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 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:** 12. Genetic Analysis of Papillomavirus Virion Morphogenesis

7. **Start and End Date of Research Project:** 1/1/2010 - 11/30/2010

8. **Name of Principal Investigator for the Research Project:** Craig Meyers, PhD

9. **Research Project Expenses**.

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

   $ $33,294

   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	Position Title	% of Effort on Project	Cost
Conway	Graduate Assistant	50%	$22,466.03

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	Position Title	% of Effort on Project
Meyers	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_____

If yes, please describe your plans:

Plan to submit a R01 application to the NIH based partially on results from this tobacco settlement funded research.

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

Plan to continue the studies exploring new avenues of interest, will do this by seeking additional funding from the NIH.

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			1	
Black				
Asian				
Other				
Unknown				
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ 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____X_____ No_____ _____

If yes, please describe the collaborations:

As of the date of submission of this report, the Grad Assistant is now an Assistant Professor at Carnegie Mellon University.

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 application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. 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.

Summary of Research Completed

The long-range goal of this project was to define genetic and molecular events of human papillomavirus (HPV) morphogenesis.

Specific Aim #1

The first aim proposed to biochemically assess the stability of the structure of native viruses (NVs) derived from temporally distinct organotypic tissues. Numerous viral preparations were harvested and purified using Optiprep density gradients and then attempts were made to assess gross morphological changes using transmission electron microscopy (TEM). In order to produce native human papillomavirus (HPV) virions continuously HPV infected human keratinocyte cell lines needed to be grown in organotypic raft epithelial tissue culture. Three raft tissues were pooled and virus was released from the tissue by dounce homogenization followed by centrifugation. This process typically provided viral stocks containing 10^8 or more viral particles per ml. Three raft tissues produced approximately 0.7 ml of viral stock. We had previously demonstrated that virus isolated from raft tissues grown for 10-days was less stable than virus isolated from raft tissues grown for 20-days. One goal was to use TEM to visually observe differences in 10-day and 20-day virus that may be correlated to the differences in stability. Unfortunately, several attempts were made, but we were unable to visualize the virus. After discussions with experts in imaging and physical analyses of virus we came to the conclusion the chemicals that are part of the Optiprep gradient may be interfering with the procedure. In the past we had used ultra-thin tissue sections or cesium chloride gradient purified virus and were able to observe particle structure by TEM. Additionally, imaging experts newly recruited to the Penn State College of Medicine have discussed with us additional techniques that we could try. Therefore we have future goals to pursue these techniques.

Along with visualizing differences in 10-day and 20-day virus by TEM, attempts were also made to analyze differences in stability via partial chemical reduction and tryptic digests followed by Western blot analyses of L1 protein expression. In a nonreducing Western blot L1 dimers and trimers were formed only in wild-type 20-day virus preparations and not in 10-day virus. Analyses of differences in stability via partial chemical reduction and tryptic digests were attempted but we have no clear results at this time. Instead of pursuing this further, time and efforts were put into pursuing goals of Aim #2, which resulted in two manuscripts.

We then decided to try to 'visualize' differences in 10-day and 20-day virus by using minor capsid protein L2 specific antibodies. Many labs are pursuing using the L2 protein as a potential wide-spectrum vaccine agent to prevent HPV infection. Presently, there are two HPV vaccines, both of which are directed against the major capsid protein L1 and do not cross-protect against HPV types not contained in the vaccine. In the process of testing for a L2-based vaccine, portions of the L2 protein have been used to raise antibody. We have taken advantage of these antibody preparations to visualize differences in 10-day and 20-day virus. Our studies have led to a manuscript "Cross-neutralization potential of native human papillomavirus N-terminal L2 epitopes", which has been accepted for publication in the journal PLoS ONE. HPV capsids are composed of 72 pentamers of the major capsid protein L1, and an unknown number of L2 minor capsid proteins. An N-terminal "external loop" of L2 contains cross-neutralizing epitopes, and native HPV16 virions extracted from 20-day-old organotypic tissues are neutralized by anti-

HPV16 L2 antibodies but virus from 10-day-old cultures are not, suggesting that L2 epitopes are more exposed in mature, 20-day virions. This study was undertaken to determine whether cross-neutralization of other HPV types is similarly dependent on time of harvest and to screen for the most effective cross-neutralizing epitope of L2 in the native virion. Although HPV16 L2 epitopes were only exposed in 20-day virions, HPV31 or HPV18 epitopes behaved differently. Conversely, HPV31 and HPV18 L2 epitopes were exposed in 10-day virions and remained so in 20-day virions. In contrast, presumably due to sequence divergence, HPV45 was not cross-neutralized by any of the anti-HPV16 L2 antibodies. We found that the most effective cross-neutralizing antibody was a polyclonal antibody named anti-P56/75 #1, which was raised against a peptide consisting of highly conserved HPV16 L2 amino acids 56 to 75.

These experiments highlight the utility of N-terminal L2 epitopes in eliciting cross-neutralizing antibodies that can neutralize infectivity of virions synthesized in stratifying and differentiating human epithelial tissue. Many antibodies neutralized 20-day HPV16 and cross-neutralized 10 or 20-day HPV31 and HPV18 in crude viral preparations, an environment which represents the physiological state of natural infection much more so than fractions of gradient-purified virus. The neutralization of virus in crude viral preparations shows that the abundant proteinaceous material in the prep does not interfere with antibody reactivity against organotypic culture-derived native virions. In addition, the failure of antibodies to neutralize HPV16 should not be a serious obstacle to the development of future L2-based vaccines. Ten-day HPV16 resides in the suprabasal compartment of stratifying and differentiating human epithelial tissue. Any transmission of these highly unstable particles would require severe damage to release these particles from deep within the tissue. Such extensive damage is likely a rare event.

The final goal of the first Aim was to utilize redox-altering reagents to determine when DNA encapsidation and formation of mature NVs is finalized. Previous studies have shown that treatment of HPV-infected organotypic raft cultures with 5mM GSSG enhances capsid maturation (1, 2). We found that a mild oxidizing agent, DMSO also gave similar results as GSSG. The reducing green tea extract, epigallocatechin gallate (EGCG), was added to raft cultures and shown to have a negative effect on the maturation of HPV. EGCG greatly lowered viral titers, again demonstrating the importance of the oxidizing environment for virion maturation.

Specific Aim #2

This aim proposed to test the role of capsid protein cysteine residues in capsid assembly. Besides observation of particle structure by TEM (for reason described above in Aim #1) we have accomplished all the goals and more of this aim. Our efforts have resulted in two manuscripts. Therefore these papers will be used to summarize the accomplishments of this aim.

<u>Manuscript #1</u>: "Differentiation-Dependent Interpentameric Disulfide Bond Stabilizes Native Human Papillomavirus Type 16"
This manuscript was published in July 2011. Genetic and biochemical analyses of human papillomavirus type 16 (HPV16) capsids have shown that certain conserved L1 cysteine residues are critical for capsid assembly, integrity, and maturation. Since previous studies utilized HPV capsids produced in monolayer culture-based protein expression systems, the ascribed roles for

these cysteine residues were not placed in the temporal context of the natural host environment for HPV, stratifying and differentiating human tissue. Here we extend upon previous observation, that HPV16 capsids become stabilized over time (10-day to 20-day) by utilizing a tissue-spanning redox gradient and by identifying temporal roles for individual L1 cysteine residues. Specifically, the C175S substitution severely undermined wild-type titers within 10 and 20-day tissue, while C428S, C185S, and C175,185S substitutions severely undermined wild-type titers only within 20-day tissue. All mutations led to 20-day virions that were less stable than wild-type and failed to form L1 multimers via nonreducing SDS-PAGE; however, only Optiprep-fractionated 20-day C428S, C175S, and C175,185S capsids appeared permeable to endonucleases in comparison to wild-type and C185S capsids. In addition, since treatment of raft cultures with oxidized glutathione (GSSG) or exposure to the natural tissue-spanning redox gradient failed to enhance infectious titers of any of the cysteine mutants compared to wild-type, we hypothesize that a complex, temporal interplay of disulfide bond formation, disruption, and reformation occurs between C175, C185, and C428 during migration of virions from the suprabasal compartment to the cornified envelope within stratifying and differentiating human epithelial tissue.

It is apparent that the C175S mutation severely limits the number of endonuclease-resistant genomes that are present within 10-day virus, while C185S and C175,185 mutations do not appear to be limiting. We hypothesize that it is possible for a capsid to contain a mixture of both C175-C428 and C185-C428 disulfide bonds with C185-C428 disulfide bonds being in the minority. In this scenario, the substitution of C175 for serine inhibits the formation of C175-C428 disulfide bonds, and allows for the formation of a larger percentage of C185-C428 interpentameric disulfide bonds. Another possibility that the data supports is that C175S mutations might alter an unknown early role for C175 in assembly. The formation of improper C185-C428 disulfides is supported by previous studies utilizing C175S mutant HPV16 VLPs and PsV which lead to the production of abnormal tubular structures via EM and the production of aberrant L1 dimers, respectively. However, nonreducing Western blot analysis of 10-day C175S virus does not depict L1 dimer formation, suggesting that if C185-C428 disulfides do take place, they are in the minority. As published previously, in this environment, a large degree of disulfide bonding would not be expected to occur. However, some level of disulfide bonding between the high molar concentration of neighboring L1 pentamers could be expected.

Analysis of 20-day viruses reveals the temporal importance of C175, C428 and C185. Titers remain low in 20-day C175S viral preparations as they had in 10-day viral preparations. Titers for C428S and C185S mutations were significantly down within 20-day viral preparations. This observation suggests that either, (i) C185 and C428 form a disulfide during maturation within tissue, (ii) that C185 is critical for the transition to more extensive C175-C428 disulfide bonds, (iii) that C185S mutations alter the environment within the E-F loop which makes it difficult for C175-C428 disulfides to form, or (iv) the potential structural effect of the serine residues could induce a shift in the loops. Due to previous structural analyses of capsids which have depicted a C175-C428 endpoint disulfide bond, we hypothesize that a transient C185-C428 disulfide bond occurs which primes final C175-C428 disulfide bonds. This hypothesis is in line with previously reported studies.

Further supporting the role of a complex interplay of disulfide bonding between C428, C175, and C185, is our finding that C428S, C175S, C185S, and C175,185S 20-day viral preparations have a significant decrease in titers compared to their 10-day counterparts. This suggests that each of these residues is important in the efficient accumulation of mature capsids. This result is supported by GSSG treatment of wild-type and mutant-infected tissues whereby virions within the mutant-infected tissues failed to respond to the oxidizing agent.

Manuscript #2: "Roles for HPV16 L1 cysteine residues 379, 161, and 229 in DNA encapsidation"
This manuscript is 'In Preparation" and should be submitted for review by the end of February 2011. Human papillomavirus (HPV) capsids are formed through a network of inter- and intra-pentameric hydrophobic interactions and disulfide bonds. 72 pentamers of the major capsid protein L1 and an unknown amount of the minor capsid protein L2 form the structure of the capsid. There are 12 conserved L1 cysteine residues in HPV16. Although C428, C175, and C185 have been implicated in the formation of a critical interpentameric disulfide bond, no structural or functional roles have been firmly attributed to any of the other conserved cysteine residues. Here we show that substitution of cysteine residues C379, C161, and C229 for serine hinders the accumulation of endonuclease-resistant genomes as virions mature within stratifying and differentiating human epithelial tissue. Further, C229S mutant virions form, but are noninfectious, which supports previous data that this residue might be involved in downstream steps in the virus life cycle. These studies add further detail to the differentiation-dependent assembly and maturation steps that occur during the HPV16 life cycle.

We showed previously that organotypic culture-derived HPV16 virions exploited a tissue-spanning redox gradient that facilitated assembly and maturation events in the context of the complete papillomavirus life cycle. Further, substitution of cysteine residues C428, C175, and C185 for serine led to a late-stage defect in the accumulation of endonuclease-resistant genomes, and made these mutant virions more fragile than wild-type virions. In this study, we wanted to determine if other conserved L1 cysteine residues were involved in the accumulation of endonuclease-resistant genomes, and if they similarly affected the stability of HPV16 capsids.

We show that although 10-day C379S virions contained as many endonuclease-resistant genomes as wild-type, C161S and C229S had less. At 20-days, C379S, C161S, and C229S virions failed to accumulate endonuclease-resistant genomes similar to wild-type virions. These results suggest that C161 and C229 are important for DNA encapsidation at both early and late stages while C379 is only important for DNA encapsidation at a late stage. Further, C229S mutant virions were noninfectious. This result supports previous studies that suggest a role for C229 in intracellular trafficking during viral entry. These studies reveal differentiation-dependent roles for capsid structural residues and incorporate data from studies using recombinant papillomavirus capsids to create a tentative model of capsid assembly and maturation within tissue.

References

1. **Conway, M. J., S. Alam, N. D. Christensen, and C. Meyers.** 2009. Overlapping and independent structural roles for human papillomavirus type 16 L2 conserved cysteines. Virology **393:**295-303.

2. **Conway, M. J., S. Alam, E. J. Ryndock, L. Cruz, N. D. Christensen, R. B. Roden, and C. Meyers.** 2009. Tissue-spanning redox gradient-dependent assembly of native human papillomavirus type 16 virions. J Virol **83:**10515-26.

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

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, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung

Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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. Cross-neutralization potential of native human papillomavirus N-terminal L2 epitopes	Conway, Cruz, Alam, Christensen, and Meyers	PLoS ONE	September, 2010	☐Submitted ☐Accepted X Published
2. Differentiation-Dependent Interpentameric Disulfide Bond Stabilizes Native Human Papillomavirus Type 16	Conway, Cruz, Alam, Christensen, and Meyers	PLoS ONE	March 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___X_____ No_____

If yes, please describe your plans:

Another manuscript "Roles for HPV16 L1 cysteine residues 379, 161, and 229 in DNA encapsidation", is in preparation and will be submitted in the next month for review.

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_____ No___X_____

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

a. Title of Invention:

b. Name of Inventor(s):

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

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

If yes, indicate date patent was filed:

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

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

If yes, how many licenses were granted?_____

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

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

Yes_____ No____X_____

If yes, please describe your plans:

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

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME **Craig Meyers**	POSITION TITLE **Distinguished Professor**
eRA COMMONS USER NAME cmeyers	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Brigham Young University, Provo, UT	B.S.	04/82	Microbiology
Brigham Young University, Provo, UT	M.S.	07/84	Microbiology
Univ. of California, Los Angeles, CA	Ph.D.	05/90	Microbiology/Immuno.
Howard Hughes Medical Institute, University of Chicago, Chicago, IL	Postdoc	07/93	Mol. Genetics/Cell Biology

A. Personal Statement

The central research goals of my laboratory are to study the differentiation-dependent life cycle of human papillomavirus (HPV) and HPV-associated oncogenesis. My studies follow six general research themes: (1) Investigation of the molecular biology and genetics of HPV morphogenesis and structure in native virus replicated under natural physiological differentiation conditions of the host tissue. The majority of the laboratories studying HPV morphogenesis and structure utilize synthetic particles such as virus-like particles (VLPs) or pseudoviral particles (PsV). Our studies have shown that the morphogenesis, maturation, and structure of native HPV differs in many significant characteristic from VLPs or PsV particles. (2) Investigation of the efficacy of disinfectants and microbicides on native HPV as compared to VLPs/PsV. Our results demonstrate that HPV is significantly more resistant to disinfectants and microbicides then our VLPs/PsV. This demonstrates the clinical importance of using native HPV for disinfectant and microbicide testing. (3) Determining the mechanism whereby cofactors such as tobacco carcinogens increase carcinogenic progression in HPV-associated cancers. We are dissecting the signaling pathways and cell cycle components that are impacted by the interaction of cofactors and HPV in host tissue. (4) Determining the interaction of highly active anti-retroviral drugs on oral tissues, HPV and HPV's infection of oral tissue. (5) Development of population science and basic science interactions to monitor, understand and influence education and behavior as they relate to a risk for acquiring an HPV-associated infection. (6) Comparative analysis of HPV infection, life cycle, and oncogenesis at different anatomical sites including the breast. In addition to HPV, I am also interested in studying the life cycle of other epitheliotropic infectious agents, such as the Herpes virus family, in three-dimensional human epithelial tissue.

B. Positions and Honors

Professional Experience:

1985-1990 Graduate Student Researcher, Dept. Microbiology & Immunology, UCLA, Los Angeles, CA

1990 Postdoctoral Appointment, Dept. of Microbiology and Immunology, UCLA, Los Angeles, CA

1990-1993	Postdoctoral Appointment, Howard Hughes Medical Institute, Department of Molecular Genetics and Cellular Biology, University of Chicago, Chicago, IL
1993-1999	Assistant Professor, Department of Microbiology and Immunology, Penn State University College of Medicine, Hershey, PA
1994-Present	Member, Graduate Faculty, Penn State University College of Medicine, Hershey, PA
1995-Present	Member, Cell and Molecular Biology Faculty, Penn State University College of Medicine
1997-Present	Member, Molecular Medicine Faculty, Penn State University College of Medicine,
1997-Present	Member, Cellular and Molecular Toxicology Faculty, Penn State Univ. College of Medicine
2002-2012	Professor, Department of Microbiology and Immunology, Penn State University College of Medicine, Hershey, PA
2003-2008	Co-Director, Molecular Medicine Graduate Program, Penn State University College of Medicine, Hershey, PA
2012-Present	Distinguished Professor, Department of Microbiology and Immunology, Penn State University College of Medicine, Hershey, PA

Honors and Awards:

1983	Amer. Soc. of Microbiology, Intermountain Region Annual Conference, Presentation Award
1985-1988	Graduate Fellowship in Genetics Mechanisms
1988	Southern California ASM Student Colloquium, Presentation Award, Doctoral Category
1988-1989	Graduate Fellowship in Microbial Pathogenesis
1995-2005	Member of the Society for *In Vitro* Biology
1997-2005	Member of the American Association for the Advancement of Science
1997-2005	Member of the American Society for Microbiology
1998-2003	Editorial Board of the Journal of Virology
2004	Society for In Vitro Biology Fellow Award, World Congress on In Vitro Biology
2004-2008	Member of the AIDS-Associated Opportunistic Infections and Cancer Study Section
2005-Present	Ad Hoc member NCI-I K-Awards Study Section
2012	Chair NCI Special Emphasis Panel ZCA1 RTRB-Z 02, AIDS
2013	Alumni Achievement Award, Brigham Young University.

C. Selected Peer-reviewed Publications

Most relevant to the current application

1. Hermonat PL, **Meyers C**, Parham GP, Santin AD. Inhibition/stimulation of bovine papillomavirus by adeno-associated virus is time as well as multiplicity dependent. *Virology*. 1998; 247(2):240-50.
2. Zhan D, Santin AD, Liu Y, Parham GP, Li C **Meyers C**, Hermonat PL. Binding of the human papillomavirus type 16 p97 by the adeno-associated virus Rep78 major regulatory protein correlates with inhibition. *J Biol Chem*. 1999; 274(44):31619-24.
3. **Meyers C**, Mane M, Kokorina N, Alam S, Hermonat PL. Ubiquitous human adeno-associated virus type 2 autonomously replicates in differentiating keratinocytes of a normal skin model. *Virology*. 2000; 272(2):338-46.
4. **Meyers C**, Alam S, Mane M, Hermonat PL. Altered biology of adeno-associated virus type 2 and human papillomavirus during dual infection of natural host tissue. *Virology*. 2001; 287(1):30-9.

5. Prasad CK, **Meyers C**, Zhan DJ, You H, Chiriva-Internati M, Mehta JL, Liu Y, Hermonat PL. The adeno-associated virus major regulatory protein Rep78-c-Jun-DNA motif complex modulates AP-1 activity. *Virology*. 2003; 314(1):423-31.
6. Sen E, McLaughlin-Drubin M, **Meyers, C**. Efficacy of two commercial preparations of interferon-a on human papillomavirus replication. *Anticancer Res*. 2005; 25(2A):1091-100.
7. Alam S, Bromberg-White J, McLaughlin-Drubin M, Sen E, Bodily JM, **Meyers, C**. Activity and therapeutic potential of ORI-1001 antisense oligonucleotide on human papillomavirus replication utilizing a model of dysplastic human epithelium. *Anticancer Res*. 2005; 25(2A):765-77.
8. Drubin DA, McLaughlin-Drubin ME, Clawson GA, **Meyers C**. A protease inhibitor specifically inhibits growth of HPV-infected keratinocytes. *Mol Ther*. 2006; 13(6):1142-8.
9. Alam S, Sen E, Brashear H, **Meyers C**. Adeno-associated virus type 2 increases proteosome-dependent degradation of $p21^{WAF1}$ in an HPV positive cervical carcinoma line. *J Virol*. 2006; 80(10):4927-39. PMCID: PMC1472069.
10. Alam S, **Meyers C**. Adeno-associated virus type 2 induces apoptosis in human papillomavirus-infected cell lines but not in normal keratinocytes. *J Virol*. 2009; 83(19):10286-92. PMCID: PMC2748052.
11. Israr M, Mitchell D, Alam S, Dinello D, Kishel JJ, **Meyers C**. Effect of the HIV protease inhibitor Amprenavir on the growth and differentiation of primary gingival epithelium. *Antivir Ther*. 2010; 15(2):253-65. PMCID: PMC3678718.
12. Israr M, Mitchell D, Alam S, Dinello D, Kishel JJ, **Meyers C**. HIV protease inhibitor lopinavir/ritonavir (Kaletra) alters growth, differentiation and proliferation of primary gingival epithelium. *HIV Med*. 2011; 12(3):145-56. PMCID: PMC3024444.
13. Alam S, Bowser BS, Conway MJ, Israr M, Tandon A, **Meyers C**. Adeno-Associated Virus Type 2 Infection Activates Caspase Dependent and Independent Apoptosis In Multiple Breast Cancer Lines But Not In Normal Mammary Epithelial Cells. *Mol Cancer*. 2011; 10:97. PMCID: PMC3199901.
14. Mitchell D, Israr M, Alam S, Kishel J, Dinello D, **Meyers C**. Effect of HIV nucleoside reverse transcriptase inhibitor, Zidovudine, on the growth and differentiation of primary gingival epithelium. *HIV Med.* 2012; 13(5):276-90. PMCID: PMC3319519.
15. Mitchell D, Israr M, Alam S, Dinello D, Kishel J, Rong J, **Meyers C**. HIV nucleoside reverse transcriptase inhibitors, Efavirenz and Tenofovir, change the growth and differentiation of primary gingival epithelium. *HIV Med*. In Press, 2013.

D. Research Support

Ongoing Research Support

1 U01 CA179724-01 08/01/13-06/31/18

"Mechanistic Investigations of Ethnic Differences in HPV Variants"
Project goal: To understand ethnic differences in HPV variants using a whole-genome analysis, including infection prevalence and carcinogenicity.
Role: PI

Merck IISP 07/18/12-02/28/14

"Monitoring of serum from vaccinated women for neutralization of vaccine and non-vaccine authentic Human Papillomavirus"
Merck Research Grant
Role: PI

Pennsylvania Breast Cancer Coalition 12/06/11-06/30/14

"AAV2 Preclinical Breast Cancer Studies"

Role: PI

Completed Research Support

1 R01 DE18305-02 (Meyers, P.I.) 08/16/07-04/30/13
Title: ART on Oral Tissue Growth, Function, and HPV Infections
Project goal: Investigate the effect of antiretroviral therapy (ART) on the life cycle of oral HPV infections in different types oral epithelium.
Role: P.I.

NIDCR-ARRA Supplement (Meyers, P.I.) 09/21/09-04/30/13
"ART on Tissue

5 R01 AI057988-03 (Meyers, P.I.) 09/01/06-08/31/11
NIH
Title: Genetic analysis of Papillomavirus Virion Morphogenesis
Project goal: Define the genetic and molecular events of papillomavirus (PV) virion morphogenesis.
Role: P.I.

CTSI 09/01/09-08/31/11
Title: HPV and Its Vaccine: Epidemiology, Education, Natural History, and Evolution Among Young Adults in Appalachia
Project goal: Perform microarray analyses to identify the global changes in gene expression induced by treating oral tissues with the ART drugs; and compare the differences and similarities induced by HPV16 infection of oral and cervical tissues providing insights into virus-specific and tissue-specific mechanisms tumorigenesis.
Role: P.I.

5 N01 AI30045-05 (Christensen, P.I.) 09/30/03-09/29/10
NIH
Title: In Vitro Antiviral Screening Program: Papillomaviruses
Project goal: In vitro testing of potential anti-papillomavirus compounds.
Role: Co-P.I.