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.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 14. Topical Application of Isoselenocyanates for the Prevention of Melanoma

7. **Start and End Date of Research Project:** 7/1/2010 - 6/30/2011

8. **Name of Principal Investigator for the Research Project:** Arati Sharma, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 37,907

   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
Chinying Chung	Grad Student	100%	18,900

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
Sharma, Arati	Assistant Professor	2%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No_____X___

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

We are planning to apply for a R21 in 2014. See below

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

ISC-4 will be combined with other small molecular inhibitors targeting various kinases involved in melanoma progression or development to test if this agent is synergizing to effectively treat or prevent melanoma progression/ development. Additionally, ISC-4 will be tested in various resistant melanoma cell lines to see if resistance can be overcome using this agent.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				
Unknown				
Total			**1**	

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

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian			1	
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_____ No_____X____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

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_____ No_____X_____

If yes, please describe the collaborations:

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.

Melanoma incidence and mortality rates continue to increase despite the use of sunscreen as well as screening programs for early surgical excision of premalignant lesions. The steady increase in melanoma incidence suggests that additional preventive approaches are needed to augment these existing strategies. One unexplored area involves targeting genes whose deregulation promotes disease development to prevent melanoma. The Akt3 signaling pathway is one key signaling cascade that plays a central role by deregulating apoptosis to promote development of approximately 70% of melanomas. Isoselenocyanate-4 (ISC-4), derived from isothiocyanates by increasing the alkyl chain length and replacing sulfur with selenium, has been developed to target this important signaling pathway in melanomas; however, its chemopreventive potential is unknown. In this study, the chemopreventive efficacy of topical ISC-4 was evaluated in a laboratory-generated human skin melanoma model containing early melanocytic lesion or advanced stage melanoma cell lines and in animals containing invasive xenografted human melanoma. Repeated topical application of ISC-4 reduced tumor cell expansion in the skin model by 80% to 90% and decreased tumor development in animals by approximately 80%. Histologic examination of ISC-4-treated skin showed no obvious damage to skin cells or skin morphology, and treated animals did not exhibit markers indicative of major organ-related toxicity. Mechanistically, ISC-4 prevented melanoma by decreasing Akt3 signaling that lead to a 3-fold increase in apoptosis rates. Thus, topical ISC-4 can delay or slow down melanocytic lesion or melanoma development in preclinical models and could impact melanoma incidence rate if similar results are observed in humans.

(1) Specific Aims

The main objective of this project was to determine the efficacy of novel isothiocyanate derivative (ISC-4) as a topical agent that prevents melanocytic lesion development by inhibiting Akt3 signaling. ISC-4 is a novel organoselenium compound developed at Penn State with significant biological activity against malignant melanoma *in vitro* and in animal models. Akt3 is a key protein kinase activated in ~70% of melanomas. No effective chemopreventive agents exist that effectively inhibits the Akt3 signaling cascade in melanomas. Thus, the *working chemoprevention hypothesis* was that synthetic isothiocyanate derivative (PBITC), and isoselenocyanate (ISC-4), which is corresponding isosteric selenium analog, would inhibit the Akt3 signaling cascade in early pre-malignant melanocytic lesions, thereby acting as effective topical chemopreventive agents. We would test our working hypothesis by using the *experimental approach* of evaluating compounds' chemopreventive effectiveness for preventing/retarding tumors in skin and in animal melanoma models. This discovery would identify and validate the functional utility of unique topical chemopreventive agents targeting one of the few signaling cascades identified as important in early melanomas**.**

(2) *Significance and Novelty of the Research:* Currently, no effective chemopreventive agents exist to prevent /retard development of early into more advanced melanomas in the skin. Thus, the research, models and technology are emerging novel concepts that constitute *significant innovation* by developing unique synthetic chemopreventive agents that target Akt3 signaling to prevent early melanoma development within skin. This innovative research was expected to yield several outcomes. First, demonstrate that inhibition of Akt3

signaling in reconstructed human skin retards development of early melanocytic skin lesions. Second, validate that topical application of a selenium analog of isothiocyanate targets Akt3 signaling and prevents melanoma development in a transgenic melanoma mouse model. Thus, a novel topical chemopreventive agent inhibiting early melanomas would be identified contributing a needed tool to the relatively small arsenal of chemopreventive agents currently available for preventing this disease. These discoveries have potential to significantly impact human health by decreasing the number of persons having melanoma, thereby directly decreasing mortality rates.

(3) *Research Design, Methods and Activities*.: The *objective of this study* was to evaluate the potential of novel synthetic isothiocyanate derivatives that target Akt3 signaling and thereby preventing early melanocytic lesion development. To achieve this objective, we would test the *working hypothesis* that varying carbon chain length in ITC derivatives and by replacing sulfur with selenium in ITC leads to potent inhibition of Akt3 signaling, which effectively prevents melanoma development. We would test our working hypothesis using the *experimental approaches* of evaluating compounds potency for preventing early melanoma lesion development using *in vitro lab-generated* skin and in animal models of melanoma. A successful outcome would validate these novel chemopreventive topical agents as an effective targeted approach to prevent melanoma.

Experiment 1: Chemoprevention of early melanoma using an *in vitro* lab-generated human skin-nevi model.

Human skin was created in a culture dish containing melanocytic cells resembling early pre-malignant lesion, which is an established technique in Dr. Sharma's laboratory. This involves embedding fibroblast cells (FF2441) in 10% reconstitution buffer, (10% DMEM (Mediatech), 2.4 ul/mL of 10 M NaOH, and 80% collagen I (Becton Dickinson), at a cell density of 3.75 x 105 cells /mL on ice followed by incubating 1.5 ml aliquots in 12-well culture plates at 37^{o}C tissue culture incubator for 3h to form dermis matrix. To each well containing dermis matrix, a 1 ml aliquot of E-medium were added and allowed to grow for 2 more days. Later, a mixture of GFP expressing early stage melanoma cells having normal human foreskin keratinocytes (1:10) re-suspended in E-medium (1ml) activated Akt were added on top of dermal matrix to produce a keratinized layer with developing melanoma tumor nodules. After 2 days of incubation, the skin reconstructs were transferred onto wire grids, and allowed to develop for 7 to 8 days in a tissue culture incubator to form a complete keratinized layer. During this period the developing skin reconstructs were fed via diffusion from E-medium (replaced every other day) below the wire grids. When at the air-liquid interface feeding of the epithelial cells is done by diffusion of the media component through the dermal equivalent, this diffusion creates a gradient mimicking the *in vivo* situation where nutrients diffuse through the dermis to epithelium. It is very important not to accidentally get media on top of the raft; this destroys the gradient and disturbs cell orientation and proper differentiation.

Experiment 2 – *Drug treatment and quantitation of melanocytic lesions in the skin reconstruct.*

Skin reconstructs containing radial or vertical growth phase melanoma tumor nodules (similar number and size) were grouped in to vehicle control (200 uL), PBITC and ISC-4 (5, 7.5, and 12.5uM) treatment groups (n=5 skin reconstructs in each group). Skin reconstructs were treated with each agent every day up to 8 days. The WM35 and WM3211 cell lines were derived from melanocytic lesions in the radial growth phase (a very early stage in melanoma melanocytic lesions) while WM115 and WM278 cell lines were derived from vertical growth phase lesions (next stage after the radial growth phase). These cells represent the state-of-the-art in terms of early stage melanocytic lesions and are used commonly as a part of early stage lesion cells in a melanoma tumor progression model (39). Histology and morphological characterization of artificial skin reconstructs: These cell lines have also been genetically engineered to express GFP making detection and quantitation of chemopreventive effects within the reconstructed skin using fluorescence microscopy. Skin reconstructs from all experiments were fixed in 4% paraformaldehyde at 4°C overnight. After fixation skin was stored in 0.5 M EDTA pH 8.0. Total average area occupied by GFP-tagged tumor nodules present in 4-6 skin images were used to quantify differences between treatment groups using IP Lab software. This approach calculates the area occupied by tumor cells through a 2- dimensional analysis strategy. Skin sections were also examined immuno-histochemically using melanoma markers HMB-45 and S100 for the presence of melanoma tumors.

Results

1. *ISC-4 kills melanocytic lesion and melanoma cells more effectively than normal skin cells.*

ISC-4 has been derived from naturally occurring isothiocyanates by increasing the alkyl carbon chain length to contain 4 carbons and replacing sulfur with selenium. ISC-4 can kill aggressive invasive advanced stage melanoma cells following systemic administration, but its effect on early melanocytic lesion and normal cells present in the skin is unknown. Human skin is composed of multiple cell types including fibroblasts, keratinocytes and melanocytes, with the latter developing into non-invasive melanocytic lesions, which can progress into invasive melanoma. Therefore, effective topical chemopreventive agents would need to kill early non-invasive melanocytic lesion or invasive melanoma cells with negligible effect on normal skin cells. To determine the appropriate concentration range and IC_{50} of ISC-4 for topical use applications, cell viability using the MTS assay was examined after exposure of melanocytic lesion, melanoma, human epidermal melanocytes or normal skin fibroblast cells to ISC-4. An ISC-4 concentration of 21.7 µM was required to kill 50% of normal human fibroblast compared to 3.9 µM or 5.0 µM for early stage WM35 or Sbcl2 cells lines derived from an early stage melanocytic lesion in the radial growth phase or 8.8 µM for invasive UACC 903 melanoma cells derived from an invasive cutaneous melanoma. Thus, ISC-4 is 2-7-fold more effective at killing melanocytic lesion or melanoma compared to normal cells, indicating potential utility for topically applications at concentrations <19 µM. PBITC served as a control to demonstrate the efficacy and importance of selenium in the

structure of ISC-4.

2. *ISC-4 decreases Akt3 activity and triggers apoptosis in melanocytic lesion cells derived from the radial growth phase and advanced stage melanoma cells.*
To measure ISC-4 inhibition of Akt3 activity in early stage and advanced stage melanomas, WM35 or UACC 903 cells were exposed to 2.5 to 15 μM of ISC-4 or control PBITC and cell lysates analyzed by Western blotting. ISC-4 decreased pAkt3 levels at lower concentrations than control PBITC with negligible effect on total Akt protein levels. Similarly, a dose dependent decrease in pAkt3 levels was also observed in UACC 903 cells. Furthermore, ISC-4 decreased levels of downstream pPRAS40 more effectively that control PBITC, which had little effect on this downstream signaling target. As a consequence of decreased Akt3 activity, cleaved PARP and caspase-3 indicating increased apoptosis rose more dramatically in ISC-4 compared to PBITC treated cells. Thus, ISC-4 functions to decrease Akt3 activity resulting in increased apoptosis in radial growth phase melanocytic lesion and advanced stage melanoma cells.

3. *SiRNA-mediated inhibition of Akt3 retards melanoma tumor development by increasing apoptosis in melanoma cells.*
To demonstrate the effect of Akt3 knockdown on melanoma tumor development and cellular apoptosis levels, siRNA was used to inhibit protein expression. Western blot analysis confirmed knockdown of Akt3 protein levels and showed corresponding increases in cleaved caspase-3 levels indicating a rise in levels of apoptosis. Decreasing Akt3 protein levels reduce tumor development by ~3-fold ($p<0.01$) by increasing cellular apoptosis by 6-7 fold ($p<0.01$; t-test). Thus, decreasing Akt3 activity reduces tumor formation by increasing levels of cellular apoptosis.

4. *Mechanism leading to death of cultured melanocytic lesion cells following ISC-4 treatment is by triggering apoptosis.*
Mechanism decreasing cultured melanocyte, melanocytic lesion WM35 or advanced melanoma UACC 903 cell survival was established by measuring Caspase3/7 activity following 24 h treatment with ISC-4, PBITC or vehicle control using MTS and Caspase 3/7 assay kits. Both ISC-4 and PBITC decreased viability by increasing apoptosis; however, ISC-4 had efficacy at lower concentrations. Thus, ISC-4 is effective at killing cultured melanocytic radial and vertical growth phase lesion cells with a lesser effect on normal human melanocytes.

5. *Topical ISC-4 treatment inhibits melanocytic and melanoma lesion development in laboratory-generated skin.*
Human skin can be generated in the laboratory containing melanocytic lesions resembling benign for WM35 or aggressive for UACC 903 tumors seen in patient skin and effects of anticancer agents evaluated on lesion development in this model. Both WM35 and UACC 903 cell lines express green fluorescent proteins (GFP) making melanocytic nodule development detectable and quantifiable using fluorescence microscopy.

A decrease in lesion development was observed in laboratory generated skin following

ISC-4 treatment compared to controls. H&E stained and fluorescent images of cross sections of skin at day 11 show dramatically fewer melanocytic lesion or melanoma cells in the skin compared to controls. In contrast to ISC-4 treated skin having little melanocytic lesion development, control skin contained nests of cells for the WM35-GFP cells line and many invading disseminating cells for the UACC 903-GFP cell line. At the end of ISC-4 treatment, most melanocytic lesion cells for both cell lines were barely detectable. ISC-4 also caused no detectable damage to the keratinocytes, fibroblasts or skin morphology present in this model, again suggesting that a topical formulation would cause negligible effect on normal skin cells.

To assess the effect of ISC-4 treatment on melanocytic lesion development over time, serial measurement were made on the same skin (n = 3 skins with 10 pictures per skin totaling 30 pictures) according to the treatment schedule. Regression in area occupied by WM35-GFP and UACC 903-GFP lesions was observed when treated with ISC-4 compared to controls. A similar trend was observed for PBITC but ISC-4 was more effective leading to an 80-90% reduction in average area occupied compared to a 50-60% decrease for PBITC at the end of the treatment. Thus, ISC-4 is effective at inhibiting melanocytic lesion development in the laboratory generated skin model containing melanocytic lesions at concentrations ranging from 7.5-25 μM, supporting topical use of ISC-4 for preventing melanocytic lesion development.

6. *Topical application of ISC-4 prevents melanocytic lesion development in the skin of nude mice with negligible changes in animal body weight.* To demonstrate that topical ISC-4 inhibits melanocytic lesion development in animals, UACC 903 melanoma cells that are tumorigenic and have high Akt3 signaling activity were injected subcutaneously and 24 h later, animals treated topically everyday with ISC-4 or acetone vehicle. WM35 cells could not be evaluated in mice since these cells do not grow or form detectable lesions in animals. Topical ISC-4 treatment led to a 77% decrease in UACC 903 tumor size compared to vehicle control ($p<0.001$). Body weights of mice treated with ISC-4 compared to control showed no significant differences between groups, suggesting negligible toxicity. Thus, use of topical ISC-4 inhibited cutaneous melanocytic lesion development without weight loss that would indicate systemic toxicity.

7. *Mechanistically, ISC-4 inhibits melanocytic lesion development in animals by inhibiting Akt3 activity to trigger tumor cell apoptosis.* To determine the mechanism causing tumor inhibition, size and time matched tumors from mice treated with ISC-4 or vehicle were compared. Western blot analysis of matched tumors lysates harvested at day 13 from animals treated with ISC-4 showed decreased active pAkt ($p<0.05$; t-test) and downstream target pPRAS40 ($p<0.001$; t-test) compared to vehicle control treated tumors.

To show that decreased Akt3 activity led to an increase in tumor cell apoptosis, rates of apoptosis (TUNEL staining) and proliferation (Ki-67 immunohistochemistry) were compared in size and time matched melanoma tumors excised from ISC-4 treated animals and compared to vehicle control. Tumors harvested at day 11 and 13 from mice treated with ISC-4, showed ~3-fold ($p< 0.01$; t-test) more TUNEL positive cells compared to

control animals treated with vehicle control. In contrast, no statistically significant difference was seen in rates of proliferation between different treatment groups. Thus, treatment of animals with ISC-4 decreased Akt3 signaling led to increased rates of tumor cells apoptosis.

8. *ISC-4 caused negligible major organ related toxicity***.** To determine whether ISC-4 would cause systemic toxicity, blood parameters (serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, blood urea, glucose, and creatinine) indicative of organ toxicity were measured following systemic administration. None of these indicators were significantly different from controls. Furthermore, histological examination of H&E-stained vital organ sections revealed that ISC-4 treatment did not significantly alter cell morphology or structure of liver, kidney, adrenal, lung, spleen, heart, pancreatic, or intestinal tissue. Thus, ISC-4 caused negligible systemic toxicity and would be effective for preventing melanocytic lesion development.

In conclusion, this study demonstrates the utility of topical ISC-4 for preventing 80-90% of cutaneous melanocytic lesion development in preclinical models by targeting the Akt3 signaling cascade. Thus, topical ISC-4 has potential to delay or slow melanocytic lesion or melanoma development in preclinical models and could impact melanoma incidence if similar results are observed in humans.

This study was presented at AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, Hynes Convention Center Boston, MA, USA, October 19-23, 2013

Topical ISC-4 for the prevention of melanoma,
Arati Sharma, Natalie Nguyen, Nhung Nguyen, Arun K. Sharma, Dhimant Desai, Sung Jin Huh, Shantu Amin and Gavin P. Robertson.
New strategies are urgently needed to prevent development of melanoma. Currently, surgical excision is the mainstay for eliminating early melanocytic lesions or preventing development of more aggressive cancer. However, use of sunscreen or body lotions containing topical agents with chemopreventive efficacy could be potentially useful for preventing cancer development. Therefore, novel topical chemopreventive agents with negligible systemic toxicity to prevent melanocytic lesion development would be a highly significant. In order to meet this unmet medical need, potent anti-melanoma agents called isoselenocyanates (ISC) have been developed from naturally occurring isothiocyanates (ITC), which are derived from plants. In these agents, sulfur has been replaced with selenium and the alkyl side chain has been lengthened from 1 or 2 to 4 or 6. The chemopreventive efficacy of ISC-4 for preventing melanoma development was evaluated in a human skin melanoma model containing early or advanced stage melanocytic lesion cell lines. Cumulative topical application of ISC-4 resulted in a >75% reduction in melanoma nodule developed in 3-D human skin reconstructs. Histological examination of drug treated skin reconstructs confirmed the chemopreventive efficacy without any obvious damage to skin cells. Furthermore, topical application of ISC-4 also effectively prevented melanoma tumor development in animals by ~70% at 3. 83 mM with negligible toxicity compared to vehicle control. Mechanistically, ISC-4 decreased

melanoma tumorigenesis causing an ~3-fold increase in apoptosis by reducing pAkt3 and pPRAS40 levels and triggered caspase-3/7 activity. Furthermore, ISC-4 treatment reduced G0-G1 phase cells (30-40% decrease), and increased G2-M population by 50-60%. A ~15-fold increase in the sub-G0-G1 cell population was also observed upon ISC-4 treatment compared to vehicle control treated cells. Thus, ISC-4 is a potent selenium-containing topical chemopreventive agent with significant potential to prevent melanocytic lesion development.

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. Melanoma Chemoprevention in Skin Reconstructs and Mouse Xenografts Using Isoselenocyanate-4	Arati Sharma, Natalie Nguyen, Nhung Nguyen, Arun K. Sharma, Dhimant Desai, Sung Jin Huh, Shantu Amin, Craig Meyers, and Gavin P. Robertson	Cancer Prevention Research	May 2010	☐Submitted ☐Accepted X Published
2. Toxicological considerations when creating nanoparticle based drugs and drug delivery systems	Arati Sharma, SubbaRao V. Madhunapantula and Gavin P. Robertson	Expert Opinion in Drug discovery	September 2011	☐Submitted ☐Accepted X 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:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes__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: "Development of Novel ISC Drugs for Inhibition of Akt Pathway Signaling in Cancer"

b. Name of Inventor(s): Gavin Robertson, Arati Sharma, Arun Sharma, Dhimant Desai, Shantu Amin

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention): .

We have developed a novel family of Akt signaling pathway inhibitors from naturally occurring compounds that demonstrate significant in vivo chemopreventive/therapeutic activity against a wide variety of cancers. We have developed both lipid and water-soluble forms of the agents and have (in vivo)

evidence of tumor inhibition at dosages significantly lower than comparable compounds. Our preliminary in vivo toxicology studies have found the toxicity of these compounds to be negligible.

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___X

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___X
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___X

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___X

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

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
Sharma, Arati	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) AKSHARMA1	

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
R.G. Shah Science College, Gujarat University, Ahmedabad	B.Sc.	04/89	Microbiology
School of Sciences, Gujarat University, Ahmedabad	M.Sc.	04/92	Life Sciences
School of Sciences, Gujarat University, Ahmedabad	M. Phil.	06/93	Life Sciences
School of Sciences, Gujarat University, Ahmedabad	Ph.D.	12/00	Life Sciences
Penn State Univ. College of Medicine, Hershey, PA	Post-Doc	01/02-07/07	Pharmacology

A. Personal Statement

The major focus of Dr. Sharma's research is identification and validation of novel targets leading to the development of melanoma, discovery & development of new therapeutic agents for melanoma and clinical evaluation of experimental melanoma therapeutics.

B. Positions and Honors

Positions

1996-2000 Teaching Assistant, School of Sciences, Gujarat University, Ahmedabad, INDIA
1997-2000 Project Assistant, Human Genetics and Reproduction Clinic, School of Sciences, Gujarat University, Ahmedabad, INDIA
2002-2007 Postdoctoral Scholar, Department of Pharmacology, Penn State University College of Medicine, Hershey, PA
2007-2008 Research Associate, Department of Pharmacology, Penn State University College of Medicine, Hershey, PA
2008-pres. Assistant Professor, Department of Pharmacology, Penn State University College of Medicine, Hershey, PA

Honors

2004-2005 Outstanding Post Doctoral Research Scholar, The Penn State University, College of Medicine, Hershey.
2001 Travel award (International travel grant) by Council of Scientific and Industrial Research, New Delhi, India to attend International Conference, USA.
1997-1999 Junior Research Fellowship, UGC, Department of Science and Technology, INDIA.
1999-2001 Senior Research Fellow, University Grants Commission, Government of India, New Delhi Sponsored Department of Special Assistance (DSA) Program, Human Genetics and Reproduction Unit, Department of Zoology, Gujarat University, Ahmedabad, India.
1999 First prize for the best oral presentation (Bio Science section), Young scientist program in XIth Gujarat Science Congress.

1999 Second prize for the best oral paper presentation in the young scientist section by Society of Reproductive Biology and Comparative Endocrinology, India,
1998,2001, Hari Ohm Ashram award for the best paper published in Zoology and Medical Science sections,
2004,1994, Sardar Patel University, Vidhyanagar.
1998-1999 Research papers were selected for an oral presentation for the young scientist of India award.

C. Selected Peer-reviewed Publications (Selected from **37** peer-reviewed publications)

1. Stahl JM, Sharma A*****, Cheung M, Zimmerman M, Cheng JQ, Bosenberg MW, Kester M, Sandirasegarane L, Robertson GP. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer Res 2004; 64(19):7002-10. **(* Shahl and Sharma contribute equally)**
2. Sharma A, Trivedi NR, Zimmerman MA, Tuveson DA, Smith CD, Robertson GP. Mutant V599EB-Raf regulates growth and vascular development of malignant melanoma tumors. Cancer Res 2005; 65(6):2412-21.
3. Sharma A, Tran MA, Liang S, Sharma AK, Amin S, Smith CD, Dong C, Robertson GP. Targeting mitogen-activated protein kinase/extracellular signal-regulated kinase kinase in the mutant (V600E) B-Raf signaling cascade effectively inhibits melanoma lung metastases. Cancer Res 2006; 66(16):8200-9.
4. Robertson GP, Sharma A. Models of Melanoma Metastasis: Using a Transient siRNA-based Protein Inhibition Strategy in Mice to Validate the Functional Relevance of Pharmacological Agents. Current Protocols in Pharmacology 2007:14.6.1-14.6.15.
5. Cheung M, Sharma A, Madhunapantula SV, Robertson GP. Akt3 and mutant V600E B-Raf cooperate to promote early melanoma development. Cancer Res 2008; 68(9):3429-39. PMCID: PMC2603082
6. Sharma AK, Sharma A, Desai D, Madhunapantula SV, Huh SJ, Robertson GP, Amin S. Synthesis and anticancer activity comparison of phenylalkyl isoselenocyanates with corresponding naturally occurring and synthetic isothiocyanates. J Med Chem 2008; 51(24):7820-6.
7. Sharma A, Sharma AK, Madhunapantula SV, Desai D, Huh SJ, Mosca P, Amin S, Robertson GP. Targeting Akt3 signaling in malignant melanoma using isoselenocyanates. Clin Cancer Res 2009; 15(5):1674-85.
8. Nguyen N, Sharma A, Sharma AK, Desai D, Huh SJ, Amin S, Meyers C, Robertson GP. Melanoma chemoprevention in skin reconstructs and mouse xenografts using isoselenocyanate-4. Cancer Prev Res (Phila). 2011;4(2):248-58. PMCID: PMC3210697. (*Nguyen **and Sharma contribute equally)**
9. Sharma A, Madhunapantula SV, Robertson GP. Toxicological considerations when creating nanoparticle-based drugs and drug delivery systems. Expert Opin Drug Metab Toxicol. 2012;8(1):47-69.
10. Singh S, Sharma A, Robertson GP. Realizing the clinical potential of cancer nanotechnology by minimizing toxicologic and targeted delivery concerns. Cancer Res. 2012;72(22):5663-8.
11. Madhunapantula SV, Sharma A, Gowda R, Robertson GP. Identification of glycogen synthase kinase 3alpha as a therapeutic target in melanoma. Pigment Cell Melanoma Res. 2013;26(6):886-99.
12. Sharma A, Madhunapantula SV, Gowda R, Berg A, Neves RI, Robertson GP. Identification of aurora kinase B and Wee1-like protein kinase as downstream targets of (V600E)B-RAF in melanoma. Am J Pathol. 2013 Apr;182(4):1151-62. Epub 2013 Feb 12.

D. Research Support

Ongoing Research Support **"**None"

Completed Research Support (all years)

(Sharma, Arati) P.I. 7/1/2008-6/30/2009
Penn State Hershey Cancer Institute
"Leelamine: A Natural Chemopreventive Agent for Melanoma"

5 R03 CA142060-02 (Sharma, Arati) 7/7/2009-6/30/2012
NIH/NCI
"Chemopreventive Efficacy of Plumbagin in Melanoma"

Dean's Feasibility Grant (Sharma, Arati) P.I. 1/1/2010-6/30/2011
PA Tobacco Settlement Fund-Dean's Feasibility Grant
"Topical application of isoselenocyanates for the prevention of melanoma"

Barsumian Trust (Sharma, Arati) P.I. 1/1/2010-12/31/2011
"Plant-derived Drug for Treatment of Melanoma"

SAP# 4100050904 (Co-Investigator) 4/21/2010-12/31/2012
PA Tobacco Settlement
"Multifunctional Nanoparticles for Melanoma and Brain Cancer"