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 717-231-2825.

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

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 - 12/31/2014

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:** 4100054865

6. **Project Number and Title of Research Project:** 4. *Sphingolipid-based Therapeutics for AML*

7. **Start and End Date of Research Project:** 5/1/2011 – 6/30/2012

8. **Name of Principal Investigator for the Research Project:** Mark Kester, 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:

   $ 250,000

   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
Barth, Brian	Post-doctoral Fellow	20% year 1	6,692.00
Bowerman, Courtney	Research Technician	23% year 1	5,787.93
Keasey, Nicole	Post-doctoral Fellow	18% year 2	7,918.00
Kale, Vijaykumar	GraduateStudent/Asst.	50% year 2	11,625.00
Wang, Xujun	Research Technician	67% year 2	29,674.00
Shanmuga, Velandy	Research Technician	23% year 2	7,553.00

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Kester, Mark	Principal Investigator	1%
Loughran, Thomas	Co-Principal Investigator	1%
Claxton, David	Co-Investigator	1%
Adair, James	Co-Investigator	1%
Amin, Shantu	Co-Investigator	1%
Desai, Dhimant	Co-Investigator	1%
Sharma, Arun	Co-Investigator	1%
Wang, Hong-Gang	Co-Investigator	1%

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:
Targeting Dysfunctional Sphingolipid Metabolism in AML Program Project Submission NIH NCI	X NIH ☐ Other federal (specify:____) ☐ Nonfederal source (specify:_)	1/13	$17,000,000	$10,000,000

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

Yes_____ No_____X_____

If yes, please describe your plans:

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

Based upon the team preliminary data generated through this Health Research Grant, we were selected as the only new NIH NCI Program Project funded in 2013. Leveraging these Health Research Funds, we were able to secure a **40 to 1** return on investment. This 5 year prestigious program project grant has allowed us to continue investigating 4 new therapeutics that target dysfunctional sphingolipid metabolism in acute myeloid leukemia. In fact, one of these nanotechnology-enhanced chemotherapeutics, the ceramide nanoliposome, which is being commercialized by Keystone Nano, Inc., is scheduled to enter clinical (human) trials in

early 2015.

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

Yes__X_____ No_____

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

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	1
Female				1
Unknown				
Total			**1**	**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	2
Unknown				
Total			**1**	**2**

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

Dr. H.G. Wang has assumed the position of Director of Pediatric Cancer Research at Penn State College of Medicine and is leveraging additional support to build his program.

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:

Yes, as a result of PA CURE funding, we have secured a NIH NCI P01 grant that includes collaborations with Memorial Sloan Kettering Cancer Center, Mt. Sinai Medical Center, the John Wayne Cancer Center and Eastern Carolina Medical Center.

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:

Keystone Nano, Inc, (State College PA) had previously licensed ceramide nanoliposome technology from Penn State Research Foundation. Keystone Nano is commercializing the technology through a first-in-man clinical trial of the ceramide nanoliposome scheduled for early 2015. Keystone Nano has completed the preclinical PK and toxicology studies in two species required by the FDA. Dr. Kester is cofounder of Keystone Nano and is their Chief Medical Officer.

Another technology, the sphingosine kinase inhibitor, developed by Dr. Jong Yun, is being commercialized through Apogee Technologies (Hershey, PA).

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.

The long term goal is to develop new therapies for the treatment of acute myeloid leukemia (AML). These funds will support three specific aims that provide additional/ new/ critical data that are essential for submission of an integrated interdisciplinary NIH P01 application in 2011 that will investigate the role of dysfunctional sphingolipid metabolism in acute myeloid leukemia. The funds will support three team projects that will benefit each of the eventual P01 projects.

We will be gathering preliminary data to strengthen the projects and cores that will be included in a P01 application. We will conduct three team based projects: Project 1 will carry out gram scale synthesis of and purification of compounds; Project 2 will complete proteomic analysis of lipid metabolic proteins, and lipidomic analysis of cellular samples and plasma samples; Project

3 will establish and examine NOD/SCID/IL2rγ^{null} animal model using primary human acute myeloid leukemia (AML) samples.

Specific Aim 1. Team Project 1 will carry out gram scale synthesis of sphingosine kinase inhibitor SKI-178, analogs of FTY-720, and C6-ceramide derivatives

Specific Aim 2. Team Project 2 will perform proteomic analysis of lipid metabolic proteins and lipidomic analysis of cellular samples and plasma samples

Specific Aim 3. Team Project 3 will establish and examine the NOD/SCID/IL2rγ^{null} animal model using primary human AML samples.

We have successfully completed all of the Specific Aims of our PA CURE grant. Based upon the team preliminary data generated through this Health Research Grant, we were selected as the only new NIH NCI Program Project funded in 2013. Leveraging these Health Research Funds, we were able to secure a 40 to 1 return on investment. This 5 year prestigious program project grant has allowed us to continue investigating 4 new therapeutics that target dysfunctional sphingolipid metabolism in acute myeloid leukemia. In fact, one of these nanotechnology-enhanced chemotherapeutics, the ceramide nanoliposome, which is being commercialized by Keystone Nano, Inc., is scheduled to enter clinical (human) trials in early 2015

Specific Progress for Specific Aim 1. We have manufactured gram scale synthesis of sphingosine kinase inhibitor SKI-178, analogs of FTY-720, and C6-ceramide derivatives. The team has also scaled up synthesis of the C6-ceramide nanoliposome. As discussed below in Aim 3, we have evaluated these proprietary sphingosine-kinase inhibitors and ceramide nanoliposomes in our humanized models of AML.

Specific Progress for Specific Aim 2. We have designed, validated and analyzed lipid metabolic proteins and lipidomic analysis of cellular samples and plasma samples from patient and cellular AML models. We have made progress in utilizing combinatorial MS-lipidomic and MRM-based proteomic approaches to evaluate AML cells after treatment with agents that alter ceramide metabolism. These data are crucial in defining the role of altered ceramide metabolism in AML. These data allowed confirmatory testing of "omics" in ~40 cooperative group samples of defined AML patient subtypes.

Specific Progress for Specific Aim 3. We have established and examined the NOD/SCID/IL2rγ^{null} animal model using primary human AML samples. We have generated NSG mice that have the unique ability to propagate human AML cells with defined cytogenetic properties. We have shown that these mice are a useful and unique tool to evaluate therapeutic efficacy of agents for treatment of AML. We have also engineered a second model that utilizes a retroviral transduction strategy as a means to induce progenitor AML populations. Moreover, using these two models, we have now validated the therapeutic approaches that are hallmarks of each of the 4 interrelated projects of the newly funded NIH NCI P01 grant.

Specifically, PA CURE funds have allowed us to establish in vivo mouse model systems of AML. AML is a genetically diverse and complex disease that no single animal model, to date, is

able to truly recapitulate. Recognizing that limitations exist for each of the currently available/established *in vivo* mouse models of AML, we have engineered and validated both **1)** the immuno-compromised NSG mouse model that allows engraftment of human AML cells, and **2)** the immuno-competent murine AML model induced by the MLL-AF9 oncogene that allows growth of leukemia stem cells (LSC). We have adapted these mouse models based on the known mechanisms of AML pathogenesis and the fact that these models, while not exact, closely mimic the complex biological processes of developing AML in human patients.

Model 1. Immuno-compromised mouse model (NOD.Cg-*Prkdc*^{scid}*Il2rg*$^{tm1/Wjl/SzJ}$), commonly known as NOD *scid* gamma (NSG), for engraftment of patient's AML cells: The NSG mouse model is a robust xenotransplantation model that allows engraftment of AML patient's primary leukemic stem cells (LSCs). We believe the use of patient's primary AML cells is obviously preferable as it directly reflects the clinical setting; whereas human AML cell lines may have acquired additional mutations/adaptations or may have undergone clonal selection in cell culture. These NSG mice lack mature T cells, B cells or functional NK cells and are deficient in cytokine signaling, leading to better engraftment of human LSCs (i.e., $CD45^+CD33^+$ or $CD34^+CD38^-$) compared to other AML mouse models (i.e., NOD/SCID/$\gamma 2m^{null}$ mouse). Evidence indicates that LSCs from human AML patients engraft into NSG mice, display self-renewal and differentiation capacity with successful serial transplantation into secondary and tertiary recipient mice. Importantly, this mouse model has a >90 weeks life expectancy, which will allow us to determine the long term effects of sphingolipid-targeted treatment on reconstitution and differentiation of AML patient's LS cells.

As a direct result of PA CURE funds, we performed "proof-of concept" studies to determine the effects of SKI-178 treatment on human primary AML progression. SKI-178, is one of the therapeutic treatment options being evaluated in the P01 and is a specific inhibitor of sphingosine kinase1. This therapeutic elevates endogenous levels of the pro-apoptotic lipid ceramide, while reducing the mass levels of sphingosine-1-phosphate a pro-mitogenic lipid-based signal. Briefly, we detected the first evidence of AML engraftment in NSG mice 8 weeks after tail vein injection of $5x10^6$ human primary AML cells into irradiated recipient NSG mice by analyzing mouse peripheral blood (PB) for human anti-CD13, hCD33, hCD45 and HLA-DR. In this study, we defined the level of AML engraftment as the percentage (%) of human AML cells (i.e., $hCD13^+$, $hCD33^+$, $hCD45^+$ and $HLA\text{-}DR^+$) in total mouse PB mononuclear cells. NSG mice with human AML engraftment (3-5%) were then treated every 3rd day with either vehicle (DMSO) or SKI-178 (60 mg/Kg) via intraperitoneal (i.p.) injection for 3 weeks. Subsequently, peripheral blood (PB), bone marrow from the femur and tibia and spleen were collected and analyzed for human AML engraftment using FACS flow cytometry. As shown in Figure 1, NSG mice treated with SKI-178 showed significantly lower levels of AML engraftment (without loss of body weights) in PB, bone marrow and spleen compared to the control DMSO treated mice. When we further examined the immunophenotypes of engrafted AML cells, SKI-178 treatment showed the most significant inhibition on $hCD33^+$ and $hCD45^+$ AML cells. Together, these preliminary results demonstrate the potential clinical utility of SKI-178 and reinforce our hypothesis that targeting SphK1 is an effective therapeutic approach in AML.

Model 2. Immuno-competent MLL-AF9 oncogene transduced AML mouse model (CD45 mice): The pathways by which MLL-AF9 oncogene causes AML in both humans and mice have been

well established. Evidence indicates that translocation of MLL gene is a common feature of many oncogenic fusion proteins that initiate myeloid leukemia. MLL normally functions in chromatin modification by catalyzing methylation, acetylation and nucleosome remodeling. Although more than 79 different fusion partners have been observed in MLL transduced leukemia, six recurring fusion partners make up the bulk (>85%) of MLL translocations and several of these MLL fusion proteins including MLL-AF9 have the ability to up-regulate the expression of stem cell self-renewal genes that induce committed progenitors (CMP's and GMPs) to form leukemia stem cells (LSCs). In fact, studies showed an efficient induction of leukemia in mouse when MLL-AF9 gene is knock-in or MLL-AF9 is retrovirally infected into bone marrow and transplanted into mice. The difference between the transgenic and retroviral transduction models lies within the expression level of MLL-AF9.

In order to model this leukemia in mice, team members developed retroviral vectors that express specific fusion proteins. Bone marrow cells transduced with these viruses, recapitulate MLL leukemia when transplanted into irradiated mice. In our studies, we used the MLL-AF9 fusion protein expressed from the MSCV viral vector. We infected bone marrow cells isolated from 5-FU treated mice (B6.SJL-*Ptprc*^{a}*Pep3*^{b}*/BoyJ* hereafter referred to as CD45.1mice), which are highly enriched for hematopoietic stem cells and multipotent progenitors, and which makes it more likely that we generate LSCs after infection. This infection protocol more closely recapitulates the human translocation. The MLL-AF9 virus also expresses GFP, which allows us to purify infected cells (GFP+) prior to transplant. GFP+ cells were transplanted into irradiated recipient C57BL/6 (CD45.2+) mice (1-5x10^5 GFP+ transduced cells). The use of CD45.1+ mice as the transduced population allowed us to use CD45.1 as a marker for leukemic cells in the periphery blood (PB), bone marrow and spleen.

As a result of PA CURE funding, we have established this MLL-AF9 murine AML model and performed preliminary "proof-of concept" studies to demonstrate the feasibility of SKI-178 treatment as a novel anti-AML agent. Briefly, all MLL-AF9 transduced mice developed signs of peripheral leukemia-significant increase in WBC counts, eight (8) weeks after initial bone marrow transplant. These MLL-AF9 mice (n=5) were then treated intravenously (IV) with either vehicle control or SKI-178 (20mg/Kg) three times per week. After two weeks of treatment with SKI-178, we observed that the WBC counts returned to normal values, which were maintained throughout the treatment period (Figure 2). Importantly, 100% of the mice treated with SKI-178 survived for an additional 5 weeks without any obvious signs of toxicity (i.e. loss of weight) (Figure 3). In contrast, all vehicle control treated mice died by 12 weeks after transplant. Together, these results further support our hypothesis that targeting SphK1 impact the progression of leukemia and SKI-178 treatment may lead to a remission of AML.

The above rationale and justification for the two new animal models for Defined AML are based upon proof of concept with just one of the therapeutic options being designed and tested in the NIH NCI P01 grant. Briefly, projects within the P01 are evaluating ceramide nanoliposomes, acid ceramidase inhibitors and glucosylceramide synthase inhibitors. In addition the sphingosine kinase inhibitor (SKI178) for AML is being tested. Specifically, our models allow us to test therapeutic efficacy in stem cell populations of AML. Moreover, these models allow us to test therapeutics in defined patient subsets and to correlate efficacy with clinical outcome. Finally, our animal models allow us to test combinatorial chemotherapeutic strategies that directly attest

to the synergistic potential of targeting sphingolipid metabolism as a treatment option in AML. The designs of these experiments are similar to the proof of concept studies with SKI178. We have already determined IC_{50} and dosing regimens for all our therapeutics and are proceeding forward with these *in vivo* studies.

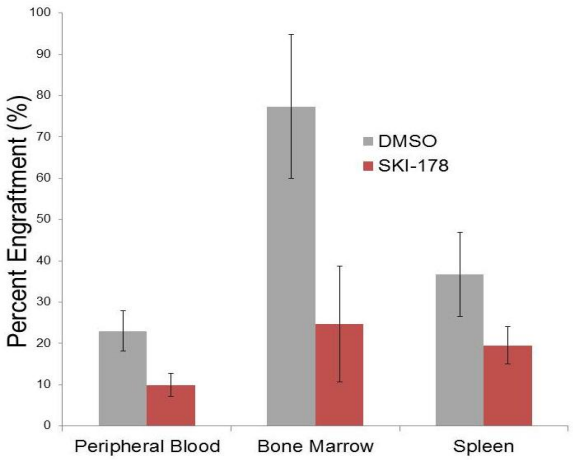

Figure 1. SKI-178 treatment inhibits human AML engraftment. NSG mice with 3-5% AML engraftment were IP injected with either vehicle DMSO or SKI-178 (60 mg/Kg) for 3 weeks every 3rd day. Subsequently, PB, bone marrow and spleen were harvested and FACS analysis were performed to determine the level of AML engraftment. AML engraftment was determined as the percentage (%) of human AML cells (i.e., $hCD13^+$, $hCD33^+$, $hCD45^+$ and $HLA\text{-}DR^+$) in total mouse PB mononuclear cells.

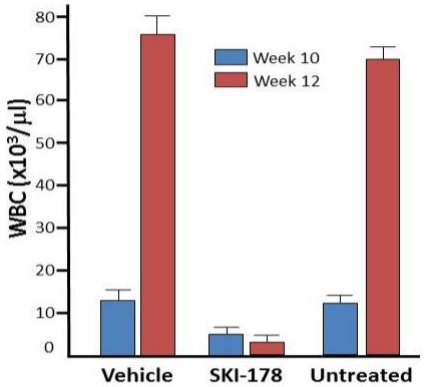

Figure 2. SKI-178 inhibits leukemic progression. Leukemic mice induced by MLL-AF9 (8 weeks post-transplant) were treated with either vehicle control, untreated or SKI-178 (20mg/Kg), three times per week. At week 10 and 12, blood samples were collected and counted for total WBC.

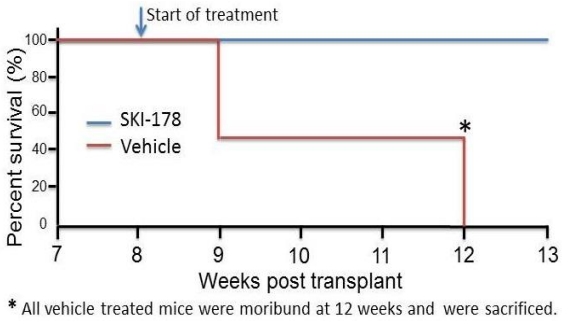

Figure 3. SKI-178 treatment promotes survival of leukemic mice. Eight weeks after transplant of bone marrow cells infected with MLL-AF9, groups of 5 mice were treated with either vehicle control or SKI-178 (20mg/Kg), three times per week.

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. None				☐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:

Yes, the sphingosine kinase inhibitor studies and the ceramide nanoliposome studies in humanized mouse models are now being written for publication.

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.

The grant has enabled us to secure additional funding to further validate therapeutics that target dysfunctional sphingolipid metabolism in AML. One of these therapeutics is scheduled to enter the clinic in 2015. Adding another potential chemotherapeutic approach to the treatment of AML is of compelling clinical significance, given that conventional chemotherapeutic approaches for this devastating disease have not appreciably changed since the 1950s.

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.

The ceramide nanoliposome has shown effectiveness in multiple *in vivo* cancer models including human xenografts of AML in murine models. The entire preclinical package to support a FDA IND application has been completed by Keystone Nano (State College PA) . Published and preliminary data support the utility of combination ceramide nanoliposomes that co-deliver vinblastine or tamoxifen in AML models. In addition, the team has developed other approaches to target dysfunctional sphingolipid metabolism including sphingosine kinase inhibitors and acid ceramidase inhibitors. Though not as advanced as the ceramide nanoliposomes, they are being evaluated and further developed in the new NCI P01 grant.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

All patents were filed before we received PA Cure funding.

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

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

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No_____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

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
Kester, Mark	Professor of Pharmacology
eRA COMMONS USER NAME (credential, e.g., agency login) Mxk38@psu.edu	

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
State University of New York at Stony Brook, NY	B.S.	05/1976	Biology, Economics
State University of New York at Buffalo, NY	Ph.D.	05/1982	Cell Biology

A. Personal Statement

I recently joined the University of Virginia as Professor of Pharmacology. I have secondary appointments as Professor of Biomedical Engineering and as Professor of Molecular Physiology and Biological Physics and am Co-Director of nanoSTAR Institute here at UVA. I was formerly the G. Thomas Passananti Professor of Pharmacology at Penn State Hershey College of Medicine. Before accepting this prestigious endowed professorship, I was a Distinguished Professor of Pharmacology as well as Interim Chair of Pharmacology. My research interests include the design, characterization and validation of nanotechnologies for targeted drug delivery. My laboratory has evaluated nanoliposomes, nanodendrimers and nanocolloids as effective drug delivery vehicles for pharmacological and molecular agents. Recent work focuses on nontoxic nanoscale systemic delivery systems for hydrophobic pro-apoptotic lipids as well as siRNAs that target mutated tumorigenic proteins. I have consulted with, or founded, several companies that have the license to these nano"Solutions". In addition, I have co-authored *Integrated Pharmacology*, published by Elsevier, Ltd., which was recognized as a "highly commended textbook" by the British Medical Society.

B. Positions and Honors

1982-1984 Research Associate, The University of Illinois at Chicago, Chicago, IL; Michael Barany, M.D., Ph.D., Professor of Biological Chemistry
1984-1986 Research Instructor, The University of Texas Health Science Center at San Antonio, San Antonio, TX; Donald Hanahan, Ph.D., Professor of Biochemistry
1986-1995 Assistant Professor, Medicine/Physiology, Case Western Reserve University,
1995-1997 Associate Professor, Medicine/Physiology, Case Western Reserve University,
1997-2001 Associate Professor, Dept. Pharmacology, Penn State Univ.
2001-2006 Professor, Department of Pharmacology, Penn State Univ. College of Medicine,
2002-2003 Interim Chair, Dept. Pharmacology, Penn State University College of Medicine,
2005-2013 Professor, Department of Cellular and Molecular Physiology, Penn State
2006-2007 Distinguished Professor of Pharmacology, Penn State University, Hershey, PA
2007-2013 G.Thomas Passananti Professor of Pharmacology, Penn State University
2010-2013 Director, Penn State Center for Nanomedicine and Materials
2011-2013 Co-Leader, Experimental Therapeutics, Penn State Hershey Cancer Institute
2012-2013 Joint appointment, Professor, Material Science and Engineering, The Pennsylvania State University
2014-pres. Professor, Department of Pharmacology, Director NanoSTAR Institute, University of Virginia, Charlottesville, VA

Honors (Recent)

2010	Nelson W. Taylor Lecture in Materials, Penn State
2011	Plenary lecture, International Sphingolipid Meeting, Villars, Switzerland
2011	NIDDK Board of Scientific Counselors
2011	Outstanding Achievement in Innovation Award, Penn State University, Inaugural Award Winner
2011	National Institutes of Health Study Section, Special Emphasis Panel (ZRG1 BDCN-W (03)
2011	NIDDK Editorial Review Board, Type 2 Diabetes Impact Award (ZDK1 GRB J 01)
2013-pres	NIH/NCI P01 Special Emphasis Panels
2014	Chair-elect, Gordon Research Conference, Glyco-and Sphingolipids

C. Selected peer-reviewed publications (in chronological order).

1. Tagaram HR, Divittore NA, Barth BM, Kaiser JM, Avella D, Kimchi ET, Jiang Y, Isom HC, Kester M, Staveley-O'Carroll KF. Nanoliposomal ceramide prevents in vivo growth of hepatocellular carcinoma. Gut 2011; 60(5):695-701. Highlighted in Nature Reviews 8:4, 2011. PMID: 21193455
2. Watters RJ, Kester M, Tran MA, Loughran TP, Jr., Liu X. Development and use of ceramide nanoliposomes in cancer. Methods Enzymol. 2012;508:89-108. PMID: 22449922
3. Young MM, Takahashi Y, Khan O, Park S, Hori T, Yun J, Sharma AK, Amin S, Hu CD, Zhang J, Kester M, Wang HG. Autophagosomal membrane serves as platform for intracellular death-inducing signaling complex (iDISC)-mediated caspase-8 activation and apoptosis. J Biol Chem. 2012;287(15):12455-68. PMCID: PMC3320995.
4. Adiseshaiah PP, Clogston JD, McLeland CB, Rodriguez J, Potter TM, Neun BW, Skoczen SL, Shanmugavelandy SS, Kester M, Stern ST, McNeil SE. Synergistic combination therapy with nanoliposomal C6-ceramide and vinblastine is associated with autophagy dysfunction in hepatocarcinoma and colorectal cancer models. Cancer Lett. 2013;337(2):254-65. PMCID: PMC3722309.
5. Barth BM, Shanmugavelandy SS, Kaiser JM, McGovern C, Altinoglu EI, Haakenson JK, Hengst JA, Gilius EL, Knupp SA, Fox TE, Smith JP, Ritty TM, Adair JH, Kester M. PhotoImmunoNanoTherapy reveals an anticancer role for sphingosine kinase 2 and dihydrosphingosine-1-phosphate. ACS Nano. 2013;7(3):2132-44. PMCID: PMC3757127.
6. Hankins JL, Ward KE, Linton SS, Barth BM, Stahelin RV, Fox TE, Kester M. Ceramide 1-phosphate mediates endothelial cell invasion via the annexin a2-p11 heterotetrameric protein complex. J Biol Chem. 2013;288(27):19726-38. PMCID: PMC3707677.
7. Watters RJ, Fox TE, Tan SF, Shanmugavelandy S, Choby JE, Broeg K, Liao J, Kester M, Cabot MC, Loughran TP, Liu X. Targeting glucosylceramide synthase synergizes with C6-ceramide nanoliposomes to induce apoptosis in natural killer cell leukemia. Leuk Lymphoma. 2013;54(6):1288-96.
8. Young MM, Kester M, Wang HG. Sphingolipids: regulators of crosstalk between apoptosis and autophagy. J Lipid Res. 2013;54(1):5-19. PMCID: PMC3520539.
9. Ryland LK, Doshi UA, Shanmugavelandy SS, Fox TE, Aliaga C, Broeg K, Baab KT, Young M, Khan O, Haakenson JK, Jarbadan NR, Liao J, Wang HG, Feith DJ, Loughran TP, Jr., Liu X, Kester M. C6-ceramide nanoliposomes target the warburg effect in chronic lymphocytic leukemia. PLoS One. 2013;8(12):e84648. PMCID: PMC3868606.
10. Brinton LT, Sloane HS, Kester M, Kelly KA. Formation and role of exosomes in cancer. Cell Mol Life Sci. 2014 Oct 22. [Epub ahead of print] PMID: 25336151