

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:** 10. Allogeneic CMV CTL for Refractory Glioblastoma Multiforme
7. **Start and End Date of Research Project:** 01/01/10-01/17/11
8. **Name of Principal Investigator for the Research Project:** Kenneth Lucas, MD
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:

\$ 16,704.45

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
Bao	Research Associate	10%	4,770.28

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
Lucas	PI	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	<input type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input type="checkbox"/> 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 PI has left Penn State University – Hershey Medical Center.

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 _____ No x _____

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

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

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

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

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

Yes _____ No x

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

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

Yes _____ No x

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

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes _____ No x

If yes, please describe the collaborations:

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

Yes _____ No x

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

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

Yes _____ No x

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

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

List the project goals, objectives and specific aims (as contained in the grant 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 (β) should not print as boxes (\square) and include the appropriate citation(s). **DO NOT DELETE THESE INSTRUCTIONS.**

Introduction. Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor affecting adults, with a median survival of approximately one year, and over 95% of patients surviving less than two years. Recent studies have shown that CMV (cytomegalovirus) DNA can be detected in colon cancer, malignant glioma, and some prostate cancers. Three groups have demonstrated that CMV pp65 and IE1, two of the most immunogenic CMV antigens, are present in GBM but not in normal brain tissue. There are no data to indicate that CMV is oncogenic but it may have an oncomodulatory role in the progression of some tumors, causing dysregulation of

key transcription factors, tumor suppressor proteins, or intracellular signaling pathways. Irrespective of the role of this virus in GBM, if immunogenic CMV antigens are expressed on these tumors, these antigens can be used as a target by adoptively transferred, antigen specific CTL. The purpose of this study was to determine the extent to which CMV pp65 and IE1 are expressed on newly diagnosed GBM, and if positive, treat patients with therapy refractory, CMV positive GBM with CMV specific cytotoxic T lymphocytes (CTL).

Immunohistochemistry. All immunohistochemical analysis was performed on 3-4 μ m thick sections from formalin-fixed, paraffin-embedded tissue. Tumors were from archived pathologic specimens of newly diagnosed patients with GBM at Penn State Hershey Medical Center from 2001-2009. The diagnosis of GBM was confirmed by a neuropathologist (CS) prior to being used for these studies. Immunohistochemistry for cytomegalovirus pp65 (NCL-CMVpp65, clone 2 and 6, Leica Microsystems, Newcastle, UK) and IE1 (MAB810, clone 8B1.2, Millipore, Temecula, CA) was performed by first using heat-induced epitope retrieval in 10 mM citrate buffer pH 6.0 (IE1) or 1 mM EDTA pH 8.0 (pp65) for 20 minutes, followed by blocking for 10 minutes with 3% peroxidase.

Immunofluorescence (IF) analysis. T98G and U251 cells (American Type Culture Collection) were maintained in serum free medium and were trypsinized and replated onto glass coverslips in 12-well tissue culture plates (1 x 10⁵ cells per well). After infection, the coverslips were harvested and rinsed in PBS. The cells on the coverslips were then fixed with 4% paraformaldehyde (in PBS) at room temperature for 5 minutes, permeabilized with 0.2% Triton X-100 (in PBS) at room temperature for 15 minutes, washed in PBS, and then blocked with 0.25% BSA in PBS at room temperature for 60 minutes. The cells on coverslips were incubated overnight with the same primary antibodies (Abs) used for immunohistochemistry. After extensive washes in PBS, coverslips were incubated with the secondary antibody (Dylight 488 AffiniPure Donkey Anti-Mouse IgG; Jackson ImmunoResearch, West Grove, PA). Following repeated washes in PBS, nuclei were counterstained with Hoechst dye, washed, and the coverslips were mounted in Aqua-Poly/Mount.

CMV Culture. Human CMV was obtained from ATCC, and virus was propagated in human skin fibroblasts (SF). The initial infection was at an MOI of 0.1. Five days after cytopathological effects appeared in more than 90% of cells, the virus-containing media were collected, passed through a 0.45-mm filter, and cryopreserved in liquid nitrogen. The viral titer was between 10⁶ and 10⁷/mL by plaque assays in human SF.

Cell lines, and cell infection with CMV. T98G (human glioblastoma cell line; ATCC CRL-1690), U251 (Human glioma, kindly provided by Dr. Song Lee at Penn State Hershey Medical Center, Hershey, PA) were grown in Dulbecco Modified Eagle Medium (DMEM; GIBCO BRL). All media were supplemented with 10% fetal bovine serum, and cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂. All cell lines were maintained in DMEM without serum for 2 days prior to infection. Cells were replated and were infected at a multiplicity of infection (MOI) of 10. The cells were incubated with virus for 12 to 16 hours, re-fed with fresh medium with serum, and harvested 72 hours after infection for analysis.

Decitabine (DAC) and Interferon- γ (IFN- γ) treatment. Prior to treatment with IFN- γ or DAC, the

cells were plated in 10-cm dishes. On the day of treatment the media was removed and replaced with media containing 1 μ M of 5-Aza-2'-deoxycytidine (DAC) (Sigma-Aldrich, St. Louis, MO) or 100 ng/ml human recombinant IFN- γ (R&D systems, Minneapolis, MN). After incubation with DAC or IFN- γ for 5 days, the cells were harvested, counted, and then prepared for flow cytometry or chromium release assay.

T Cell Culture. CMV specific CTL were generated using CMV pp65 and IE1 pools of peptides (15mers overlapping by 11 amino acids) as described previously. Nonadherent peripheral blood lymphocytes (PBLs) were stimulated with adherent cells pulsed with either CMV pp65 or IE1 peptide mixes (JPT Peptide Technologies, Berlin), consisting of 138 and 120 overlapping 15mers, respectively. CMV specific CTL were analyzed by chromium release assays and flow cytometry for intracellular IFN- γ production.

Chromium Release Assays. Targets for chromium release assays (CRA) included autologous B cell blasts (BB; used as a negative control) and BB pulsed with the pp65 peptide mix. To determine whether these effector cells recognized naturally processed and presented pp65 epitopes, we infected GBM tumor cell lines with HCMV strain AD 169, and a portion of the cells were treated with DAC or IFN- γ . Targets were labeled overnight with ^{51}Cr (100 $\mu\text{Ci}/10^6$ cells; PerkinElmer Life and Analytical Science, Boston, MA), washed in PBS, and dispensed in triplicate into 96-well V-bottom plates (ICN, Costa Mesa, CA) at 4×10^3 cells/well, as previously described. CTLs were added at different responder:target ratios, and after pelleting and incubation for 4 hours, the supernatant was analyzed in a gamma counter.

Flow cytometry. Flow cytometry was performed with a FACScan (BD Biosciences, San Jose, CA) for MHC class I and Class II expression. MHC Class I and Class II expression was determined by staining with directly conjugated monoclonal antibodies (mAb), including PE conjugated anti-human HLA-ABC mAb to detect MHC class I and FITC conjugated anti-human HLA-DR, DP, DQ mAb (BD Biosciences, San Jose, CA) to detect MHC class II.

Results

Immunohistochemical analysis of patient tumors. We analyzed 49 paraffin sections of primary GBM from newly diagnosed patients at Penn State Hershey Medical Center for the presence of CMVpp65 and IE1 by immunohistochemistry. There were 26 males and 23 females, with a mean age of 67.6 years (range 38-79 years). Figure 1 depicts the expression of both pp65 and IE1 from two representative tumors testing positive for these antigens. There was no staining from CMV non-infected tissues or with isotype control antibodies. These tumors had both cytoplasmic and nuclear staining for these antigens, as reported by other groups. The incidence of CMV pp65 and IE1 in our subjects was 25/49 (51%) positive for pp65 and 8/49 (16%) positive for IE1. Not all cells within a given tumor were positive for pp65 or IE1, possibly reflecting variability in the infection of GBM cells. In nearly all of the tumors, the presence or absence of staining for pp65 correlated with IE1, but 1 specimen was positive for IE1 and negative for pp65.

CMV infection of GBM cell lines. Cells that are permissively infected by CMV, such as SF, have prominent nuclear staining for these antigens, but several CMV positive GBM in this series had prominent staining for pp65 and IE1 in the cytoplasm. CMV pp65 and IE1 nuclear staining

was only seen in 16% of GBM with immunohistochemical evidence for these proteins in this series. To determine the extent to which pp65 and IE1 are expressed after CMV infection of GBM cells, CMV strain AD169 was used to infect two GBM cell lines, and the expression of pp65 and IE1 were analyzed by fluorescent microscopy. Approximately 30% of the cells were positive for pp65 and IE1 at three days post-infection. Figure 2 depicts staining for both antigens from two CMV infected GBM tumors, showing that most staining for pp65 was cytoplasmic, whereas there was peri-nuclear localization of IE1 in some tumor cells.

CTL killing of CMV-infected GBM cells. Several tumor cell lines, including GBM, have been shown to have decreased expression of MHC Class I and II molecules, which could preclude tumor cell recognition by CMV pp65/IE1 specific CTL. DAC, a demethylating agent, and IFN- γ have also been shown to increase the expression of tumor antigens and MHC Class I and II molecules on tumor cells. We cultured GBM tumor cell lines with IFN- γ or DAC, and CMV infected and non-infected GBM cells were used as target cells in CRA with partially HLA matched CMV specific CTL derived from healthy donors (figure 3). Donor 1 CMV CTL recognized B cell blasts (BB) infected with vaccinia encoding pp65 as well as BB from a partially matched donor sharing HLA A 0201 (Figure 3a). Donor 1 CMV CTL recognized the pp65 peptide pulsed tumor cell line T98G (sharing HLA A0201 with this donor), demonstrating the ability of a GBM cell line to present CMV antigens. We also tested the capacity of these CTL to lyse CMV infected T98G, and found that there was enhanced cytotoxicity when the tumor cell was treated with IFN- γ or DAC, which is presented in Figure 3b. Cytofluorometric analysis of this GBM tumor cell line following exposure to DAC and IFN- γ showed increased expression of MHC class I and II antigens. IFN- γ treatment had a greater effect on MHC Class II expression on this tumor cell line.

CMV CTL study in GBM patients. With these data and having developed a technique for expansion of CMV pp65/IE1 CTL, we began a clinical trial of allogeneic CMV CTL in patients with refractory, CMV positive GBM. We screened 16 subjects for this study of whom 7 were positive for CMV antigens. Due to the nature of their relapsed disease, the majority of eligible subjects progressed prior to the treatment phase of the study. One patient was treated with HLA identical CMV pp65/IE1 CTL following a regimen of fludarabine and cyclophosphamide, the latter 2 drugs to induce donor CTL chimerism. This patient had rapid progression of her tumor after study entry and died one month later due to brainstem involvement with her tumor. Due to her immunocompromised state, there was no evidence of CMV specific immune reconstitution. Due to the fact that the majority of these subjects were requiring corticosteroids for tumor control and were rapidly progressing, we closed the study.

Our efforts have resulted in two publications which unfortunately didn't acknowledge the Tobacco CURE funding:

Lucas KG, Bao L, Bruggeman R, Dunham K, Specht C.; "The detection of CMV pp65 and IE1 in glioblastoma multiforme"; Journal of Neuro-Oncology; 2010 Sept

Lei Bao, MD, PhD, Kimberly Dunham, Kenneth Lucas, MD; MAGE-A1, MAGE-A3, and NY-ESO-1 can be upregulated on neuroblastoma cells to facilitate cytotoxic T lymphocyte-mediated tumor cell killing; Cancer Immunology and Immunotherapy; 2011 Sept

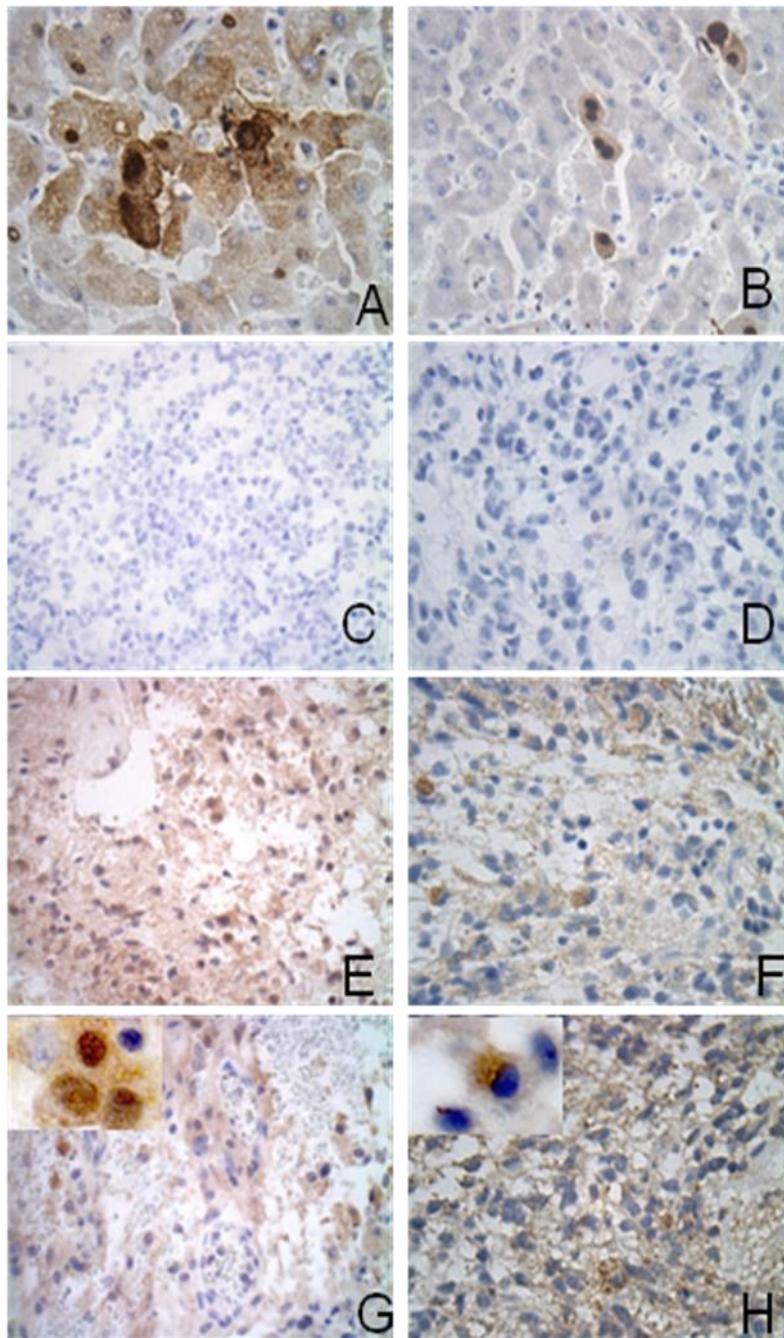


Figure 1. Immunohistochemical detection of CMV pp65 and IE-1 in glioblastomas (GBM) and control tissues. CMV infected liver stained with (A) pp65 and (B) IE-1; GBM stained with (C) pp65 and (D) IE-1 isotype control antibodies; (E, and G) GBM 1 and 2 stained with pp65; (F and H) GBM tumors 1 and 2 stained with IE1 .

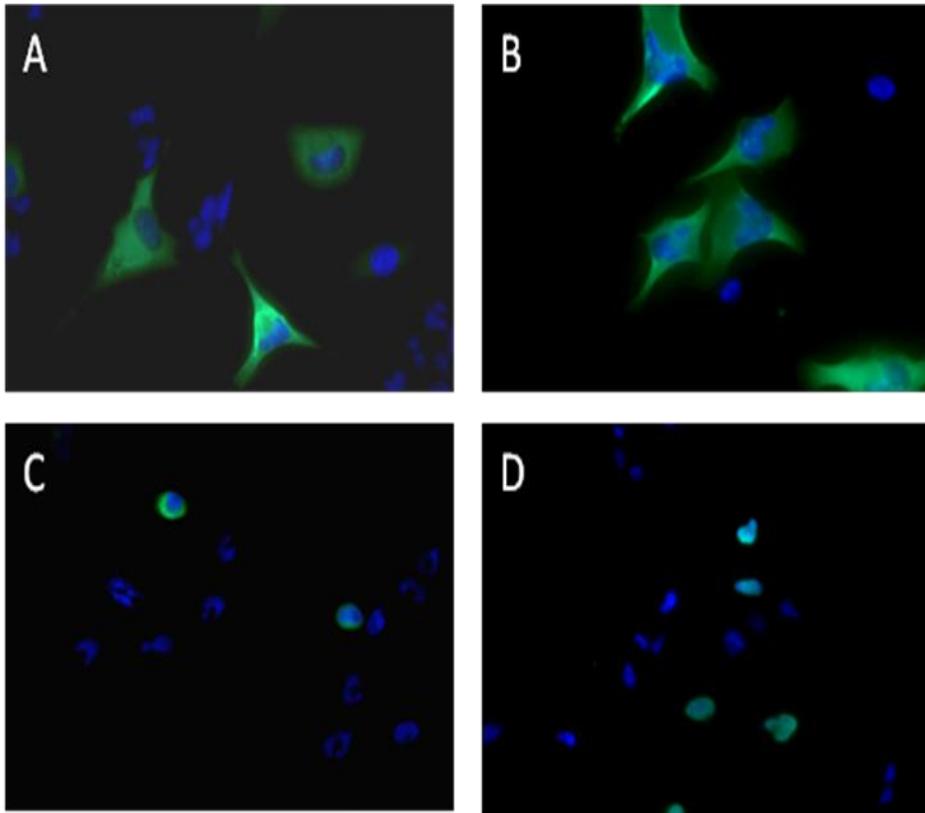


Figure 2. Immunofluorescence analysis of pp65 and IE-1 in CMV infected glioblastoma (GBM) cell lines. Nuclei are counterstained with a blue fluorescent dye. GBM cell lines (A) T98G and (B) U251 stained with an anti-pp65 monoclonal antibody, showing cytoplasmic localization of pp65 (green). Cell lines T98G (C) and U251 (D) were also stained with a murine anti-IE-1 (green), which showed some peri-nuclear localization.

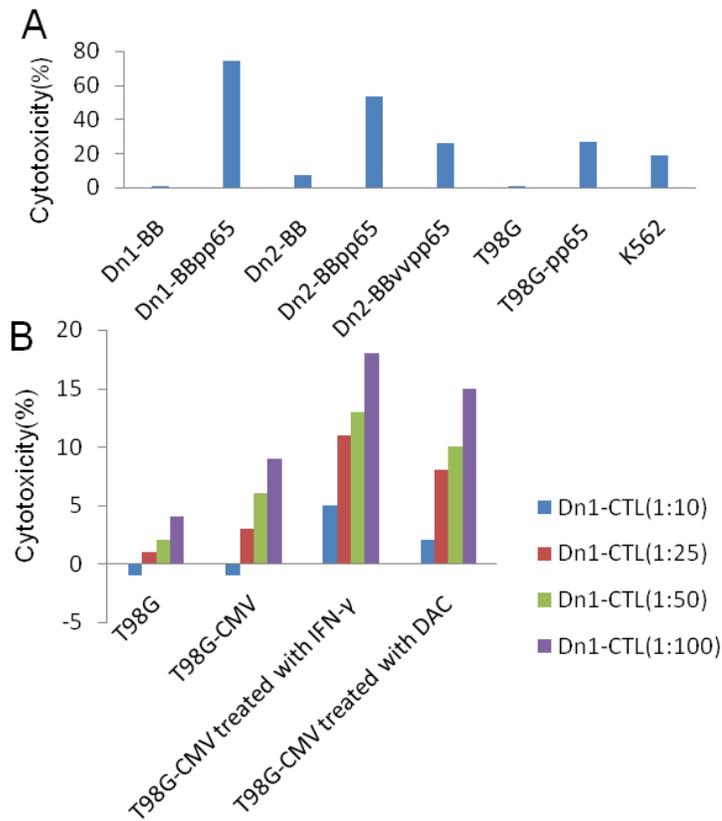


Figure 3 A: Cytotoxicity data from ^{51}Cr release assay: CMV pp65 specific CTL from a healthy donor 1(HLA-0201)(Dn1) against autologous B blasts(BB) pulsed with pp65 PepMix, an allogeneic (HLA 0201)(Dn2) BB pulsed with pp65, as well as a glioblastoma cell line (T98G, also HLA 0201) pulsed with pp65 peptides at target to effector ratio of 1:25. **B:** T98G cells infected with CMV and tested in ^{51}Cr release assays at different target to responder (CTL) ratios (1:10, 1:25, 1:50, 1:100). T98G cells infected with CMV were treated with IFN- γ (100U/ml) or 5-Aza-2'-deoxycytidine (DAC)(1uM/ml) for 3 days. CMV pp65 specific CTL from HLA-0201 positive Dn1 were able to kill CMV infected glioblastoma cells, and the cytotoxicity was increased by treating these cells with IFN- γ and DAC.

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
 No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

Yes
 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?

 2 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?

 10 Number of subjects originally targeted to be included in the study
 1 Number of subjects enrolled in the study

Note: We opened the clinical study and screened 16 subjects of whom 7 were determined to be eligible (positive for CMV pp65). Unfortunately the majority of these subjects rapidly progressed or were on corticosteroid doses that would not make this treatment regimen feasible. One subject received CMV CTL on study but her disease rapidly progressed and she died one month after receiving these cells. This experience led us to conclude that the proposed regimen is not suitable for this patient population due to the time it takes to identify a cell donor and grow the cells, and the fact that the majority of patients who are referred to a study of this type with GBM have rapidly progressive disease. The study was closed early.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
 Males
 1 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.)

Dauphin County- Penn State Hershey Medical Center

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
 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. None				<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes _____ No X _____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.

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

None

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

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes _____ 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 Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Kenneth G. Lucas, MD	POSITION TITLE Professor of Pediatrics and Microbiology / Immunology		
eRA COMMONS USER NAME (credential, e.g., agency login) kglucas			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
Siena College, Loudonville, New York	BS	5/1985	Biology
SUNY Health Science Center, Syracuse, NY	MD	6/1989	Medicine
Children's Hospital of Pittsburgh, PA		6/1992	Residency, Pediatrics

Positions and Honors:

- 1992-1995 Fellow, Pediatric Hematology/Oncology, Memorial Sloan Kettering Cancer Center, New York, NY.
- 1995-1997 Assistant Professor, Pediatric Hematology/Oncology Stem Cell Transplantation, Indiana University Medical Center, Indianapolis, IN.
- 11/1997-2000 Assistant Professor, Division of Hematology/Oncology, Department of Microbiology; Associate Scientist, Comprehensive Cancer Center, University of Alabama at Birmingham.
- 2000-9/2003 Associate Professor of Medicine/Pediatrics, and Microbiology, University of Alabama at Birmingham
- 9/2003-5/2008 Associate Professor of Pediatrics, Microbiology and Immunology, Penn State Hershey Medical Center
- 6/08- Professor of Pediatrics, Microbiology and Immunology, Penn State Hershey Medical Center

Peer-Reviewed publications or manuscripts in press

1. **Lucas KG**, Small TN, Dupont B, O'Reilly RJ: The development of EBV-specific cellular immunity following allogeneic bone marrow transplantation. Blood 1996; 87:2594-2603.
2. **Lucas KG**, Emanuel DJ, Cornetta K, Zimmerman S, Lee CH. Semi-quantitative EBV PCR for the determination of patients at risk for EBV induced lymphoproliferative disease following stem cell transplantation. Blood 1998; 91:3654-3661.
3. Sun Q, Pollok KE, Burton RL, Dai LJ, Britt W, Emanuel DJ, **Lucas KG**. Simultaneous ex vivo expansion of cytomegalovirus and Epstein-Barr virus specific cytotoxic T-lymphocytes using B lymphoblastoid cell lines expressing CMV pp65. Blood 1999; 94:3242-3250
4. **Lucas KG**, Burton RL, Pollok KE, Emanuel DJ. CD4+, Epstein Barr Virus specific cytotoxic T lymphocytes from human umbilical cord blood. Cellular Immunology 1999; 195:81-88.
5. Sun Q, Burton RL, Dai LJ, Britt WJ, **Lucas KG**. B-lymphoblastoid cell lines as efficient antigen-presenting cells to elicit CD8+ T cell responses to a cytomegalovirus antigen. Journal of Immunology 2000; 165:4105-4111.

6. Sun, Q., Burton, R.L. and **Lucas KG**. Cytokine production and cytolytic mechanism of CD4+ cytotoxic T lymphocytes in ex vivo expanded therapeutic EBV-specific T cell cultures. Blood 2002; 99:3302-3309.
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