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 Wistar Institute

2. **Reporting Period (start and end date of grant award period):** 01/01/09 – 06/30/10

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):**
   Russel E. Kaufman, M.D.

4. **Grant Contact Person's Telephone Number:** 215-898-3926

5. **Grant SAP Number:** SAP #4100047657

6. **Project Number and Title of Research Project:** Project 2: Impact of Latent Cytomegalovirus Immediate Early Protein Expression on Embryonic Development

7. **Start and End Date of Research Project:** 01/01/09 – 06/30/10

8. **Name of Principal Investigator for the Research Project:** Gerd Maul, Ph.D.

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:

   $ 298,102.73

   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 (Salary & FB)
Maul	Principal Investigator	85%	$ 183,040

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
Negorev	Research associate	75%
Vladimirova	Senior technician	100%

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 ____X____ No ____ _____

If yes, please indicate the source and amount of other funds: NIH grant; $250,000

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:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐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_____ No ____X_____

If yes, please describe your plans:

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

The transgenic mouse generated is presently being transferred to two medical research laboratories that have funds to continue the research, that of Dr. William Britt in Alabama and Dr. Jonjic in Croatia.

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				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				1 (Russian)
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 ___X____ No_____

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

Retaining one position allowed the completion of the project and enhanced the research success of an additional collaborator within The Wistar Institute (Dr. Nadia Dahmane).

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:

Collaborations were established with Drs. William Britt of the University of Alabama and Stipan Jonjic at the University of Rijeka Croatia. The transgenic mouse generated is presently being transferred to Drs. Britt and Jonjic for continued research.

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.

Specific Aims, Goals and Objectives (Overview)

Specific Aim 1. Development of a mouse model of inducible IE1 expression.
Specific Aim 2. Analyze the effect of IE1 expression on mouse brain development.

The objective of this research project was to determine whether inactivation of the host's repressors and gene-silencing proteins by the human cytomegalovirus (HCMV) IE1 and 2 and equivalent mouse (M) MCMV IE1 and 3 gene products affect differentiating neuronal cells of the developing embryo. Based on our identification of specific molecular interactions of IE proteins that lead to suppression of intrinsic nucleus-based defense systems, such as the gene-silencing machinery and other DNA modification mechanisms, we hypothesized that intrinsic host cell defenses effectively limit or completely abrogate spread of CMV infection, unless impeded by substantial viral countermeasures (high viral input). We further hypothesized that viral proteins able to perturb the cell's gene-silencing machinery can alter the cell's differentiation program, even in the absence of viral replication.

To test our hypotheses, we would determine whether inactivation of HDAC and abrogation of Daxx-mediated pro-apoptotic activity by the major immediate early proteins alters the differentiation program to induce abnormal neuronal development during embryogenesis.

Specifically, we would (i) construct and test the IE1/3 vectors for use in transgenic mouse experiments, produce embryonic stem cells with an integrated IE transcription unit, and develop

transgenic mice; and (ii) analyze GFP expression of founder-derived homozygotic mice during embryogenesis, generate double transgenics, and analyze brain structures.

Results

Construction of the Cre-lox stop IE1/3 vector and embryonic stem cell production. HCMV immediate early protein IE1 prevents cells from passing through the G2 phase of the cell cycle. We had to assume that the equivalent MCMV IE3 might also have detrimental properties for the replication of mouse cells. The strategy in the construction of the knock-in vector was to place transcriptional and translational stop sequences proximal to and in line with exon 2 and a GFP reporter so that, after differential splicing, both IE1 or IE3 would be labeled. The final plasmid, RGiSTOP, was tested by transfection into 3T3 cells and cyclisation recombinase (Cre) expression from an adenovirus to determine its ability to express the respective viral proteins using the ND10 dispersive function of IE1 as a read-out. Cells transfected with the GFP-expressing control and tested for PML distribution retained ND10, i.e., the PML aggregates we refer to as nuclear dots. Cells marked by GFP from the experimental vector alone dispersed PML showing that the stop sequences had been eliminated and, therefore, that the IE1 function was preserved.

Since the 14.3 LoxP flanked STOP-lox cassette containing MCMV IE1/3 transcriptional unit was too large to amplify, it was transferred into the low copy HPRT targeting vector (based on a BAC plasmid). The final vector has been validated by restriction enzyme digestion and sequencing, and the linearized 32.9 kb targeting fragment transfected into E14 ES cells according to genOway's electroporation procedure. Positive clones were verified by Southern blotting, which also confirmed that only a single insert at the appropriate site had been integrated through a homologous recombination event. Due to the X-chromosomal location and male ES cells, one allele only was detected (data not shown).

ES cells with the appropriate insert were tested again as to the production of functional IE1 by adenovirus transduced Cre enzyme. The results obtained were the same as those described above for the vector alone, i.e., dispersion of PML aggregates, giving assurance that the large insert had not accumulated mutations during construction and that at least IE1 was functional. Mice with the transmitted floxed IE1/3 insert were bred to homozygosity and then crossed with timed Cre expressing mice.

Effect of IE1/3 expression in the embryonic mouse. We assumed that expression of IE1/3 would lead to ablation of brain cells that expressed these proteins and thus result in a recognizable phenotype. To activate the stop codon containing IE1/3 transcription unit, we crossed mice carrying the brain specific Nestin promoter activated Cre-ER with IE1/3 mice to obtain homozygotic distribution of IE1/3 and heterozygotic distribution of Cre-ER. Mating either IE1/3-Cre-ER males with IE1/3 females or IE1/3-Cre-ER females with IE1/3 males should produce embryos, half of which after Tamoxifen injection could induce expression of IE1/3 in Nestin expressing brain cells. Tamoxifen injections were made at 10 or 12 p.c. We received 50 pups from 10 matings and found neither a sex ratio difference nor any apparent problems during nursing or adolescence. A single mouse showed movement problems by running in circles.

We expected low penetration of Cre enzyme might have been the reason that we could not observe a specific phenotype. Because we could not test the penetration aspect of our induction instead we made the same crosses with a PAX3-Cre-expressing mouse since all PAX3 expressing cells should remove the stop codons, i.e., all cells should express IE1/3 from day 1 in the developing brain. Again from 10 litters, we found no recognizable phenotype nor any changes in the expected ratios of live births and survival between the PAX-Cre-ER expressing and non-expressing progeny. Either all MCMV IE1/3 promoters in the Pax3 expressing cells were silenced or the expression of IE1/3 had no affect on the mouse embryos.

We then proceeded to make the appropriate crosses with ubiquitin-Cre-ER mice as they allowed us to test for penetration and successful removal of the stop sequences using tail snips. Timed pregnant mice were injected with Tamoxifen on the 2nd day and individually in successive days until 14 days p.c. The female mice showed a substantial penetration of Cre enzyme activity, although the stop sequences were not excised in all cells as shown by a faint band indicating the existence of the stop sequences in the IE1/3 sequence.

We obtained no live births when tamoxifen had been administered in the first 8 days p.c. while apparently, normal litters were obtained between days 9 and 14. Injection later than that resulted in several premature litters, and 2 litters that were 2-3 days late resulting in large pups.

Control injected mice demonstrated that the effect seen on live births was not a consequence of IE1/3 expression, but due to tamoxifen exposure. Apparently, live births could only be achieved when injection of tamoxifen was administered before embedding the embryo into the uterus. However, the experiment seemed to show that, even with potential expression at day 8 forward, live births had no recognizable phenotype.

The unlikely possibility the there is no detectable embryonic phenotype even if the cells express IE1/3 from the time of conception was also tested by producing mice in which the expression is possible from the time of conception. This genotype was constructed by injecting IE1/3/Cre-ER males with tamoxifen, then waiting 38 days to recycle the sperm toward those that went through meiotic division after tamoxifen treatment. These males were mated with IE1/3/Cre-ER females. Genotyping of female pups identified a few in which the stop sequences had been removed from one of the X-chromosomes. This result showed that such females could survive.

Since it was possible that the X-chromosome with the open reading frame might have been the silenced X chromosome, we crossed the heterozygotic females with the IE1/3 open X chromosome with the male IE1/3 open genotype. Genotyping of the resulting pups revealed a near equal distribution of the IE1/3 open genotype, hence the ability to produce the two immediate-early proteins did not affect embryogenesis and later breeding success.

The breeding results raised the question whether GFP-IE1/3 was actually expressed during embryogenesis in which we used embryos (E14) and developed mouse embryonic fibroblasts (MEFs). Fluorescent microscopy showed a very low number of cells expressed low concentrations of IE1/3 (< 0.1 %) and only rarely at high concentrations. Those cells were tested as to whether the expressed IE1 was functional by staining the cells with PML antibodies. No

ND10 bodies were found in any of the GFP-containing nuclei; thus, the IE1 is functional as ND10 dispersal was detected (data not shown).

We assumed that, in most cells, the CMV major immediate early promoter was silenced. The MEFs were treated with the acetylation inhibitor Trichostatin A (TSA) to determine whether silencing was achieved through acetylation. Comparison of untreated with treated cultures showed a substantial increase of nuclei exhibiting green fluorescence and high nuclear concentrations. This indicates that some cells were silenced by acetylation, but also that the majority of cells were silenced by other mechanisms.

Since MCMV can be reactivated with cytokines such as TNFa, we treated the cultures with this factor for 24 hours and did observe activation of the MIEPE and protein expression in a much higher frequency than controls, but not more than with TSA (data not shown). However, the number of cells activated was still low. Combined administration of TSA and TNFa did not increase the number of GFP containing cells as determined by cell sorting. Cells did show the potential to activate, but a large number seemed impervious to reactivation with the methods used. We suspected that methylation of the MIEPE may be the cause of silencing for the majority of the cells. Attempts to reactivate the cells with Arc failed because the cells lifted off from the substrate and probably died at several concentrations used. Direct assays for promoter methylation are now underway. The fibroblast cells may be useful to study other means of silenced MIEPE reactivation.

We also examined whether protein expression of only IE1 from the knocked-in viral transcripts, i.e., biased splicing, may have prevented any recognizable phenotype. This possibility might be detected by the different distribution of the two major immediate early proteins of MCMV. In the uninduced MEFs, we find mostly diffuse GFP distribution indicating IE1 presence, whereas in the TSA- or TNFa-exposed cultures we find cells that showed diffuse and aggregated GFP distribution. These results suggest that the cells might have different splicing ratios and that lower expression of IE3 might be the cause for the absence of a recognizable phenotype. In addition, we do not know if IE3 is functional in our construct.

The major immediate transactivator of HCMV, IE2, results in the block of cell cycle progression, which in the embryo's brain could lead to damage even in the absence of viral replication. In order to test whether IE3 is functional in our transgenic animals, we tested whether this protein can activate the early viral promoter of E1. We produced 3T3 cells with E1 under control of its own promoter and fused them with the nesting controlled IE1/3 expressing cells by exposure to ethylene glycol 1000. The readout displayed enhanced expression of E1 as monitored through immunofluorescence. Fused cells could be recognized when they have acquired E1into the nuclei of IE1/3 expressing cells. The intensity of E1 in GFP-producing cells vs. the red staining in surrounding cells without GFP was evaluated by immunomicroscopy and showed that IE3 is functional at activating the early viral promoter.

The possibility that the attachment of GFP at the N-terminal of IE1 and IE3 has a detrimental effect on the functions of these proteins was investigated through the generation of viruses in which GFP was expressed on the N-terminal of IE1 and IE3 and by fusion of exon 3 and 5 to generate a mutant virus expressing only IE3. No recognizable diminishment in virus propagation

was found. We also tested the replication time of the first round infection by time lapse photography. It showed that the GFP-wt-virus took about 23 hours to produce infectious virus (judged by the appearance of GFP in the neighboring cells) and the IE1 deleted virus 24 hours – probably not a significant difference.

This analysis produced the surprising additional finding that many infected cells which had started to produce immediate early proteins did not proceed to productive viral replication. To test whether this was a GFP-dependent effect, we infected 3T3 cells or second passage MEFs with wild type virus. Coverslips were fixed 6 hours p.i. and stained with IE1/3 reacting antibodies, and the positive cells estimated. First round viral release was counted when miniplaques had formed in parallel cultures after 24 or 30 hours p.i. We found that the overwhelming number of IE1/3 positive cells did not produce infectious virus. Though variable between repeated experiments, the approximate ratio was one miniplaque for every 60 IE1/3 producing cells. Our results of initial IE1/3 activation using time lapse imagery were therefore not due to the addition of GFP to either IE1 or IE3.

Some of the infected GFP-IE1/3 producing cells divided several times in the presence of the GFP-tagged immediate early proteins without producing infectious virus. Since IE3 is associated with the immediate transcript environment of one or very few individual viral genomes, we could follow the dilution of the viral genomes over four cell cycles. The number of IE3 aggregates diminished over an individual cell cycle, but increased again in the following G1-phase. Most often the original number was not reached in the equivalent daughter cells. One of the cells eventually produced infectious virus, but most did not. Whether or not any new viral genomes had been produced in those cells that did not produce infectious virus cannot be determined, but clearly the cell could silence the genomes in their respective nuclei; and a few could be reactivated at the beginning of the cell cycle, assuming that an immediate early transcription environment harbors a minimum of one viral genome. The initial expected possibility that GFP at the N-terminal of IE1 or IE3 might inhibit those proteins functions as measured by viral replication can therefore be rejected. Also, no cytotoxic effects were noticed; rather, a surprising cellular inhibitory function, even at genome loads of about eight, had to be postulated, suggesting a sequential shutdown or silencing of viral genomes in some cells independent of the stage in the cell cycle.

Expression of IE1/3 in the adult mouse. The low frequency expression in MEFs might, if also present in the adult mouse, be the reason that we do not see an obvious negative phenotype. To evaluate the presence of IE1/3 producing cells, we fixed mice by transfusion and sectioned the different frozen organs to determine where IE1/3 is expressed. We find a most specific expression in the mouse cerebellum. GFP-expressing cells were found between the granular and molecular layer in a pattern consistent with Purkinje cell distribution. However we did not find that their nuclei contain GFP-IE1/3. The position the GFP labeled nuclei in relation to the Bermann cells confirms previous results that b-galactosidase from the MCMV IE1/3 promoter enhancer is expressed there. Bermann cells surround the Purkinje cells, but only a few express the viral proteins. Any possible disturbance in the few positive Bermann cells may, therefore, not have a strong influence on the Purkinje cell's sensorimotor output.

Also expressing IE1/3 were cells surrounding the ventricular cavity, but these cells had a substantially lower nuclear concentration of GFP-IE1/3. Treatment with TNFa concentrations, which reactivated MCMV, did not have a significant effect on the cerebellum, although cells in the meninges did become activated. In the lower part of the brain, a higher concentration of an as-yet unidentified cell type had a relative high expression level. Very few cells in the cortex showed IE1/3 expression (not shown). No expression was found in the spinal cord.

These results demonstrate that only very specific cell types express IE1/3 and that, within these cell types, the expression is activated in only a low percentage similar to the explanted MEFs. Since kidney transplantation often results in reactivation of CMV, we evaluated the expression in this organ. We find GFP-labeled nuclei only rarely in the untreated control. Contrary to untreated control, GFP-positive nuclei were found in the distal tubule after treatment with TNFa for 24 hours and the autofluorescence outlines the tubules. It is the distal tubules that carry nearly all the GFP-IE1/2 expressing nuclei and there is no GFP signal in the proximal tubules. Still, the positive nuclei are much lower than expected from reports where MIEPE activated b-galactosidase was evaluated. In the untreated control, only a few nuclei of the proximal tubules were found; but a surprisingly high number of nuclei expressed the GFP-labeled viral protein in a presently undefined tissue beside the kidney.

We also examined the eye. We found only a very few cells express IE1/3, except for a ring around the lens; and at higher magnification, it is obvious that only the cells closest to the lens are activated. In the heart, we find cells expressing the viral proteins in high concentrations located in the septum and the valve and there at higher magnification, we find that some cells show either smooth distribution or aggregated distribution. The same differential distribution is found in the lungs and bronchi where the GFP-tagged protein is either distributed diffusely throughout the chromatin area except for the nucleolus or as aggregates excluding the DAPI stain of region of the chromatin. In muscle cells, only rare nuclei are found to express the viral protein. No expression was found in liver, stomach and gut tissue (not shown). Though not exhaustive, the evaluation of different tissues shows that the viral proteins are expressed in a limited number of tissues; and, in those where it is expressed, only a few cells express specific cell types. Furthermore, the same cell types that express the viral proteins show either a pattern reminiscent of the dispersed distribution of IE1 or the aggregated distribution of IE3. The expression of aggregated protein in lung epithelial cells was high relative to IE1, whereas in the brain the diffuse distribution pattern was dominant. Whether this difference was due to different ratios of the respective message was determined by quantitative PCR comparing both organs.

Discussion

The impetus to investigate the expression of the UL122/123 gene of MCMV in the appropriate host had been the hypothesis that the IE1 protein would modify embryonic development through modification of HDAC availability, i.e., through the brain's ontogenic silencing mechanism. Similarly, IE3 like the HCMV IE2 was thought to interfere with the progression of the cell cycle, providing another detrimental effect in brain development. With these potential effects in mind and expecting embryological lethal effects, we designed the transgenic knock-in vector so that the transgene could be activated by Cre-ER through crossing the floxed IE1/3 knock-in mouse with those that express the Cre enzyme at different gestation times or in different specific cell

types during embryonic development. Finding no recognizable overt phenotype was surprising. One reason for this finding may be because too few cells express the transgene. However, the transgenic mouse model that we have generated and partially characterized is an important resource that can now allow the search for those activating signals which may be responsible for reactivation of the virus. In addition, it provides a model system that will allow one to search for the next steps that must be required for the sequential reactivation of the virus by crossing with transgenic mice where E1 can be activated.

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.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐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:
A manuscript is in the process of being written and will be submitted to the Journal of Virology shortly: *Characterization of the Full MCMV Major Immediate Early Gene Expression in the Adult Mouse*, Gerd G. Maul, Olga V. Vladimirova, Dmitri G. Negorev and Nadia Dahmane.

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.

At present, there is no impact.

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
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NAME	POSITION TITLE
MAUL, Gerd G.	
eRA Commons Username: **GERDMAUL**	**Professor**

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Technical University of Darmstadt, Germany	B.S.	1964	Biology
Technical University of Darmstadt, Germany	M.S.	1965	Biology
University of Texas, Austin, TX	Ph.D.	1967	Zoology
M.D. Anderson Hospital, Houston, TX	Postdoc	1968	Biochemistry

A. Positions and Honors:

1967-1968 Postdoctoral Fellow, M.D. Anderson Hospital, Houston, TX
1968-1969 Instructor, University of Texas, Department of Pathology, Galveston, TX
1969-1973 Assistant Professor, Temple University School of Medicine, Department of Pathology, Philadelphia, PA
1973-1985 Associate Professor, The Wistar Institute, Philadelphia, PA
1986-present Professor, The Wistar Institute, Philadelphia, PA
1987-present Professor of Laboratory Medicine, University of Pennsylvania, Philadelphia, PA
1992-present Professor of Rheumatology, University of Pennsylvania, Philadelphia, PA

Honors:

1966-1967 Rosalie B. Hite Predoctoral Fellowship, University of Texas, Austin, TX
1967-1968 Rosalie B. Hite Postdoctoral Fellowship, M.D. Anderson Hospital, Houston, TX
1973-1978 Faculty Research Award from the American Cancer Society
1987 Lupus Foundation, The S.N. Hirsch Award

B. Selected Research Publications out of 150:

1. Ascoli, C.A. and **Maul, G.G.** 1991. Characterization of a novel family of nuclear structures. J. Cell Biol. 112: 785-796.
2. Dyck, J., **Maul, G.G**., Miller, W.H., Chen, J.D., Kakizuka, A., and Evans, R.M. 1994. A novel macromolecular structure is a target of the PML-RAR oncoprotein. Cell 76: 333-343.
3. Everett, R.D. and **Maul, G.G**. 1994. HSV-1 IE protein Vmw110 causes redistribution of PML. EMBO J. 13: 5062-5069.
4. Korioth, F., Griffers, C., **Maul, G.G**., and Frey, J. 1995. Molecular characterization of NDP52, a novel protein of the nuclear domain 10, which is redistributed upon virus infection and interferon treatment. J. Cell Biol. 130: 1-13.
5. **Maul, G.G**., Yu, E., Ishov, A.M., and Epstein, A. 1995. Nuclear domain 10 (ND10) associated proteins are also present in nuclear bodies and redistribute to hundreds of nuclear sites after stress. J. Cell Biochem. 59: 498-513.
6. Doucas, V., Ishov, A.M., Romo, A., Juguilon, H., Weitzman, M.D., Evans, R.M., and **Maul, G.G**. 1996. The adenovirus replication is coupled with the dynamic properties of the PML nuclear structures. Gene Dev. 10: 196-.
7. **Maul, G.G**., Ishov, A.M., and Everett, R.D. 1996. Nuclear domain 10 as preexisting potential replication start site of herpes virus type 1. Virology 217: 67-75.
8. Ishov, A.M. and **Maul, G.G**. 1996. The periphery of nuclear domain 10 (ND10) as site of DNA virus deposition. J. Cell Biol. 134: 815-826.
9. Ishov, A.M., Stenberg, R.M., and **Maul, G.G**. 1997. Human cytomegalovirus immediate early interaction with host nuclear structures. Definition of an immediate transcript environment. J. Cell Biol. 138: 5-16.
10. **Maul, G.G.** 1998. Nuclear domain10, site of NDA virus transcription and replication. BioEssays 20: 660-67.
11. **Maul, G.G**., Jensen, D. E., Ishov, A. M., Herlyn, M., and Rauscher, F. J. 1998. Nuclear redistribution of BRCA1 during viral infection. 1998. Cell Growth Diff. 9: 743-755.
12. Ishov, A.M., Sotnikov, A.G., Negorev, D. Vladimirova, O.V., Neff, N., Kamitani, T., Yeh, E.T.H., Strauss III, J.F., and **Maul, G.G**. 1999. PML is critical for ND10 formation and recruits the PML interacting protein Daxx to this nuclear structure when modified by SUMO-1. J. Cell Biol. 147: 221-233.
13. Bell, P., Lieberman, P., and **Maul, G.G.** 2000. Latent Epstein Barr virus Distribution and Reactivation in Cultured Cells. J. Virol. 74: 11800-11810.

14. Negorev D., Ishov A. M., and **Maul G.G**. 2001. Evidence for separate ND10-binding and homo-oligomerization domains of Sp100. J. Cell Sci. 104: 59-68.
15. Bell, P., Montaner L., and **Maul, G.G.** 2001. Accumulation and intranuclear distribution of unintegrated HIV-1 DNA. J. Virol. 74: 11800-11810.
16. Schultz, D.C., Ayyanathan, K., Negorev, D., **Maul, G.G.**, and Rauscher, F.J. 3rd. 2002. SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev. 16: 919-932.
17. Ishov, A.M., Vladimirova, O.V. and **Maul, G.G.** 2002. Daxx-mediated accumulation of human cytomegalovirus tegument protein pp71 at ND10 facilitates initiation of viral infection at these nuclear domains. J Virol 76: 7705-7712.
18. Nefkens, I., Negorev D., Ishov, A.M.,Yeh, E.T.H., Tanguay, R.M., Muller, W.E.G. and **Maul G.G**. 2003. Stress by heat shock or Cd^{++} exposure regulates ND10-associated protein release by different mechanisms. J Cell Sci. 116: 513-524.
19. Tang, Q. and **Maul, G.G.** 2003. Mouse Cytomegalovirus Immediate-Early Protein 1 Binds with Host Cell Repressors To Relieve Suppressive Effects on Viral Transcription and Replication during Lytic Infection. J Virol 77: 1357-1367.
20. Tang, Q., Li, L., Ishov, A.M., Revol, V., Epstein, A.L., and **Maul, G.G**. 2003. Determination of minimum herpes simplex virus type 1 components necessary to localize transcriptionally active DNA to ND10. J Virol. 77: 5821-5828.
21. Davido, D.J., Von Zagorski, W.F., **Maul, G.G**., and Schaffer, P.A. 2003. The differential requirement for cyclin-dependent kinase activities distinguishes two functions of herpes simplex virus type 1 ICP0. J Virol. 77: 12603-12616.
22. Becker, K.A., Florin, L., Sapp, C., **Maul, G.G**., and Sapp, M. 2004. Nuclear localization but not PML protein is required for incorporation of the papillomavirus minor capsid protein L2 into virus-like particles. J Virol. 78: 1121-1128.
23. Ishov, A.M., Vladimirova, O.V., and **Maul, G.G**. 2004. Heterochromatin and ND10 are cell-cycle regulated and phosphorylation-dependent alternate nuclear sites of the transcription repressor Daxx and SWI/SNF protein ATRX. J Cell Sci. 117: 3807-3820.
24. Tang, Q., Li, L., and **Maul, G.G**. 2005. Mouse Cytomegalovirus Early M112/113 Proteins Control the Repressive Effect of IE3 on the Major Immediate-Early Promoter. J Virol. 79: 256-263.
25. Lechner, M.S., Schultz, D.C., Negorev, D., **Maul, G.G.**, and Rauscher, F.J. III. 2005. The mammalian heterochromatin 1 binds diverse nuclear proteins through a common motif that targets the chromoshadow domain. 2005. Biochem Biophys Res Commun. 331: 929-937.
26. Kuo, H.Y., Chang, C.C., Jeng, J.C., Hu, H.M., Lin, D.Y., **Maul, G.G.**, Kwok, R.P., and Shih, H.M. 2005. SUMO modification negatively modulates the transcriptional activity of CREB-binding protein via the recruitment of Daxx. Proc Natl Acad Sci U S A. 102:16973-16978.
27. Greger, J.G., Katz, R.A., Ishov, A.M., **Maul, G.G.**, and Skalka, A.M. 2005. The cellular protein Daxx interacts with avian sarcoma virus integrase and viral DNA to repress viral transcription. J Virol. 79: 4610-4618.
28. Tang Q. and **Maul, G.G**. 2006. Immediate Early Interactions and Epigenetic Defense Mechanisms Cytomegaloviruses: Molecular Biology and Immunology; Edited by: Matthias J. Reddehase, Caister Academic Press. pp131-150.
29. Negorev, D.G., O.V. Vladimirova, A. Ivanov, F. Rauscher, 3rd, and G.G. Maul. 2006. Differential role of Sp100 isoforms in interferon-mediated repression of herpes simplex virus type 1 immediate-early protein expression. *J Virol*. 80:8019-29.
30. Tang Q. Murphy E. and **Maul G.G**. 2006. Experimental Confirmation of Global Murine Cytomegalovirus Open Reading Frames by Transcriptional Detection and Partial Characterization of Newly Described Gene Products. J. Virol; 80: 6873-82.
31. Tang Q. and **Maul G.G**. 2006. Mouse Cytomegalovirus Crosses the Species Barrier with Help from a Few Human Cytomegalovirus Proteins J. Virol. 80: 7510-21
32. Del Galdo, F., **G.G. Maul**, S.A. Jimenez, and C.M. Artlett. 2006. Expression of allograft inflammatory factor 1 in tissues from patients with systemic sclerosis and in vitro differential expression of its isoforms in response to transforming growth factor beta. *Arthritis Rheum*. 54:2616-25.