase inhibitors and screened clinically relevant drugs for off-target effects against the DNA damage checkpoint. This led to the discovery of Bostunib, an inhibitor of Src/Abl kinases that is FDA approved for CML. We hope to test Bosutinib for off-label use as a chemosensitizer in pancreatic cancer patients.

2. Our discovery of the vitamin D receptor to be a critical determinant for gemcitabine sensitivity in PCa cells opens up new ways to improve treatment outcomes. VDR binds ligand such as vitamin D, and is thus a druggable target. If a compound can be identified to bind and inhibit VDR's functions in PCa cells, it would provide a new treatment given that VDR function appears to be essential for viability of these cancer cells. In addition, that inhibition of VDR blocks homologous recombination also opens up the possibility of using PARPi to treat PCa.

   In summary, these are significant translational discoveries that came about from solid basic research. This validates the importance of basic research in leading the way to advancing medical breakthroughs.

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__X_____ No_____

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:
   1. Patent: US2013/031344. Sensitization of cancer cells to DNA damage by inhibiting kinases essential for DNA damage checkpoint control.
   2. Patent: US2014/0163087A1 Combination inhibition of the Vitamin D Receptor and DNA replication in the treatment of cancer.

b. Name of Inventor(s):
   1. Yen, TJ and Beeharry, N.
   2. Yen, TJ. and Bhattacharjee, V.

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):
   1. The invention provides methods for overriding cell cycle arrest in a tumor cell, which comprise inducing DNA damage in the cell, and contacting the cell with an amount of bosutinib or a bosutinib isomer effective to inhibit one or more kinase constituents of a DNA damage checkpoint pathway. The invention also provides novel bosutinib isomers, as well as compositions of the novel isomers and the bosutinib isomer 3,5-dichloro-4-methoxyaniline.

2. Methods for treating tumors comprising cells expressing the vitamin D receptor are provided, and comprise inhibiting the expression or the biologic activity of the vitamin D receptor in the tumor cells, and/or inhibiting the expression or the biologic activity of a constituent of the vitamin D receptor signaling pathway in the tumor cells, and administering to the tumor cells an effective amount of gemcitabine.

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

   If yes, indicate date patent was filed:
   1. Patent: US2013/031344. Filed Mar. 14. 2013.
   2. Patent: US2014/0163087A1 Filed Dec. 26. 2013.

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?
   1. No
   2. Pending

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant?
   No

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale?
   No

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____X_____ No_____

If yes, please describe your plans:

We would like to develop the Bosutinib-isomer as a new drug for chemosensitization. This requires that we receive support to conduct animal studies to determine pharmacokinetics of this drug. This would then hopefully lead to production of the drug for Phase 1 clinical trials. For Bosutinib, we are submitting an application to Pfizer (maker of this drug) to request drug for a Phase 1 clinical trial to test dose escalation in Pancreatic patients.

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

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Yen, Tim J., Ph.D.	Professor
eRA COMMONS USER NAME Drtjyen	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of California, Santa Barbara,	B.S.	06/78	Biochemistry
University of California, Santa Barbara,	M.A.	06/81	Biochemistry
University of California, Santa Barbara,	Ph.D.	06/85	Biochemistry/ Molecular Biochemistry
Johns Hopkins University School of Baltimore, MD	Postdoctoral Fellow	06/90	Molecular Cell

A. PERSONAL STATEMENT

I am a tenured professor with over 20 years of experience as an independent PI. My research in fundamental mechanisms of cell division is directly relevant to understanding the biology of cancer as well as its treatment. My current interests are to apply basic knowledge about fundamental biological process towards translational research. My focus on Pancreatic cancer stems from my interest in understanding how these cells are able to survive after treatment with drugs that should in principle be incompatible with life. Using institutional pilot funds we conducted a chemical and siRNA synthetic lethal screen to identify how tumor cells survive gemcitabine treatment. This has led to identification of novel chemosensitization agents, as well as a new role of the vitamin D receptor (VDR) in promoting DNA repair in pancreatic cancer cells. I have established working collaborations with pancreatic cancer researchers as well as medical oncologists. My efforts are therefore to pursue these two important discoveries so that they can be used in the clinic.

B. POSITIONS

Associate Member, Fox Chase Cancer Center, Philadelphia, PA	1990-1995
Member with tenure, Fox Chase Cancer Center, Philadelphia, PA	1995-2001
Professor with tenure, Fox Chase Cancer Center, Philadelphia, PA	2001-present

HONORS

Postdoctoral Fellowship Award, American Cancer Society	1985-1988
Young Investigator's Award for research performed as a postdoctoral fellow at The Johns Hopkins University School of Medicine, Baltimore, MD	1988
Lucille P. Markey Scholar	1989-1995
Adjunct Professor in the Department of Genetics, University of Pennsylvania, Philadelphia, PA	1991
Leukemia Society of America Scholar	1995-2000
NIH Study Section, CDF2	2000-2004

C. SELECTED PEER-REVIEWED PUBLICATIONS

1. Liu, S.-T., Rattner, J.B., Jablonski, S.A., Yen, T.J. Mapping the assembly pathways that specify formation of the trilaminar kinetochore plates in human cells. J. Cell Biol. 175:41-

53, 2006.
2. Huang, H., Feng, J., Famulski, J., Rattner, J.B., Liu, S.T., Kao, G.D., Muschel, R., Chan, G.K., Yen, T.J. Tripin/hSgo2 recruits MCAK to the inner centromere to correct defective kinetochore attachments. J. Cell Biol. 177:413-424, 2007.
3. Eytan, E., Braunstein, I., Ganoth, D., Teichner, A., Hittle, J.C., Yen, T.J., Hershko, A. Two different mitotic checkpoint inhibitors of the anaphase-promoting complex/cyclosome antagonize the action of the activator Cdc20. Proc. Natl. Acad. Sci. USA 105:9181-9185, 2008.
4. Zhang, X.D., Goeres, J., Zhang, H., Yen, T.J., Porter, A.C., Matunis, M.J. SUMO-2/3 modification and binding regulate the association of CENP-E with kinetochores and progression through mitosis. Mol. Cell 29:729-741, 2008.
5. Huang, H., Hittle, J., Zappacosta, F., Annan, R.S., Hershko, A., Yen, T.J. Phosphorylation sites in BubR1 that regulate kinetochore attachment, tension, and mitotic exit. J. Cell Biol. 183:667-680, 2008.
6. Wan, X., O'Quinn, R.P., Pierce, H.L., Joglekar, A.P., Gall, W.E., DeLuca, J.G., Carroll, C.W., Liu, S.T., Yen, T.J., McEwen, B.F., Stukenberg, P.T., Desai, A., Salmon, E.D. Protein architecture of the human kinetochore microtubule attachment site. Cell. 137:672-84. 2009.
7. Tipton, A.R., Wang, K., Link, L., Bellizzi, J.J., Huang, H., Yen, T.J., Liu, S-T. BUBR1 and closed MAD2 (C-MAD2) interact directly to assemble a functional mitotic checkpoint complex (MCC). J. Biol. Chem. 286:21173-21179, 2011.
8. Ratushny, V., Pathak, H.B., Beeharry, N., Tikhmyanova, N., Xiao, F., Litwin, S., Connolly, D., Yen, T.J., Weiner, L.M., Godwin, A.K., Golemis, E.A. Dual inhibition of SRC and Aurora kinases induces postmitotic attachment defects and cell death. Oncogene 31:1217-1227, 2012.
9. Beeharry, N., Rattner, J.B., Bellacosa, A., Smith, M.R., Yen, T.J. Dose dependent effects on cell cycle checkpoints and DNA repair by bendamustine. PLoS One 7(6)e40342, 2012.
10. Martz, M.K., Grabocka, E., Beeharry, N., Yen, T.J., Wedegaertner, P.B. Leukemia-associated RhoGEF (LARG) is a novel RhoGEF in cytokinesis and required for the proper completion of abscission. Mol. Biol. Cell 24:2785-2794, 2013.
11. Beeharry, N., Rattner, J.B., Caviston, J.P., Yen, T. Centromere fragmentation is a common mitotic defect of S and G2 checkpoint override. Cell Cycle 12:1588-1597, 2013.
12. Lal, S., Burkhart, R.A., Bhattacharjee, V., Beeharry, N., Londin, E.R., Cozzitorto, J.A., Kimbo, M., Norris, Z.A., Yeo, C.J., Sawicki, J.A., Rigoutsos, I., Yen, T.J., Brody, J.R. HuR post-transcriptionally regulates WEE1: implications for the DNA damage response in pancreatic cancer cells. Cancer Res. In Press (January; doi: 10.1158/0008-5472.CAN-13-1915.
13. Beeharry, N., Banina, E., Hittle, J., Skobeleva, N., Khazak, V., Deacon, S., Andrake, M., Egleston, B.L., Peterson, J.R., Astsaturov, I., Yen, T.J. Re-purposing clinical kinase inhibitors to enhance chemosensitivity by overriding checkpoints. Cell Cycle 13: (Epub ahead of print), 2014.
14. Wang, K., Sturt-Gillespie, B., Hittle, J.C., Macdonald, D., Chan, G.K., Yen, T.J., Liu, S.T. Thyroid hormone receptor interacting protein 13 (TRIP13) AAA-ATPase is a novel mitotic checkpoint silencing protein. J Biol Chem. 2014 [Epub ahead of print]
15. Eytan, E., Wang, K., Miniowitz-Shemtov, S., Sitry-Shevah, D., Kaisari, S., Yen, T.J., Liu, S-T., Hershko, A. Disassembly of mitotic checkpoint complexes by the joint action of the AAA-ATPase TRIP13 and p31comet. Proc. Natl. Acad. Sci. 2014 [published ahead of print]