Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** Trustees of the University of Pennsylvania

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

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Gearline Robinson-Hall, BSF

4. **Grant Contact Person's Telephone Number:** 215-746-6821

5. **Grant SAP Number:** 4100054874

6. **Project Number and Title of Research Project:** 9- Personalized Cancer Vaccines and Somatic Tumor Mutations

7. **Start and End Date of Research Project:** 7/1/2011 -12/31/2014

8. **Name of Principal Investigator for the Research Project:** George Coukos, MD, PhD

9. **Research Project Expenses**.

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

   $400,238.06

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc..of the project (x% Yr 1; z% Yr 2-3

Last Name, First Name	Position Title	% of Effort on Project	Cost
Coukos, George	Clinical Associate	1%Yr2-4; 2%Yr3	6323.96
Robert, Annalisa	Research Specialist	100%Y1-2	63,306.40

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

Last Name, First Name	Position Title	% of Effort on Project
Zhang, Lin	Research Associate	4%Yr.1; 6%Yr2-3

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 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_____ No____X_____

If yes, please describe your plans:

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

The project has been transferred to Switzerland, where I moved recently.

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 agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Personalized Cancer Vaccines and Somatic Tumor Mutations - The purpose of this project is to understand whether personalized cancer vaccines can be based on the identification of somatic mutations on individual tumors.

In the last two decades, intense clinical experimentation has maximized the benefits of chemotherapy for most solid tumors. However, cancer mortality remains considerably high, especially in adults, creating a pressing need for additional therapies.

Cancer immunotherapy has made significant advances in the last two decades, with three randomized, multi-center, double-blind, controlled phase III clinical trials reporting positive results this year in follicular non-Hodgkin's lymphoma, melanoma and prostate cancer. However, additional optimization is needed. One limitation of currently used monovalent vaccines targeting a single cancer protein is that they can select for immune escape tumor variants. Whole tumor antigen vaccines have consistently outperformed single-target immunotherapy in early phase clinical trials, but they carry an overwhelming amount of "self" antigens, which could dampen immunogenicity, or vice versa, markedly increase the risk of autoimmunity, if immune therapy becomes potent enough with the advent of immune modulation. Developing polyvalent tumor vaccines comprised uniquely of cancer-specific proteins should thus maximize cancer vaccine efficacy *and* safety. However, the specific tumor-rejection antigens remain to date unknown for most solid tumors.

Recent deep sequencing analyses have revealed that solid tumors harbor numerous somatic mutations, most of which differ among tumor specimens even within the same tumor type. We hypothesize that somatic missense point mutations acquired by tumor cells result in the expression of aberrant 'non-self' protein products, which are tumor rejection antigens. We further hypothesize that effective therapeutic immunization with whole tumor antigen vaccines is mediated in part through a potent response against these unique, mutated proteins. We proposed the following aims:

Aim 1. Identify and validate somatic missense point mutations in advanced ovarian cancer using next generation high throughput sequencing.

The subset of the mutations that alters the protein coding sequence creates neoantigens that may elicit cytotoxic T-cell reactivity. From a therapeutic prospective, the definition of the tumor associated epitopes on a patient-specific basis will provide the basis for enhancing T-cell targeting antigen therapy.

In this study we analyzed the tumor samples of 11 patients with recurrent high-grade ovarian carcinoma that were previously enrolled in a dendritic cell (DC)-based autologous whole tumor vaccination and achieved antitumor immune response and clinical benefits. DNA and RNA samples were harvested from tissue specimens and Next Generation Sequencing (NGS) was used to define the patient-specific set of potential tumor neo-antigens that could have specifically stimulated the antitumor T-cell immunity. We performed exome capture and paired-end sequencing using HiSeq2000 Illumina platform. As the definition of antigens, we selected for non-synonymous mutations that result in the expression of non-germ line protein sequences. In order to discriminate between germ line and tumor-specific mutations we performed whole-

exome sequencing on tumor DNA (same DNA used for vaccine) and from matched blood for each patient. Consistent with published results of others, we annotated 838 (~77 per tumors) somatic mutations in 690 protein coding genes. Although, we identified some recurrently mutated genes, the overall analysis confirmed the high heterogeneity in the ovarian cancer mutational spectrum as each patient has a unique set of tumor-specific aberrations. (Figure 1)

From an immunological point of view, mutations in genes that are not expressed cannot lead to the formation of neo-antigens. Thus, we combine the transcriptome profiling (RNA-Sequencing) with the whole-exome sequencing analysis to exclusively focus on the expressed protein coding mutations. We found that of the 838 somatic mutations in coding genes, 460 occured in expressed genes. The remaining 378 arose in silent or low expressed genes, which mutations cannot be considered as potential neo antigens (Figure 1). RNA sequencing data were furthermore used to analize the expression level of the mutated genes. As a confirmation, we found that the 460 Somatic Expressed Mutations (SEM) occurred in actively transcribed genes.

Although it has already been confirmed by two independent experiments (RNA sequencing and tumor exome sequencing), we decided to further validate the SEMs by targeted resequencing before being screened for T cell recognition. We generated a multiplexed panel of 384 amplicons specifically targeting each SEMs. Primers were specifically generated to spanning the exact chromosome position annotated with Illumina sequencing platform. In the primers design we did not included the SNPs that have been already described in Single Nucleotide Polymorphism Database (dbSNP). In order to confirm the somatic origin of the SEMs, for each patient we analyzed both tumor and blood DNA. Sequencing was performed on a PGM sequencer (Ion Torrent). Torrent Suite software (Ion Torrent) was used to align reads to the reference genome, and to generate run metrics and the variants were identified with Variant Caller software (Ion Torrent). An average of 70% of the mutations were validated by Ion Torrent platform, almost all of them were confirmed to be tumor-specific, only 9 on 471 mutations resulted to be germ line as the mutations were annotated in the matching blood sample. (Figure 2)

By altering the protein coding sequence the somatic expressed mutations might generate patient-specific neo antigens. We are currently performing high-resolution HLA genotyping for each patient by using a newly developed next-generation sequencing technology. By providing a deeply HLA typing for Class I and II alleles, this new technology will improve the reliability of the *in silico* prediction of peptide-MHC binding affinity.

Discrepancies between DNA and RNA sequences are likely due to RNA editing mechanisms. Identifying true editing sites is still affected by bioinformatics bias due to the difficulty in properly mapping RNA-Seq reads. We developed a new bioinformatics approach that strongly reduces false-discovery rate. By comparing exome- and RNA-sequencing data we annotated 406 potential editing sites among the 11 samples. Although still under validation, we are refining the lists of RNA somatic editing sites in ovarian cancer expressed genes.

In contrast with the high heterogeneity found in the DNA somatic mutations, alterations in RNA

editing seem to be a shared mutational mechanism in ovarian cancer. Currently, we are investigating the oncogenic function of two new ovarian cancer correlated editing mutations.We have found that both hypo- and hyper-RNA editing events occur in ovarian cancer patients and

we have demonstrated that these alterations are shared among samples (Figure 3). Presently, we are investigating the possible clinical correlation between the editing events and ovarian cancer. We are also studying the biological function of these alterations in order to understand if they might confer or enhance tumorigenicity *in vitro*.

RNA "editing" mutated proteins can also act as tumor-associated antigens and this represents an additional level of complexity in tumor biology and a completely new prospective in cancer immunotherapy.

Aim 2:
After we identified and validated the spectrum of the somatic expressed mutations specific for each of the ovarian cancer patients, we tested the immunogenicity of the potential tumor neo-antigens that could have stimulated the antitumor T-cell immunity in the 11 patients under investigation.

1) Identification of potential neo-epitopes based on HLA Peptide Binding Predictions algorithms.
By using the NetMHC algorith, we derived a list of candidate epitopes (nonamer and decamer), predicted to bind with high affinity (<500nM) to the HLA-I alleles for each patient. An average of 3 to 7 potential neo-epitopes was predicted for each mutation in each patient. Based on the prediction model a total of 263 HLA-matched peptides were synthesized (with a median number of 26 peptides per patient), in order to cover the whole spectrum of predicted mutations among the patients analyzed.

2) Confirmation of peptides HLAs binding.
Although, the accuracy of *in silico* T-cell epitope prediction continuously improves, this toll is exclusively based on bioinformatic algorithms without biochemical experimental evidence. In order to validate the binding of a large set of predicted peptides to their cognate class I HLA alleles we used a recent cell-free assay developed by TcMetrix. This method allows evaluating the ability of a peptide to drive the refolding of HLA class I molecules and to assess the stability of HLA-peptide complexes in physiological condition. In brief, microtiter plates coated with HLA-peptide complexes are washed with acidic buffer to remove the original peptide and β2-microglobulin (β2m) and immediately incubated with biotinylated β2m and peptides of interest.

After staining with PE-streptavidin, effective peptide binding is determined by reading PE fluorescence. Furthermore, the kinetic of the signal duration (*i.e.* decay rate) reflects the stability of HLA-peptide complexes. Of interest, using this functional assay, more than 90% of *in silico* prediction was confirmed by the effective binding of peptide to HLA molecules, additionally this experiment also provided a specific assessment for the peptide-HLA complexes stability.

3)Immuno-assays to screen for the presence of neoantigen-specific T cells in PBMC from vaccinated patients .
Currently, we have performed functional cellular assays to screen T cells for their reactivity against mutated epitopes. Two major experimental settings are pursued in parallel. Unlike viral antigens, the unambiguous *ex vivo* detection of TAA-specific T cells in peripheral blood is well known to be challenging due to the very low frequency of circulating cells. We have been developing strategies to overcome this limitation by specifically expanding cells in short-term *in*

vitro stimulation assay (IVS), in order to increase the detection sensitivity.

Initial set-up experiments were performed using cryopreserved PBMC from healthy donors with known reactivity against immunodominant viral epitopes derived from cytomegalovirus (CMV), Epstein-Barr virus (EBV) and influenza (Flu) tested individually or pooled (CEF peptide pool). In addition, HLA-A*0201 healthy donors or melanoma patients with known reactivity against Flu ($M1_{58–66}$ GILGFVFTL), EBV ($BMLF1_{280-288}$ GLCTLVAML), CMV ($pp65_{495-503}$ NLVPMVATV) or the TAA Melan-A_{26-35} (ELAGIGILTV) epitope, respectively, were also analyzed. Preliminary data indicated that, as hypothesized, *in vitro* T-cell expansion significantly increased (up to 10-fold) the frequency of antigen-specific T-cell responses, thus improving detection sensitivity.

The experimental set-up was then further validated in HLA-A2 patients with a set of HLA-A2-restricted peptides from known TAA. Using the above mentioned set-up method, total PBMCs from the 11 ovarian cancer patients are currently being tested for their specific reactivity against the 263 HLA-matched mutated peptides that were predicted with the *in silico algorithm.*

An alternative strategy is currently also under investigation. In this approach, T-cell stimulation is not provided by exogenous peptides, but rather by autologous DC (*i.e.* professional antigen-presenting cells, APC) transfected with mutated mRNA encoding for the predicted SNPs; this allows the generation of multiple epitopes from individual or several proteins. The rationale of this alternative strategy is the possibility to bypass epitope prediction based on HLA haplotypes.

Aim 3. Extend the approach for the prediction and validation of patient-specific tumor antigens to the entire cohort of patients vaccinated with autologous whole-tumor-cell vaccine.

We demonstrated that immunization with whole tumor antigen in ovarian cancer patients is mediated in part through a potent response against specific somatic missense mutations.
As a consequence of the intertumour heterogeneity (genetic diversity among the same tumor type), we confirmed that $CD8^+$ T cells are able to recognize one or more neoepitopes from mutant proteins unique to that specific ovarian cancer patient.

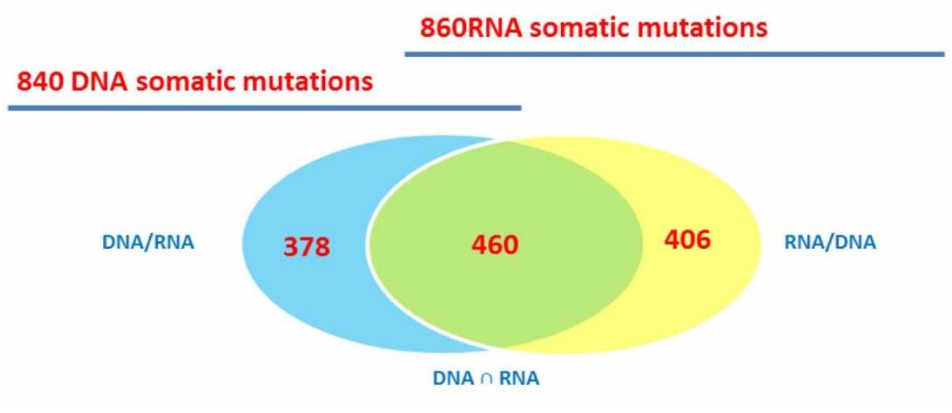
These results emphasize the need for personalized strategies for cancer immunotherapy that are compatible with mutational heterogeneity and the requirement of polyvalent tumor vaccines comprised uniquely of cancer-specific proteins in order to maximize cancer vaccine efficacy. After we validated the efficacy of our approach for discovering, prediction and validation of tumor neoantigens derived from individual tumors we decided to expand the cohort of patients analyzed and to correlate T cell immunoreactivity (pre and post vaccination) against somatic missense mutations with clinical response.

Following the same experimental approach as was used for the first set of samples, we analyzed 15 additional advance ovarian cancer patients that were previously enrolled in the whole tumor antigen clinical trial. We identified mutated candidate epitopes through sequential application of whole exome sequencing followed by HLA-binding prediction and screen of T-cell reactivity against mutant peptides.

In order to map all the expressed somatic mutations, we combined whole exome sequencing of matching PBMC and tumor DNA with gene expression data from tumor RNA (RNA-sequencing). Our analysis confirmed the high degree of heterogeneity in the spectrum of ovarian cancer somatic mutations. As well documented, somatic mutations in p53 gene were found to be the most recurrent in our cohort, although none of theme were predicted to be T-cell epitope candidates. BRAC1 and BRAC2 genes have been described as recurrently mutated in ovarian cancer; however, mutations in these genes are mainly germ line and consequently removed in our approach for antigen discovery.

We are currently performing high-resolution HLA genotyping for each patient by using a newly developed next-generation sequencing technology. We will then follow the experimental approach, as described in Aim 2, for prediction and validation of mutated neo-antigens.
By using a major histocompatibility complex-binding algorithm, we will derive candidate peptide epitopes encoded by nonsynonymous mutations in tumor-expressed genes predicted to bind with high affinity (<500nM) to the HLA-I alleles for each patient.

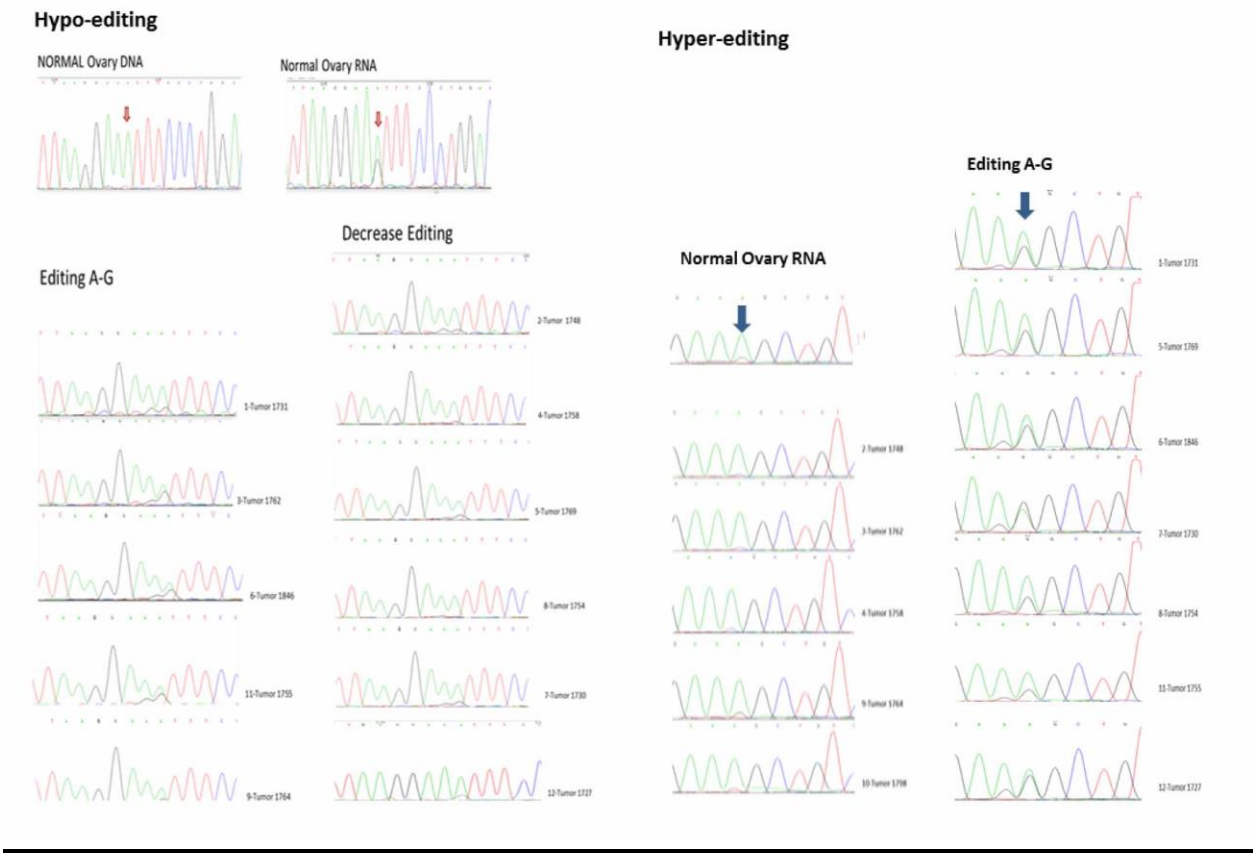
<u>Figure 1:</u> Schematic representation of exome sequencing and RNA-sequencing result (Illumina platform).



<u>Figure 2:</u> Results of targeted resequencing (Ion Torrent) of Somatic Expressed Mutations.

Patient		Mapped Reads	Illumina Variants	dbSNPs	Amplicons	Ion Torren Variants	Not Validated	Germ line	%
1		328,127	35	3	32	26	6	0	81.25
2		385,484	43	10	33	25	8	2	75.75757576
3		405,04	59	7	52	31	21	1	59.61538462
4		377,567	63	6	57	42	15	0	73.68421053
5		358,751	9	2	7	5	2	0	71.42857143
6		316,88	47	14	33	25	8	3	75.75757576
7		303,575	20	7	12	8	4	0	66.66666667
8		498,08	62	9	53	40	13	0	75.47169811
9		408,65	29	4	24	13	11	1	54.16666667
10		309,67	37	7	30	20	10	0	66.66666667
11		389,567	59	8	51	35	16	2	70

Figure 3: Hypo and hyper RNA-editing in ovarian cancer.



18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

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

_____Yes
___X__No

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

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

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

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?

_____Yes

___X__No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

_____Yes

_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20.Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

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

Yes_____X____ No_____

If yes, please describe your plans:

We are in the process of submitting the publication

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

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

NAME

George Coukos, MD, PhD

POSITION TITLE

Associate Professor, Division of Gynecologic Oncology

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

University of Modena, Modena, Italy MD 1979-1987 Medicine
University of Patras, Patras, Greece PhD 1986-1990 Reproductive Biology

A. POSITIONS AND HONORS

2000 - 2004 Assistant Professor, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Pennsylvania
2000 - present Director, Gynecologic Oncology Research, CRRWH, University of Pennsylvania
2000 - 2004 Director of Gynecology Service, Department of Surgery, Veteran's Administration Hospital, Philadelphia
2003 - present Leader, Gynecologic Cancer Research, Abramson Family Cancer Research Institute,University of Pennsylvania
2004 - 2005 Cèlso Ramon Garcia Assistant Professor, Division of Gynecologic Oncology, University of Pennsylvania
2006 - present Cèlso Ramon Garcia Associate Professor, Division of Gynecologic Oncology, University of Pennsylvania
2007 - present Associate Chief, Division of Gynecologic Oncology, Dept of Obstetrics & Gynecology, University of Pennsylvania
2007 - present Director, Ovarian Cancer Research Center, University of Pennsylvania

Honors / Awards

2000 - ABOG Scholar, American Association of Obstetricians and Gynecologists Foundations ;
2001 - Bristol- Myers Immunology-Oncology Award, Gynecologic Cancer Foundation;
2002 - Translational Science Award, Sidney Kimmel Foundation;
2003 - Schwid Award, Gynecologic Cancer Foundation;
2003 - Liz Tiberis Award, Ovarian Cancer Research Fund;
2003 - Mary Kay Ash Foundation Award;
2004 - Celso Ramon Garcia Chair in Reproductive Biology;
2005 - Greenfield Award for "Excellence in Ovarian Cancer Research", Gynecologic Cancer Foundation;
2006 - Judah Folkman Award, Alliance for Cancer Gene Therapy;
2006 - Sir William Osler Award for Excellence in Patient Oriented Research, University of Pennsylvania;
2007 - Philadelphia Crystal Ball Award for Research Excellence in Ovarian Cancer;
2007-2008 - Sidney-Kimmel Scholar Award;
2008 - New Jersey National Ovarian Cancer Coalition, Teal Magnolias

B. Selected peer-reviewed publications (from 148 peer-review articles and book chapters)

G. Coukos, A. Makrigiannakis, S. Montas, L.R. Kaiser, I. Benjamin, S.M. Albelda, S.C. Rubin, K.L. Molnar-Kimber. Multi-attenuated herpes simplex virus-1 mutant G207 exerts cytotoxicity against epithelial ovarian cancer but not normal mesothelium, and is suitable for intraperitoneal oncolytic therapy. *Cancer Gene Ther,* 7:275-283, 2000.

E. Y. Woo, C.S. Chu, T.J. Goletz, K. Schlienger, H. Yeh, **G. Coukos,** S. C. Rubin, L.R. Kaiser, C.H. June. Regulatory CD4+ CD25+ T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res*, 61:4766-4772, 2001

L. Zhang, N. Yang, J-R. Conejo-Garcia, A. Mohamed-Hadley, F. Benencia, S.C. Rubin, D. Allman, **G. Coukos.** Generation of a Syngeneic Mouse Model to Study the Effects of Vascular Endothelial Growth Factor in Ovarian Carcinoma. *Am. J. Pathol.,* 161:2295-2309, 2002.

K. Schlienger., C.S. Chu, E.Y. Woo, P. Rivers, A..J. Toll, B. Hudson, M.V. Maus, J.L. Riley, Y. Choi, **G. Coukos,** L.R. Kaiser, S. C. Rubin, C.H. June. TRANCE and CD40L-matured dendritic cells reveal MHC Class-I restricted T cells specific for autologous tumor in late-stage ovarian cancer patients. *Clin. Cancer Res.*, 9:1517-1527, 2003.

G. Coukos, M.C. Courreges, F. Benencia. Virus-based Intraperitoneal Oncolytic and In Situ Tumor Vaccination Therapy: the HSV Paradigm *Curr. Gene Ther.*, 3:113-125, 2003.

L. Zhang, J.R. Conejo-Garcia, D. Katsaros, P. Gimotty, M. Massobrio, G. Regnani, A. Makrigiannakis, K. Schlienger, H. Gray, M. Liebman, S.C. Rubin, **G. Coukos**. Intratumoral T cells, recurrence and survival in epithelial ovarian cancer. *New Engl. J. Med*., 348:201-211, 2003.

L. Zhang, N. Yang, A. Mohamed-Hadley, S.C. Rubin, **G. Coukos.** Vector-based RNAi, a Novel Tool for Isoform-specific Knock-down of VEGF and Anti-angiogenesis Gene Therapy of Cancer. *Biophys.Biochem. Res. Commun*., 303:1169-1178, 2003.

L. Zhang, N. Yang, J.W. Park, D. Katsaros, S. Fracchioli, A.. O'Brien-Jenkins, T.C. Randall, J.R. Conejo-Garcia, S.C. Rubin, **G. Coukos**. Tumor-derived VEGF Upregulates Angiopoietin-2 in Host Endothelium and Destabilizes Host Vacsulature, Supporting Angiogenesis in Ovarian Cancer. *Cancer Res.* 15:3403-3412, 2003.

J.R. Conejo-Garcia, F. Benencia, M.C. Courreges, E. Khang, L. Zhang, A. Mohamed-Hadley, J. Vinocur, R.J. Buckanovich, C.B. Thompson, B. Levine, **G. Coukos**. LETAL, a tumor-associated NKG2D immunoreceptor ligand, induces costimulatory expansion of immune effector cells. *Cancer Biol. Ther*. 2:446-451, 2003.

J.R. Conejo-Garcia, F. Benencia, M.C. Courreges, E. Khang, L. Zhang, A. Mohamed-Hadley, J. Vinocur, R.J.Buckanovich, C.B. Thompson, B. Levine, **G. Coukos**. Ovarian cancer expresses the NKG2D ligand LETAL and promotes the survival and intratumoral expansion of anti-tumor cytotoxic T cells. *Cancer Res*.64:2175-2182, 2004.

J.R. Conejo-Garcia, F. Benencia, M.C. Courreges, E. Kang, A. Mohamed-Hadley, R.J. Buckanovich, D. Holtz, A. Jenkins, H. Na, L. Zhang, D.S. Wagner, D. Katsaros, R. Caroll, **G. Coukos**. Tumor-infiltrating dendritic cell precursors recruited by beta-defensin contribute to vasculogenesis under the influence of VEGF. *Nature Med*., 10:950-958, 2004.

T. J. Curiel, **G. Coukos**, L. Zou, X. Alvarez, (11 authors), L. Myers, A. Lackner, M.L. Disis, K. Knutson, L. Chen, W. Zou. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nature Med.,* 10:942-949, 2004.
J. R. Conejo-Garcia, R.J. Buckanovich, F. Benencia, M.C. Courreges, R.G. Carroll, **G. Coukos**. Vascular Leukocytes Contribute to Ovarian Tumor Vascularization. *Blood*, 105:679-681, 2004.
M.T. Lotze, E. Wang, F. M. Marincola, N. Hanna, P.J. Bugelski, C.A. Burns, **G. Coukos,** (10 authors), J.N. Weinstein, T. L. Whiteside. Workshop on Cancer Biometrics: Identifying Biomarkers and Surrogates of Cancer in Patients: A Meeting Held at the Masur Auditorium, National Institutes of Health. *J. Immunother*., 28:79-119, 2005.
G. Coukos, F. Benencia, R. Buckanovich, J.R. Conejo-Garcia. The role of dendritic cell precursors in tumour vasculogenesis. *Br. J. Cancer*. 92:1182-1187, 2005.
F. Benencia, M.C. Courréges, J.R. Conejo-García, R.J. Buckanovich, L. Zhang, R.G. Carroll, M.A. Morgan, **G.Coukos**. Oncolytic HSV Exerts Direct Antiangiogenic Activity in Ovarian Carcinoma. *Human Gen Ther.* 16:765-778, 2005.
G. Coukos, J.R. Conejo-Garcia, T.C. Wu. Immunotherapy for Gynecologic Malignancies. *Exp. Op. Biol. Ther.*5:1193-1210, 2005.
F. Benencia, **G. Coukos**. T regulatory cell depletion can boost DC-based vaccines. *Cancer Biol. Ther.* 4:628-630, 2005.
F. Benencia, M.C. Courrèges, J.R. Conejo-García, N. Fraser**, G. Coukos**. Dendritic Cell-derived Interferoninducible Chemokines Induce Tumor Infiltration by Immune Effector Cells Following HSV Oncolytic Therapy. *Molec. Ther*. 13:182–193, 2005.
M.C. Courrèges, F. Benencia, J.R. Conejo-García, L. Zhang, **G. Coukos**. Preparation of apoptotic tumor cells with replication-incompetent HSV augments the efficacy of dendritic cell vaccines. *Cancer Gene Ther*.13:182–193, 2006.
R.J. Buckanovich, D. Sasaroli, A. O'Brien-Jenkins, J. Botbyl, J.R. Conejo-Garcia, F. Benencia, L.A. Liotta,P.A. Gimotty, **G. Coukos.** Use of Immuno-LCM to Identify the in Situ Expression Profile of Cellular Constituents of the Tumor Microenvironment. *Cancer Biol Ther*, 5(6):635-642, 2006.
R. Savani, G. Cao, M. Fehrenbach, C. Lyons, L. Zhang, **G. Coukos**, H.M. DeLisser. Involvement of endothelial CD44 during in vivo angiogenesis. *Am J Pathol*, 69(1):325-336, 2006.
F. Benencia, M.C. Courrèges, J.R. Conejo-García, A. Mohamed-Hadley, **G. Coukos**. Direct vaccination with tumor cells killed by replication-incompetent HSV*d*120 elicits effective antitumor immunity. *Cancer Biol Ther*, 5(7):867-874, 2006.
V. Espina, J.D. Wulfkuhle, V.S. Calvert, A. VanMeter, W. Zhou, **G. Coukos**, D.H. Geho, E.F. Petricoin, L.A. Liotta. Laser-capture microdissection. *Nature Protocols*, [published on line] 1: 586-603, 2006.
D.H. Sterman, C.T. Gillespie, R.G. Carroll, C.M. Coughlin, E.M. Lord, J. Sun, A. Haas, A. Recio, L.R. Kaiser, **G. Coukos,** C.H. June, S.M. Albelda, R.H. Vonderheide Interferon beta adenoviral gene therapy in a patient with ovarian cancer. *Nature Pract Clin Oncol*, 3(11):633-639, 2006.
C.F. Hung, Y.C. Tsai, L. He, **G. Coukos**, I. Fodor, H. Levitsky, T.C. Wu. Vaccinia virus preferentially infects and controls established human and murine ovarian tumors in mice. *Gene Ther* 14(1):20-29, 2007.

R.J. Buckanovich, D. Sasaroli, J. Botbyl, F. Benencia, A. O'Brien-Jenkins, G. Danet-Desnoyers, D. Katsaros, L.A. Liotta, P.A. Gimotty, **G. Coukos**. Tumor vasculature proteins as biomarkers in ovarian cancer. *J Clin Oncol*, 1:25(7):852-861, 2007.
B.J. Czerniecki, G.K. Koski, U. Koldovsky, S. Xu, R. Mick, H. Nisenbaum, T. Pasha, K.R. Fox, S. Weinstein, S.G. Orel, R. Vonderheide, **G. Coukos**, A. DeMichele, M. Xu, L. Araujo, F.R. Spitz, M. Rosen, P.A. Cohen, B. Levine, C. June, P. Zhang. Induction of Lymphocytic Infiltrates, Humoral Immunity, and Tumor Immunoediting in Women with Early Breast Cancer Following HER-2/neu Pulsed-Dendritic Cell Vaccines. *Cancer Res*, 67(4):1842-1852, 2007.
G. Coukos, J.R. Conejo-Garcia, R. Buckanovich, F. Benencia. Vascular leukocytes: a population with angiogenic and immunossuppressive properties highly represented in ovarian cancer. *Adv Exp Med Biol*. 590:185-193, 2007.
M.D. Atkins, D. Carbone, **G. Coukos**, M. Dhodapkar, M.S. Ernstoff, J. Finke, T.F. Gajewski, J. Gollob, M.T. Lotze, W. Storkus, L.M. Weiner. Report on the ISBTC mini-symposium on biologic effects of targeted therapeutics**.** *J Immunother.* 30(6):577-590, 2007.
G. Coukos. Monocytes TIE(2)d up in Murky Business. *Blood*, 109:5076, 2007. (Comment on Venneri et al, 109:5276-5285).
I. Kryczek, S. Wei, G. Zhu, L. Myers, P. Mottram, P. Cheng, L. Chen, **G. Coukos**, W. Zou. Relationship between B7-H4, Regulatory T Cells, and Patient Outcome in Human Ovarian Carcinoma. *Cancer Res*.67(18):8900-8905, 2007.
P.A. Gimotty, L. Zhang, I. Alagkiozidis, S. Kim, M. Cadungog, S. Adams, C.S. Chu, D. Katsaros, **G. Coukos**. Immune Prognostic Factors in Ovarian Cancer: Lessons from Translational Research**.** *Disease Markers*. 23(5-6):445-452, 2007
R.J. Buckanovich, A. Facciabene, S. Kim, F. Benencia, D. Sasaroli , K. Balint, D. Katsaros, A. O'Brien-Jenkins, P.A. Gimotty, **G. Coukos**. Endothelin (B) Receptor Mediates the Endothelial Barrier to T-cell Homing to Tumors and Disables Tumor Immune Therapy. *Nature Med*, 14:28-36, 2007.
R.T. Krafty, P.A. Gimotty, D.O. Holtz, G. Coukos, W. Guo. Varying-Coefficient Model with Unknown Within-Subject Covariance for Analysis of Tumor Growth Curves. Biostatistics, 00:1-9, 2007.
C.S. Chu, S.H. Kim, C.H. June, **G. Coukos**. Immunotherapy Opportunities in Ovarian Cancer. *Expert Rev Anticancer Ther*, 8(2):243-257, 2008.
D.O. Holtz, R.T. Krafty, L. Zhang, I. Alagkiozidis, B. Leiby, W. Guo, P.A. Gimotty, **G. Coukos**. Should Tumor VEGF Expression Influence Decisions on Combining Metronomic Chemotherapy with Antiangiogenic Therapy? Preclinical Modeling in Ovarian Cancer. *J Transl Med*, 6(2):1-10, 2008. PMCID: PMC2235830
F. Benencia, M.C. Courreges, **G. Coukos**. Whole tumor antigen vaccination using dendritic cells: Comparison of RNA electroporation and pulsing with UV-irradiated tumor cells. *J Transl Med,* 6(1):21, 2008.PMCID: PMC2408561
F. Benencia, M.C. Courrèges, N.W. Fraser, **G. Coukos**. Herpes Virus Oncolytic Therapy Reverses Tumor Immune Dysfunction and Facilitates Tumor Antigen Presentation. *Cancer Biol Ther,* 7(8), 2008.
S. Wei, T. Curiel, **G. Coukos,** R. Liu, W. Zou. Inhibitory B7 family members in human ovarian carcinoma. *Adv Exp Med Biol.* 622;261-271, 2008.

B.G. Barnett, J. Ruter, I. Kryczek, M.J. Brumlik, P.J. Cheng, B.J. Daniel, **G. Coukos**, W. Zou, T.J. Curiel. Regulatory T cells: a new frontier in cancer immunotherapy. *Adv Exp Med Biol*. 622:255-60, 2008.
K. Balint, J.R. Conejo-Garcia, R. Buckanovich, **G. Coukos**. Role of vascular leukocytes in ovarian cancer neovascularization. *Adv Exp Med Biol*. 622; 273-280, 2008.
F. Benencia, **G. Coukos**. Biological therapy with oncolytic herpesvirus. *Adv Exp Med Biol*., 622:221-233, 2008.
L. Zhang, N. Yang, **G. Coukos**. MicroRNA in human cancer: one step forward in diagnosis and treatment. *Adv Exp Med Biol*. 622:69-78, 2008.
R.G. Carroll, C. Carpenito, X. Shan, G. Danet-Desnoyers, R. Liu, S. Jiang, S.M. Albelda, T. Golovina **G. Coukos**, J.L. Riley, Z.L. Jonak, C.H. June. Distint effects of IL-18 on the engraftment and function of human effector CD8 T cells and regulatory T cells. *PLoS ONE*. 3(9):e3289, 2008.
M.M. Markiewski, R.A. DeAngelis, F. Benencia, S. K. Ricklin-Lichsteiner, A. Koutoulaki, C. Gerard, **G. Coukos**, J.D. Lambris. Modulation of the antitumor immune response by complement. *Nature Immunology*, 9 (11):1225-1235, 2008.
B. Clarke, A.V. Tinker, C.H. Lee, S. Subramanian, M. van de Rijn, D. Turbin, S. Kalloger, G. Han, K. Ceballos, M.G. Cadungog, D.G. Huntsman, **G. Coukos**, C.B. Gilks. Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. *Mod Pathol*., Dec. 5 2008 [Epub ahead of print] Mar; 22(3):393-402, 2009.
L.H. Butterfield, M.L. Disis, B.A. Fox, P.P. Lee, S.N. Khleif, M. Thurin, G. Trinchieri, E. Wang, J. Wigginton, D. Chaussabel, **G. Coukos**, M. Dhodapkar, L. Hakansson, S. Janetzki, T.O. Kleen, J.M. Kirkwood, C.Maccalli, H. Maecker, M. Maio, A. Malyguine, G. Mascucci, A.K. Palucka, D.M. Potter, A. Ribas, L. Rivoltini, D. Schendel, B. Seliger, S. Selvan, C.L. Slingluff, D. F. Stroncek, H. Streicher, X. Wu, B.Zeskind, Y. Zhao, M.B. Zocca, h. Zwierzina, F.M. Marincola. A systematic approach to biomarker discovery; preamble to “the iSBTc-FDA taskforce on immunotherapy biomarkers.” *J Transl Med*. Dec 23;6:81, 2008. PMCID: PMC2630944.
J. Ruter, B.G. Barnett, I. Kryczek, M.J. Brumlik, B.J. Daniel, **G. Coukos**, W. Zou, T.J. Curiel. Altering regulatory T cell function in cancer immunotherapy: a novel means to boost the efficacy of cancer vaccines. *Front Biosci*. (1)14: 1761-1770, 2009.
N. A. Christian, F. Benencia, M.C. Milone, G. Li, P.R. Frail, M.J. Therien, **G. Coukos**, D.A. Hammer. In Vivo Dendritic Cell Tracking Using Fluorescence Lifetime Imaging and Near-Infrared-Emissive Polymersomes. *Mol Imaging Biol*., Feb. 5 [Epub ahead of print] 2009.

C. Active Research Support

NIH - R01-CA116779 09/27/06 - 07/31/11 PI: George Coukos
Vascular Leukocytes Induce Treg in Tumors

NCI - P50-CA083638 9/01/03 – 6/30/09
SPORE PI: Ozols; Proj# 5 PI: George Coukos
Immunotherapy of Ovarian Cancer: taking down the barriers

Alliance for Cancer Gene Therapy 10/01/06 - 09/30/09 PI: George Coukos

Development of T-body Immune-gene Antivascular Therapy for Cancer

FDA - R01 FD003520-01 9/20/08 – 7/31/12 PI: George Coukos
Phase I/II Randomized Trial of Adoptive Lymphocyte Transfer in Ovarian Cancer

Ovarian Cancer Research Fdn 1/1/09 – 12/31/11 PI: George Coukos
Engineering T Cells to Augment Natural Immunity for Ovarian Cancer

Completed Research
DOD - OC-050314 12/1/05 – 12/31/08 PI: George Coukos
Targeting a Novel and Critical Vasculogenesis Pathway in Ovarian Cancer

OCRF - Liz Tiberis Award 01/01/07 - 12/31/08 PI: George Coukos
Tumor-host Interaction in Ovarian Carcinoma

NIH P50-CA083638 09/01/06 – 08/31/08
PI: Robert Ozols, Pilot Projetc PI: George Coukos
Development of Antibodies for Early Detection of Ovarian Cancer

NCI R01-*CA098951-05* *09/19/03 - 06/30/08* PI: George Coukos
Endothelial Regulation of T cell Homing in Ovarian Carcinoma