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:** Magee-Womens Research Institute and Foundation

2. **Reporting Period (start and end date of grant award period):** 1/01/2009-12/31/2009

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Cheryl Richards, MBA

4. **Grant Contact Person's Telephone Number:** 412-641-8932

5. **Grant ME Number or SAP Number:** 4100047639

6. **Project Number and Title of Research Project:** Project 8 -Immunity to MUC1 Tumor Antigen in Conditional and Transplantable in vivo Models for Ovarian Cancer

7. **Start and End Date of Research Project:** 01/01/2009-12/31/02009

8. **Name of Principal Investigator for the Research Project:** Anda M. Vlad, MD PhD

9. **Research Project Expenses**.

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

   $ $253,654.17

   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
Vlad	Principal Investigator	50%	$46,020.76
Budiu	Post-doctoral Associate	100%	$49,863.78
Brozick	Research Associate	50%	$32,237.52

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

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

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____x_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____x_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Disease Heterogeneity and Immune Biomarkers in Preclinical Mouse Models of Ovarian Carcinogenesis	☐NIH ☒ Other federal (specify: Department of Defense (DoD) ☐ Nonfederal source (specify: _____)	05/2009	$839,080- (direct + indirect costs)	Under negotiation
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

The DoD grant application was submitted in May 2009. Several preliminary results derived by research performed under this grant demonstrated our ability to 1. perform survival surgery and model ovarian carcinogenesis in mice; 2. measure tumor-specific immunity in mice with conditional ovarian tumors. Taken together, this data provided solid support for our grant application and likely contributed to its success.

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

Yes____x_____ No_____

If yes, please describe your plans:

The PI intends to use the results obtained from this granting period for one NIH R01 application, to be submitted in October 2010.

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

Future studies will focus on:
1. Exploring new therapies, including novel vaccines and/or immune biologics, in the conditional mice described here.
2. Translate into the clinic those therapies rendered effective in our preclinical studies.

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____x_____ No_____

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

The impact of this funding opportunity was twofold:
1. It allowed the PI, to perform experiments that provide the basis for grant applications at governmental and non- governmental funding agencies. One of these applications (to the Department of Defense) was approved for funding and will bring indirect costs to MWRI.

2. It provided salary support for one flow cytometrist, who in addition to contributing to the successful completion of this proposal, is also providing the technical support for the flow cytometry core at MWRI. This core facility is used by an increasing number of MWRI researchers, with faster and more satisfying results.

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:

This grant made possible the initiation of fruitful collaborations with clinicians (oncology surgeons, pathologists etc) who provide surgical care for women with ovarian cancer in the UPMC medical system and with basic scientists from the Immunology Department.

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.

Project overview. Despite progress in therapy, ovarian cancer continues to be associated with high mortality rate, and is the most lethal gynecologic cancers. The lesions are thought to originate in the ovarian surface epithelial (OSE) cells, although the exact mechanisms of disease pathogenesis are not well understood. The OSE in humans express low levels of MUC1 glycoprotein; in contrast, the epithelial tumors derived from them overexpress a modified form of MUC1. Because the immune system of ovarian cancer patients can recognize MUC1 when expressed by tumors, MUC1 has been considered a tumor-associated antigen and shows promise as a cancer vaccine candidate. Furthermore, MUC1 is an oncoprotein with important roles in transformation, migration and metastasis. However, changes in MUC1 biology and immunogenicity during natural progression to ovarian cancer have not been addressed and a better understanding of MUC1 roles requires adequate in vivo animal models. By combining mouse genetics and survival surgery in mice, we proposed the following specific aims:

Aim 1. To generate and characterize a conditional, triple transgenic mouse model for MUC1-expressing endometrioid ovarian cancer.

Aim 2. To generate and characterize a syngeneic, transplantable ovarian tumor model expressing MUC1.

Aim 3. To assess MUC1 expression and immunogenicity changes in mice either progressing to ovarian cancer or with transplantable IP tumors.

Results**.** Our specific aims set forth in the original grant application have been completed and details of our research achievements are described below.

Aim 1. To generate and characterize a conditional, triple transgenic mouse model for MUC1-expressing ovarian endometrioid ovarian cancer.

We have completed this aim and are reporting below the genotypic and phenotypic characteristics of the newly obtained triple transgenic (Tg) $MUC1^{+/-}Kras^{G12D/+}Pten^{loxP/loxP}$. In order to obtain triple Tg mice, we implemented complex mouse breeding protocols, using previously generated as well as newly derived, single and double transgenic mice. The breeding regimen we implemented (Fig. 1) allowed us to first generate novel double Tg $MUC1^{+/-}Pten^{loxP/loxP}$ mice (MUC1Pten, Schedule A-Fig.1), after going through several intermediate steps. The newly obtained MUC1Pten mice were next crossed with the previously described KrasPten mice (1) (generously provided to us by Dr Dinulescu and maintained as breeding colonies in our animal facility- Schedule B, Fig. 1). All litters were screened for the presence of the 3 transgenes. Selection of pups heterozygous for the MUC1 and KrasG12D transgenes and homozygous for floxed Pten (triple Tg MUC1KrasPten mice), was performed by PCR, using tail-extracted DNA, as detailed in our application. A typical PCR screening results for detection of MUC1 and engineered loxP $Kras^{G12D/+}$ and $Pten^{loxP/loxP}$ is shown in Fig. 2**.** The profile of one $Pten^{loxP/loxP}$ homozygous mouse is shown in lane 1; one wild type is shown in lane 3 and one heterozygous ($Pten^{loxP/+}$) is shown in lane 2. In order for tumors to occur, the Kras oncomutation (G to D at position 12) and the Pten deletion need to be activated in the OSE. This “activation” occurs via Cre-loxP recombination, with twofold consequences: 1. it removes the Stop codon before the Kras promoter, thus rendering the KrasG12D oncomutation active; 2. it removes the phosphatase domain in the Pten gene, thus triggering homozygous Pten deficiency. In order to deliver Cre recombinase the ovaries, we

used replication-defective adenoviral vectors (AdCre), administered under the ovarian bursa, during survival surgery, as described by us and others (1, 2).

Briefly, we used "synchronized" 8 week old female mice, injected with 5U of pregnant mare serum gonadotropin (PMSG) and 2 days later with 5U of human chorionic gonadotropin (hCG) in order to ensure ovulation. Forty-eight hours later (time point corresponding to increased proliferation of epithelial cells) the mice were injected with Ad-Cre. We injected 2.5×10^7 plaque forming units (p.f.u.) of AdCre/mouse in the bursa of only one of the ovaries (keeping the contra-lateral as negative control). The injection of 5 μl AdCre/mouse was performed according to a previously described calcium phosphate precipitate (0.86 mM phosphate and 12mM calcium) method. All surgeries were completed in 4 mouse groups, as originally proposed: 2 experimental groups (MUC1KrasPten triple Tg and KrasPten double Tg mice) and 2 control groups (MUC1Pten and MUC1 single Tg mice) ten mice per group.

The double Tg KrasPten mice were previously demonstrated to progress to ovarian tumors after intrabursal AdCre injection (1). Once we obtained the breeding pairs, we set up large scale breeding colonies and female double Tg KrasPten mice were readily identified and used for survival surgery and AdCre injections in the ovarian bursa. Our results from the first half of our granting period demonstrate that we were able to successfully reproduce the phenotype previously reported (1) and to induce ovarian carcinogenesis in KrasPten mice (Fig. 3) after intrabursal AdCre injection. The tumors developed in 100% of the injected mice, after an average of 11 weeks post-disease induction. Necropsy inspections showed that the tumors were widely spread in the peritoneal cavity, often present on the upper abdomen and on the diaphragm and were frequently accompanied by hemorrhagic ascites. Tumorigenesis occurred in the injected ovary only and not in the control (contra-lateral) ovary (Fig. 3). Overall, these results demonstrated our ability to target AdCre to the OSE, consequently turning on the Kras oncogenic mutation and inducing Pten deletion in the infected cells, ultimately triggering ovarian tumor formation.

Having established the versatility of the double Tg model, we next proceeded with tumor induction in the triple Tg mice, which we obtained in the second six-month funding period. Similarly to experiments in double Tg mice, we used the newly obtained triple Tg mice for survival surgery and AdCre injections, using identical tumor-induction protocols. All injected animals developed tumors, confirming the 100% disease penetrance seen in double Tg mice, (1). We sacrificed the diseased mice and harvested at necropsy the ovarian as well as other abdominal tumors, diaphragm, lungs, liver, pancreas and kidneys. All tissues were formalin-fixed and embedded in paraffin. Four micron sections were subjected to immunohistochemistry (IHC) staining. All tumor sections from triple and double (control) Tg mice were subjected to IHC staining for the following markers: MUC1, cytokeratin 7, estrogen receptor (ER), pErk, pMEK, snail, WT1. We also harvested ascites fluid, plasma, regional (para-aortic) lymph nodes and spleen and used them for immune assays (flow cytometry and ELISA), as described in aim 3. Taken together, our aim 1 studies revealed several phenotypic similarities but also differences between the double and triple Tg tumors:

Similarities:

1. The tumors in both double and triple Tg mice were accompanied by hemorrhagic ascites and numerous metastases in the peritoneal cavity and on the diaphragm (Fig. 3 and 4), mimicking the biology of late stage human tumors.

2. The hematoxyllin-eosin (HE) staining showed similar histology in the triple and double Tg tumors (Fig.5). However, as expected, MUC1 was present in the triple Tg tumors only (Fig. 5). The pathology analysis of tumors was performed in collaboration with Esther Elishaev, MD a pathologist with extensive expertise in gynecologic tumors. Interestingly, the tumors from both double and triple Tg mice showed papillary formations, some of which displayed clear cell-like morphology. The potentially mixed endometrioid-clear cell histology will be further addressed by our collaborator pathologists and may provide a previously unreported trait of this genetic mouse model.

3. Both double and triple tumors were cytokeratin 7 + and showed increased pMEK and pERK by IHC, consistent with the enhanced signaling via the Kras and Akt pathways in these genetically engineered mice (not shown).

Differences:

1. The triple Tg mice developed tumors with a higher kinetics (average of 37 in triple Tg versus 77 days in double Tg mice, $p<0.05$), suggestive of a more aggressive phenotype in mice carrying the transgene encoding for the human MUC1-associated antigen, a known oncoprotein involved in cell transformation (3-7).

2. MUC1 is a heterodimeric protein made of an elongated and highly glycosylated extracellular domain (MUC1-ED), and a short cytosolic tail (MUC1-CT) with intracellular signaling properties. The human MUC1-ED is involved in adhesion and metastasis and shares little homology with its less studied mouse counterpart. The MUC1-CT however, is highly conserved among species and plays important roles in transformation and anti-apoptotic pathways.

Because in the MUC1 Tg mice used here MUC1 is driven by the endogenous mouse mucin 1 promoter, both the double and triple Tg mice are expected to express normal levels of endogenous mucin 1 (adnotated Muc1, to differentiate it from its human MUC1 counterpart). However, since human MUC1 protein is only expressed in the triple Tg mice, carrying the MUC1-encoding transgene, human MUC1 protein is expressed in triple Tg mice only (Fig 6). We also confirmed that the anatomical distribution in healthy triple Tg mice is consistent with the one seen in the single Tg MUC1 mice and with the one seen in humans (Fig 6**)**. In the female genital tract MUC1 is expressed in a polarized manner on the uterine lining and in endometrial glands, facing the lumen. MUC1 is also discretely expressed on the human ovarian surface epithelium (OSE, the originating site for most ovarian epithelial tumors) and is overexpressed in all types of ovarian carcinomas (not shown). The MUC1 staining pattern we observed in tumor-bearing triple Tg mice shows increased MUC1 expression in the OSE of injected ovaries and marked overexpression in tumors, consistent with the profile seen in

human tumors. The human-like MUC1 expression pattern provides further support for this mouse tumor model and its relevance to the human disease and further endorses its suitability for preclinical studies.

We also explored the murine Muc1 expression pattern in both healthy and tumor-bearing ovaries from both triple and double Tg mice. Unlike MUC1, an intensely studied protein, Muc1 biology and immunogenicity have not been described. Based on the expression pattern we observed for human MUC1 (showing its presence in healthy OSE and marked overexpression in ovarian tumors), we hypothesized that murine Muc1 will behave similarly to MUC1 during tumor progression and will be abundantly present on late stage ovarian tumors. To test this, we performed IHC on tumor sections from both double and triple transgenic mice, using an antibody that detects the cytosolic tail of murine 1 molecule (clone CT2, Fig. 7). However, since the murine 1 cytosolic tail (CT) is highly conserved among species, the CT2 antibody is cross-reactive to both human and mouse murine 1. Interestingly, our staining results show low/no mouse Muc1 in double Tg ovarian surface epithelium (OSE, Fig. 7C) and double Tg ovarian tumors (Fig. 7E). However, CT2 staining is positive in endometrial glands (Fig 7A, B), demonstrating the ability of CT2 antibody to properly identify positive cells. Furthermore, unlike the double Tg tumors, the triple Tg ovarian tumors were CT2 positive, given the presence of the MUC1-associated CT epitopes (Fig. 7F).

Several studies have previously explored the MUC1 roles in tumor formation and demonstrated MUC1 is an oncogene (7-9). Consistent with these, our findings show increased MUC1 but not Muc1 expression in ovarian tumors and an accelerated tumor progression (and thus a more aggressive tumor phenotype) in triple but not double Tg tumors and suggest that MUC1 may drive the more aggressive ovarian tumor phenotype seen in the triple Tg mice. We note, however, that while MUC1 may indeed alter tumor biology and may influence its invasive potential, the tumor formation is not dependent upon MUC1 presence, since the double Tg (non-MUC1 expressing but Muc1 positive) mice also developed ovarian tumors (albeit with a delayed kinetics) and the disease penetrance is 100% in both mouse genotypes. Nevertheless in light of our findings, we postulate that MUC1 may play an important role in ovarian tumor pathogenesis and may influence OSE transformation, and cell migration. In human cancers, MUC1-CT blocks apoptosis and induces transformation (4, 7, 10). In contrast to the human mucin 1, the murine molecule (Muc1) has been little studied and no involvement of Muc1-CT in mouse cellular transformation has been so far reported. In the light of our results showing that Muc1-CT Is not detectable in the OSE and is only present in triple Tg ovarian tumors, we conclude that the human and mouse mucin 1 have different in vivo biology, with different consequences on OSE cell transformation and tumor pathogenesis. Furthermore, we postulate that these differences are likely due to the fact that the extracellular domains of human and murine mucin 1 are engaged in different interactions in vivo and bind to different ligands, with different consequences on intracellular signaling via CT. Our finding that triple Tg tumors develop much faster and are more aggressive is now prompting the question whether and how MUC1 influences the tumor phenotype and what are quantitatively and qualitatively the differences in intracellular signaling mechanisms via MUC1-CT versus Muc1-CT. The mouse models presented here are extremely useful to address these questions and to identify MUC1-sepcific roles in ovarian cancer and create the

platform for future studies aimed at identification of intracellular mechanisms triggered by MUC1. Uncovering these mechanisms will not only advance our basic understanding of ovarian carcinogenesis but will also potentially unveil new therapeutic targets and disease biomarkers.

In summary, we successfully completed aim 1 and succeeded in our attempt to generate and characterize a triple transgenic, human MUC1 antigen-expressing ovarian cancer mouse model. These mice progress to tumors with a human-like biology and express the human MUC1 tumor-associated antigen, mimicking even closer the human disease. The mice reported in these studies will greatly advance our capacity to address in the future important questions related to ovarian tumor pathogenesis and to monitor preclinical testing of novel MUC1-based immune therapeutics.

Aim 2. To generate and characterize a syngeneic, transplantable, MUC1-expressing ovarian tumor model.

We have also successfully completed this aim. Unlike the in vivo model characterized in aim 1, using mice that progress to ovarian (orthotopic) tumors, mimicking the phenotype seen with primary disease, developing in the ovaries, the transplantable tumors, using IP injected ovarian cancer lines represent the prototype of recurrent ovarian cancer, mostly involving the peritoneal cavity.

In order to complete this aim, we worked with primary cells derived from the ovarian surface epithelium (OSE) of MUC1Kras mice via gentle trypsinization. The cultured cells were subsequently passaged multiple times until they became transformed, in accordance to results similarly reported by Roby et al (11). The transformation was assessed by the ability of cells to lose the contact inhibition of cell growth and by changes in their morphology (from typical cobblestone to more irregular shape). The novel cell line (which we named MKOSE) expressed increasing MUC1 levels. Although the MKOSE line shows the characteristic of an immortalized line, pre-selection based on agar growth is a necessary step in order to identify clones with increased in vivo tumorigenesis potential. We performed a limiting dilution in agar plates and selected cells from one fast agar-growing colony (MKOSE clone B). The agar colony consisting of monoclonal cells was further expanded in vitro, until adequate numbers were obtained. The monoclonal MKOSE cells were then injected in 5 healthy MUC1Kras female mice (syngeneic recipients). We injected 5 x 10^6 cells/200 μl cDMEM medium, in the peritoneal cavity. The mice were monitored for tumor growth detection for 24 weeks. No tumors were observed during this time period, suggesting that further selection of tumorigenic clones is needed. However, to circumvent this problem and in the interest of time, we transfected another syngeneic cell line (IG10) (11) with a MUC1-expressing plasmid. IG10 cells have been previously reported to form IP tumors in immune competent, syngeneic mice, 8-12 weeks post IP injection. The MUC1-encoding plasmid used for transfection (pcDNA-MUC1) encodes for 22 tandem-repeat MUC1 (12), driven by the CMV promoter. The electroporated IG10 cells were rested in culture media (cDMEM-10), overnight. Twenty-four hours later, the selection agent (G418 1mg/ml) was added to the culture media. The growing cells were later stained for MUC1 expression using a FITC-labeled anti-MUC1 antibody (clone HMPV) and MUC1 positive cells sorted with a

FACSArea sorter. The newly obtained IG10-MUC1 cells expressed high MUC1 levels (Fig. 8)**,** in line with levels seen in human ovarian cancer lines, and were able to generate MUC1+ tumors in vivo. Numerous tumors were visible throughout the peritoneal cavity, ranging in size from 1 mm to 5-6 mm. The tumors were accompanied by hemorrhagic ascites, a profile similar to the one observed in some of the conditional mice with tumors (Fig 9). However, unlike the tumors developing in the double and triple Tg mice the transplantable IG10-MUC1 tumors have undefined histology (Fig 9 E and F). Importantly, however, the tumors maintained MUC1 expression in vivo (Fig. 9F) and are adequate models for studies on MUC1 specific immunity in transplantable ovarian tumors. We also acknowledge that the IP transplantable model lacks the versatility and superiority of the triple Tg inducible model in which tumors, in addition to being MUC1 positive also have defined endometrioid/clear cell histology.

In summary, despite several limitations, the model developed in aim 2, based on IP injection of IG10-MUC1 is the only one currently available for MUC1 studies in immune competent mice with transplantable ovarian tumors expressing a human tumor antigen and therefore constitutes a resourceful tool for future preclinical studies on natural and vaccine-induced MUC1-specific immunity in ovarian cancer.

Aim 3. To assess MUC1 expression and immunogenicity changes in mice either progressing to ovarian cancer or with transplantable IP tumors.

This aim has also been completed and some of our most significant findings are summarized below.

After having established in aims 1 and 2 the two in vivo models for ovarian tumorigenesis (conditional primary and transplantable recurrent, respectively), we next analyzed the immune regulation in these mice. Specifically, we analyzed ovarian MUC1 expression in normal and tumor-bearing mice, MUC1-specific immunity and measured the presence of immune suppressive CD4+Foxp3+ regulatory T cells (Treg) in lymphoid organs of tumor-bearing mice and in control, healthy mice. We sacrificed the diseased mice (the tumor-bearing MUC1KrasPTEN mice and the IG10-MUC1 injected mice) and harvested at necropsy the tumors, ascites fluid, plasma spleen (SN) and regional, para-aortic (PLN) and inguinal lymph nodes (ILN). We used the tumor tissue sections for HE and IHC staining, plasma and ascites fluid for ELISA measurements of MUC1-specific antibodies and the SN and PLN for multicolor flow cytometry.

Our results from aim 1 showed that MUC1 is low on OSE and its expression increases considerably in tumor cells at the primary site and metastases (Figures 5 and 6 and data not shown). Based on this observed increased tumor MUC1 expression, we hypothesized that there will also be increased soluble (serum) MUC1 antigen levels and this circulating antigenic overload in the host will trigger MUC1-specific antibodies. However, despite detected increases in soluble serum MUC1 (also known as carcinoma antigen CA 15-3) in 50% of the tumor-bearing mice (several examples shown in Table 1**)** the antibody ELISA measurements showed that no detectable IgM or IgG MUC1-specific antibodies could be identified at any time before or after tumors occurred (Fig. 10).This finding is in contrast to

our results from ovarian cancer patients (not shown), many of whom have detectable levels of IgM and in some instances IgG antibodies at the time of primary debulking. The suppressed humoral responses in tumor bearing mice are suggestive of the fact that immune surveillance in tumor-bearing mice may be overwhelmed by the rapid progression to disease (likely more accelerated than in women) and by the increased tumor-associated immune suppression, which inhibits the naturally occurring humoral immunity to tumor antigens.

To address immune suppression in the host, we explored the extent of immune suppressive T cell (Treg) accumulation in the spleen and regional lymph nodes of tumor-bearing mice. Our results (Fig. 11) from multicolor flow cytometry using antibodies to CD3, CD4, CD8 and Foxp3 show a dramatic increase in Treg population in the secondary lymphoid organs (where 5-8% Treg are expected), similarly to reports from cancer patients (13). Similar increases were also noted in triple Tg tumor-bearing mice (not shown). Interestingly however, despite significant increases in Treg percentages in tumor bearing double and triple Tg mice over healthy controls, we could not detect any significant differences in Treg percentages in triple versus double Tg mice. Overall, these results suggest that although increased Treg percentages may be responsible for the lack of spontaneous humoral immunity against the MUC1 antigen, they are not responsible for the more aggressive phenotype seen in triple Tg mice. Our future studies will focus on the identification of MUC1-mediated mechanisms involved in the accelerated tumor progression seen in triple Tg mice.

In summary, we have performed experiments according to the proposed research design, successfully achieved all milestones and reported here several important findings from this project. The genetically engineered mice employed in our work are superior to the preclinical models currently available, mostly based on immune compromised mice challenged IP with human xenogeneic tumors or in immune competent mice with tumors of undefined histology. The novel triple Tg mice characterized here are immune competent, develop tumors with endometrioid histology, express the human MUC1 antigen and will be instrumental to future studies in several key research areas:

- MUC1 vaccine design and development;
- In vivo testing of new and improved Kras and/or Pten inhibitors;
- Identification of new MUC1 targets and their role in ovarian cancer pathogenesis;
- Identification of new ovarian cancer biomarkers.

Results from our work will be submitted for publication and will create the basis for a R01 grant submission to the NIH National Cancer Institute (NCI). Our success in working with these preclinical models of ovarian cancer has already been recognized in a recent successful application to the Department of Defense, Congressionally Directed Medical Research Programs.

Figures and Legends.

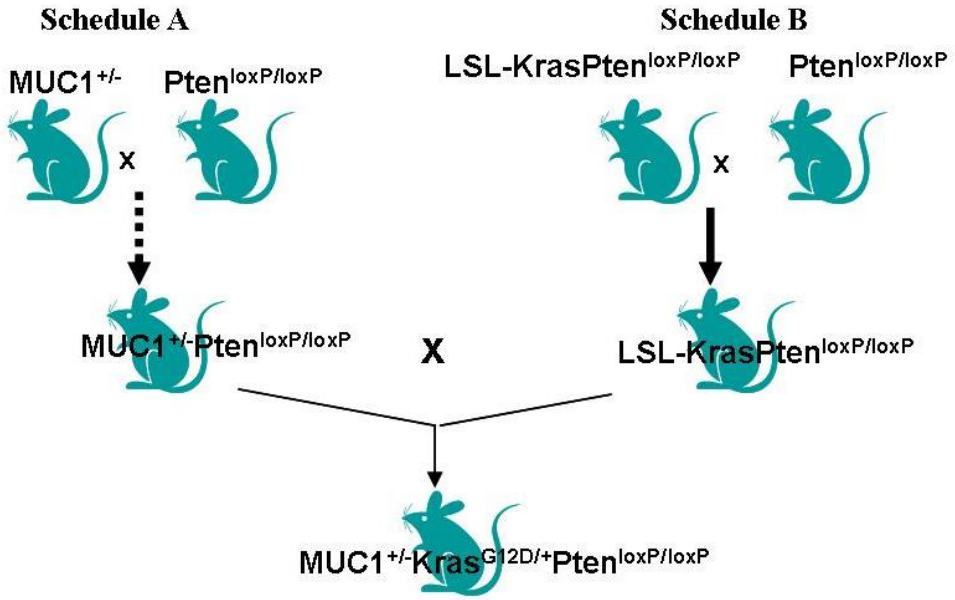

Fig. 1. Breeding diagram. The triple Tg mice were generated by crossing the MUC1$^{+/-}$Pten$^{loxP/loxP}$ mice (breeding schedule A) with the LSL-Kras$^{G12D/+}$Pten$^{loxP/loxp}$ (or briefly KrasPten) mice (schedule B). The MUC1$^{+/-}$Pten$^{loxP/loxP}$ mice were obtained after a series of intermediate breedings (Schedule A, dotted arrows), to ensure loxP/loxP homozygosity at the Pten locus. The triple Tg MUC1KrasPten female mice are identified via PCR and used for induction of MUC1-positive tumors. Breeding schedule B ensures continued generation of KrasPten mice which we used for breeding (males) as well for disease induction (females). Intrabursal AdCre injection in female KrasPten mice triggers formation of MUC1-negative ovarian tumors.

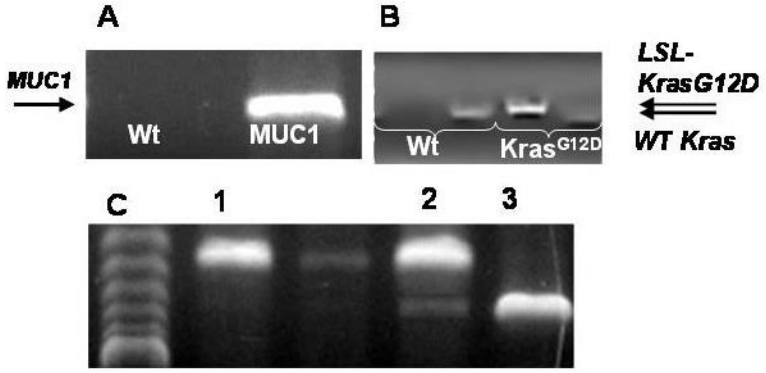

Fig. 2. PCR identification of MUC1 (A) loxP-engineered KrasG12D (B) and Pten$^{loxP/loxP}$ (C). The mice with a desired genotype are selected based on the heterozygous presence of the *MUC1* transgene (Fig. 2A, right lane), the mutant *LSL-Kras*$^{G12D/+}$ (Fig. 2B) and homozygous Pten (panel C). One Pten$^{loxP/loxP}$ homozygous mouse is shown in panel C, lane 1; one wild type is shown in lane 3 and one heterozygous (Pten $^{loxP/+}$) is shown in lane 2. (Abbreviations used: Wt=wild type; LSL=LoxP-Stop-LoxP).

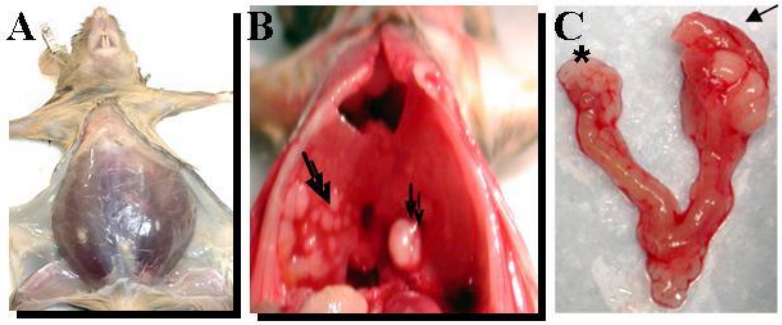

Fig. 3. Phenotype of double Tg (KrasPten) tumors injected in the ovarian bursa with Cre-encoding adenovirus, during survival surgery. The tumors developed in 100% of the injected mice, after an average of 11 weeks post-disease induction. Mice developed enlarged abdomens (A) and the necropsy inspections showed that the tumors were widely spread in the peritoneal cavity, often present on the upper abdomen and on the diaphragm (B, arrows) and were frequently accompanied by hemorrhagic ascites (A). Tumorigenesis occurred in the injected ovary only (C, arrow) and not in the control (contra-lateral) ovary (C, asterisk). One mouse, representative of more than 20 mice with tumors, is shown.

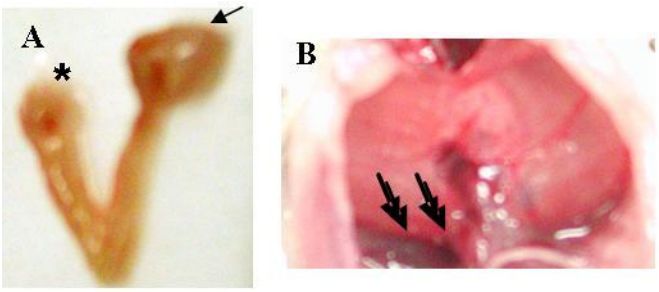

Fig. 4. Phenotype of triple Tg (MUC1KrasPten) tumors injected in the ovarian bursa with Cre-encoding adenovirus, during survival surgery. The tumors developed in 100% of the injected mice, after an average of 5 weeks post-disease induction. Tumorigenesis occurred in the injected ovary only (A, arrow) and not in the control, contra-lateral ovary (A, asterisk). The tumors were widely spread in the peritoneal cavity, often present on the upper abdomen and on the diaphragm (B, arrows) and were frequently accompanied by hemorrhagic ascites (not shown).

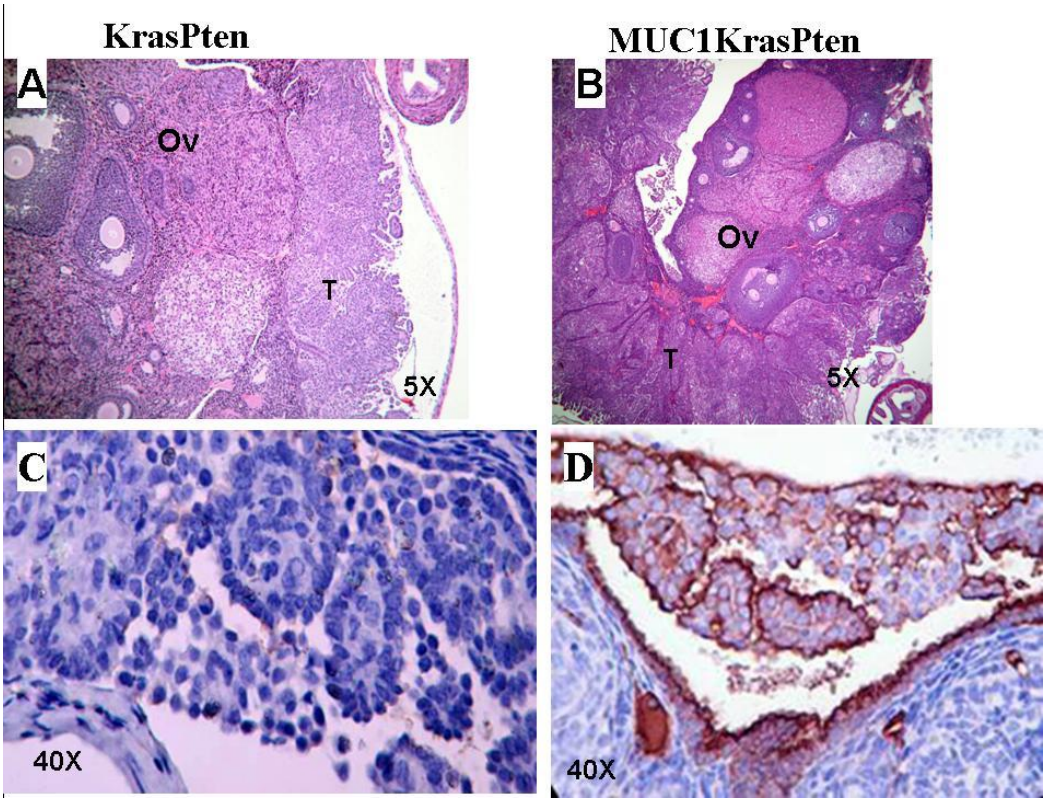

Fig. 5. The hematoxyllin-eosin (HE) staining showed similar histology in the double (A) and triple (B) transgenic tumors. (A and B: Ov-ovary; T-tumor). C and D: Immunohistochemistry (IHC) staining for MUC1 (clone HMPV) shows MUC1 presence in triple (D) but not double Tg tumors (C). All images were acquired with either 5x (A and B) or 40x (C and D) objective lens.

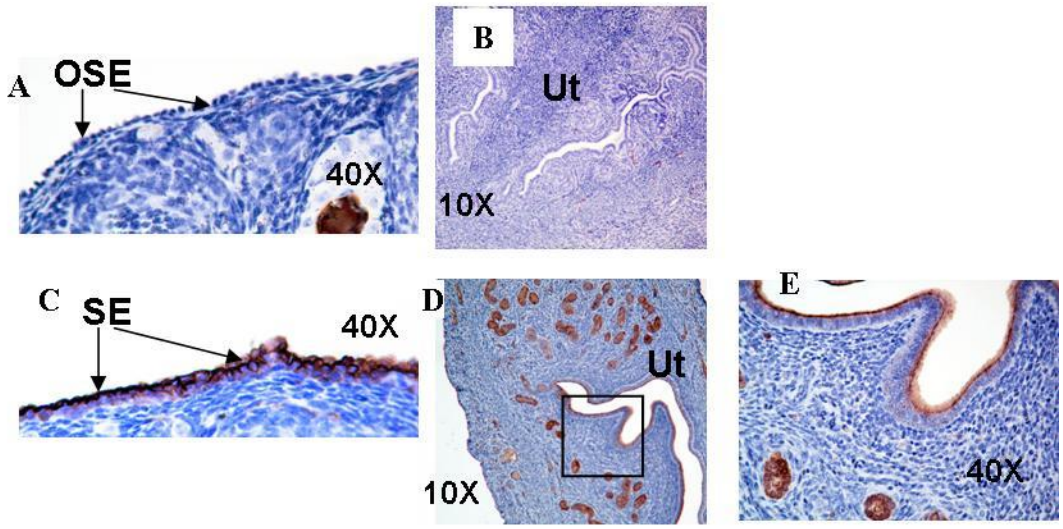

Fig. 6. Human MUC1expression detected by IHC in the normal ovaries (C) and uterus (D, E) of triple (C,D,and E) but not double transgenic mice (A,B). Staining was performed with a monoclonal antibody (clone HMPV) that specifically detects the extracellular domain of the human and not mouse mucin 1. As expected the triple Tg (lower panels) and not double Tg mice express the human MUC1 and the distribution is similar to the one seen in the single Tg MUC1 mice and reproduces the anatomical distribution seen in the genital tract of patients. Image in E is a magnification of the detail seen in D (box).

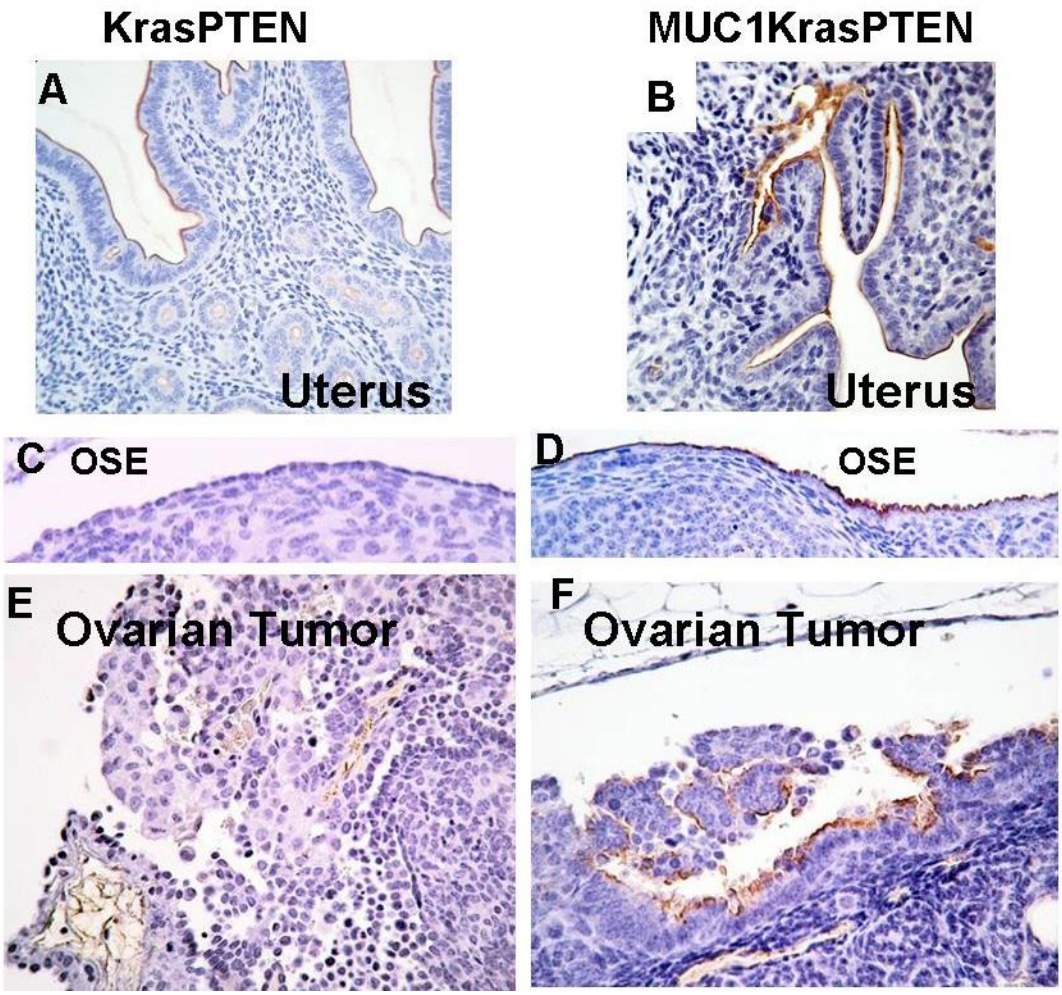

Fig.7. Detection of mucin 1-cytosolic tail (CT) in tissues from double (A,C,E) and triple (B,D,E) transgenic mice by IHC. Staining was performed with a monoclonal antibody (clone CT2) that detects MUC1-CT as well as Muc1-CT. Staining patterns seen in the uterus (A,B) ovarian surface epithelium (OSE, C,D) and ovarian tumors (E,F). In double Tg mice CT2 antibody detects Muc1-CT while in triple Tg mice CT2 detects both murine (Muc1-CT) and human (MUC1-CT) molecules. These results suggest that in double Tg mice (A, C, E), the endogenous (murine) mucin1 is present in the uterine glands (A), but absent from the OSE (C) and from the ovarian tumors (E). In contrast, when human MUC1 is present as a transgene (B, D, F), the CT2 antibody stains not only the uterine glands (B) but also the OSE (D) and the ovarian tumor (F). This staining pattern demonstrates differences in mouse and human mucin 1 biology in vivo and points to a (human) MUC1-specific role in ovarian tumorigenesis.

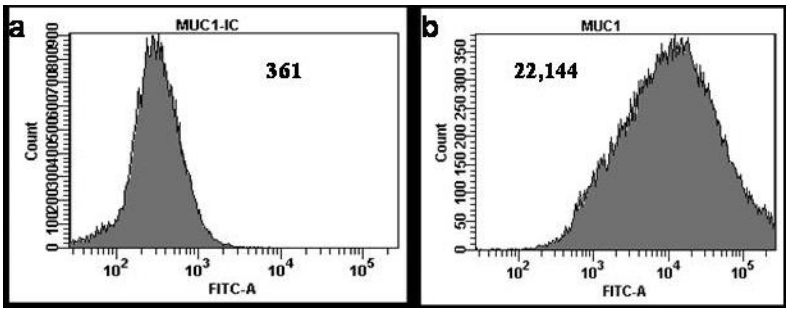

Fig. 8. Stable in vitro MUC1 expression upon transfection of IG10 cells.
IG10 cells were subjected to transfection with a MUC1-encoding plasmid via electroporation, as described in the Materials and Methods section. The stable transfect ants show very high levels of MUC1 expression, as demonstrated by flow cytometry, with the FITC-labeled, MUC1-specific monoclonal antibody, clone HMPV. Histogram in panel a represents the isotype (negative) control staining and the mean fluorescence intensity is 361. The HMPV staining (histogram b) shows a mean fluorescence intensity of 22,144, demonstrating the abundant MUC1 expression in the transfected cells.

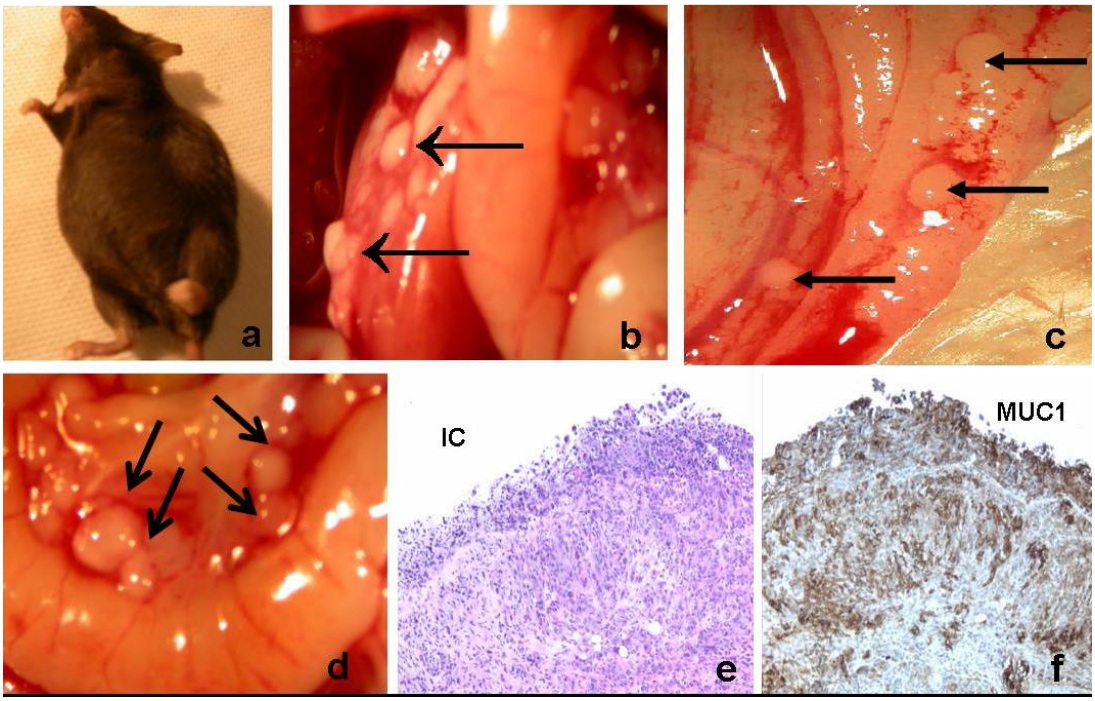

Fig. 9. Stable in vivo MUC1 expression in transplantable IG10-MUC1 syngeneic tumors. Five MUC1 mice were subjected to intraperitoneal (IP) injection of 5 x 106 IG10-MUC1 syngeneic cells. The injected cells induced tumor formation, accompanied by the accumulation of ascites (a). Representative pictures from one of the injected mice are shown. The tumors were spread throughout the peritoneal cavity and were often seen in the liver (b) peritoneum (c) and mesenteric (d), as pointed by arrows. Tumors were harvested and analyzed for MUC1 expression by IHC (e, f). Negative staining with an isotype control (IC) antibody is shown in (e). MUC1 presence appears as brown staining (f).

CA15-3 level		
	OD	[U/ml]
2344 MUC1KrasPten	0.193	140.54
2345 MUC1KrasPten	0.125	undetectable
2347 MUC1KrasPten	0.126	undetectable
2370 MUC1KrasPten	0.205	201.63
2299 KrasPten	0.051	0

Table 1.Soluble MUC1 detection in the serum of tumor-bearing mice using the CA 15-3 ELISA test. Normal circulating values are lower than equal to 35 U/ml. Despite all mice having tumors, only 50% of the triple Tg mice tested only 2 of which are presented here (2344 and 2370) had above normal serum levels. As expected, the MUC1-negative control sera from double Tg tumor-bearing mice (2299) had no detectable serum MUC1. The mice shown in this table match the serum antibody detection graphs in Fig. 10.

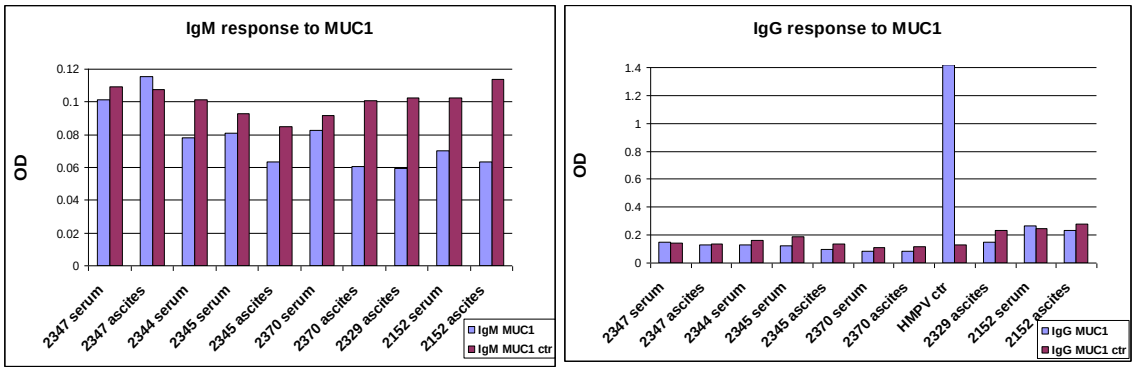

Fig. 10. No antibodies to MUC1 of IgM (right) or IgG isotype could be detected in serum and/or ascites of double Tg mice, despite some mice (2344, 2370) having high levels of circulating MUC1 (Table 1). The blue bars represent OD readings from experimental (MUC1-coated wells); burgundy bars represent OD from control (bovine serum albumin, BSA) coated wells. The readings were low (OD<0.12) and no significant increases were detected in any of the experimental wells over the control wells. The only above-control readings came from the IgG control antibody (HMPV, right panel).

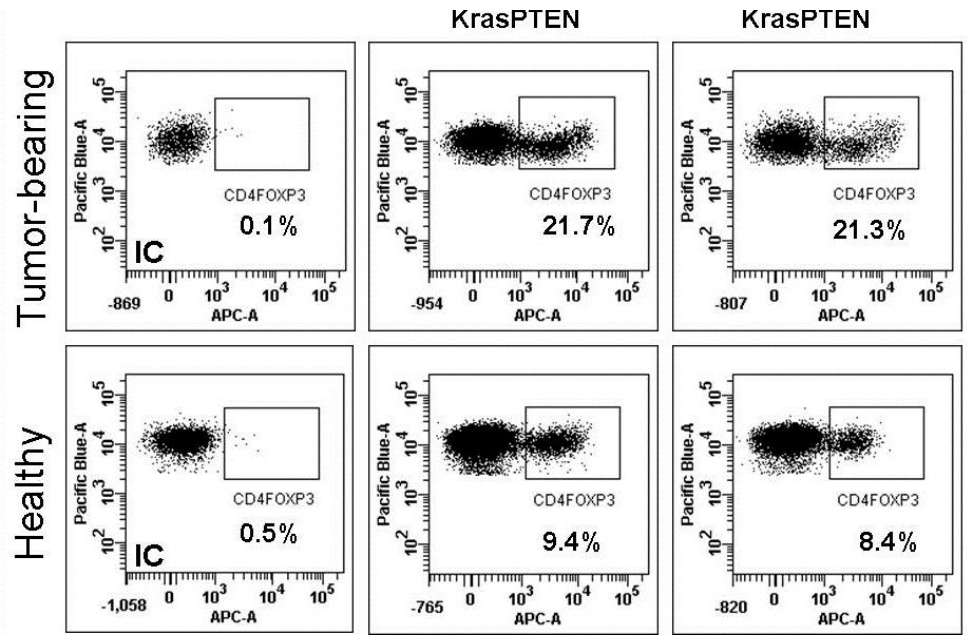

Fig. 11. Multicolor flow cytometry shows increased accumulation of CD4 Foxp3 T cells in the spleens of tumor-bearing but not healthy mice. Examples from two tumor-bearing KrasPten mice (upper panel) and two healthy (non-AdCre injected) mice (lower panels) are shown. The left dot plots in each row show staining with isotype control antibody (IC), based on which the gates were set and the shown percentages were calculated. The spleen cells were first gated on CD3 + cells and then sub gated on CD4 + T cells.

References

1. Dinulescu, D. M., T. A. Ince, B. J. Quade, S. A. Shafer, D. Crowley, and T. Jacks. 2005. Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. *Nat Med* 11:63-70.
2. Budiu, R. A., I. Diaconu, R. Chrissluis, A. Dricu, R. P. Edwards, and A. M. Vlad. 2009. A conditional mouse model for human MUC1-positive endometriosis shows the presence of anti-MUC1 antibodies and Foxp3+ regulatory T cells. *Disease models & mechanisms* 2:593-603.
3. Wu, R., Y. Zhai, E. R. Fearon, and K. R. Cho. 2001. Diverse mechanisms of beta-catenin deregulation in ovarian endometrioid adenocarcinomas. *Cancer research* 61:8247-8255.
4. Wei, X., H. Xu, and D. Kufe. 2005. Human MUC1 oncoprotein regulates p53-responsive gene transcription in the genotoxic stress response. *Cancer cell* 7:167-178.
5. Vlad, A. M., J. C. Kettel, N. M. Alajez, C. A. Carlos, and O. J. Finn. 2004. MUC1 immunobiology: from discovery to clinical applications. *Adv Immunol* 82:249-293.
6. Vlad, A. M., I. Diaconu, and K. R. Gantt. 2006. MUC1 in endometriosis and ovarian cancer. *Immunol Res* 36:229-236.
7. Ren, J., N. Agata, D. Chen, Y. Li, W. H. Yu, L. Huang, D. Raina, W. Chen, S. Kharbanda, and D. Kufe. 2004. Human MUC1 carcinoma-associated protein confers resistance to genotoxic anticancer agents. *Cancer cell* 5:163-175.

8. Yin, L., Y. Li, J. Ren, H. Kuwahara, and D. Kufe. 2003. Human MUC1 carcinoma antigen regulates intracellular oxidant levels and the apoptotic response to oxidative stress. *The Journal of biological chemistry* 278:35458-35464.
9. Packer, L. M., S. J. Williams, S. Callaghan, D. C. Gotley, and M. A. McGuckin. 2004. Expression of the cell surface mucin gene family in adenocarcinomas. *Int J Oncol* 25:1119-1126.
10. Li, Y., D. Liu, D. Chen, S. Kharbanda, and D. Kufe. 2003. Human DF3/MUC1 carcinoma-associated protein functions as an oncogene. *Oncogene* 22:6107-6110.
11. Roby, K. F., C. C. Taylor, J. P. Sweetwood, Y. Cheng, J. L. Pace, O. Tawfik, D. L. Persons, P. G. Smith, and P. F. Terranova. 2000. Development of a syngeneic mouse model for events related to ovarian cancer. *Carcinogenesis* 21:585-591.
12. Kai, S., Z. S., G. Luo, Z. Cao, X. lu, and Z. YE. 2005. Lethal effect of T cells activated by dendritic cells with MUC1 gene transfection on BIU-87 cells in vitro. *The Chinese-German Journal of Clinical Oncology* 4:347-350.
13. Zhang, L., J. R. Conejo-Garcia, D. Katsaros, P. A. Gimotty, M. Massobrio, G. Regnani, A. Makrigiannakis, H. Gray, K. Schlienger, M. N. Liebman, S. C. Rubin, and G. Coukos. 2003. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *The New England journal of medicine* 348:203-213.

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:

Results from our work during this granting period, reinforced by further studies currently in progress, will be incorporated in a manuscript that will be submitted for publication in 2010.

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

BIOGRAPHICAL SKETCH	
NAME Anda Mioara Vlad, M.D., Ph.D.	POSITION TITLE Assistant Professor
eRA COMMONS USER NAME	

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
University of Craiova, Romania	M.D.	1993	Medicine
University of Craiova Medical Center,		1993-1994	Internship, Medicine
"Fundeni" Hospital, Bucharest,		1994-1995	Residency, Medicine
University of Pittsburgh, Pittsburgh	Ph.D.	2002	Immunology
University of Pittsburgh, Pittsburgh		2002-2004	Post-doctoral training

A. Research and Professional Experience

1996-1997 Teaching Assistant, Duquesne University School of Nursing, Pittsburgh, PA.
1997-2002 Graduate Student/Teaching Assistant, Graduate Program in Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA.
2002-2004 Research Associate, University of Pittsburgh School of Medicine, Department of Immunology, Pittsburgh, PA.
2004-2007 Research Assistant Professor, University of Pittsburgh School of Medicine, Department of Immunology, Pittsburgh, PA.
2007-present Assistant Professor, Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine and Magee Women's Research Institute, Pittsburgh PA.

Professional Membership

American Association of Immunologists (trainee member 2000-2005; full member 2005-present); American Association for Cancer Research (2004-present);
Romanian College of Physicians (1994-present).

Honors and Awards

2010-2015 Early Career Investigator Award- Department of Defense Ovarian Cancer Academy Award
1989-1992 Romanian Governmental Fellowship for Academic Merit
2000-2002 Susan G. Komen Breast Cancer Foundation Predoctoral Dissertation Award
2006 "American Association of Immunologists Junior Faculty Travel Award"

B. Publications (in chronological order)

1. Barrat-Boyes, S., **Vlad A.**, and O. Finn. Immunization of chimpanzees with tumor antigen MUC1 mucin tandem repeat peptide elicits both helper and cytotoxic T cell responses. Clinical Cancer Research, vol 5: 1918-1924, 1999.

2. **Vlad A.*,** Hiltbold E.***,** Ciborowski P., Watkins S., and O. Finn. Mechanism of T cell tolerance to glycosylated tumor antigen MUC1 is a block in intracellular sorting and processing by dendritic cells. Journal of Immunology, 165(7):3730-41, 2000. (*Equal contributions).
3. **Vlad A.**, Muller S., Cudic M., Paulsen H., Otvos L., Georg-Hanisch F. and O. Finn. Complex glycans remain intact during the processing of MUC1 glycopeptides by dendritic cells and affect T cell recognition of MHC class II-restricted peptides. Journal of Experimental Medicine, 196(11): 1435-46, 2002.
4. Correa I., Plunkett T., **Vlad A**., Mungul A., Candelora-Kettel J., Burchell J. M., Taylor-Papadimitriou J., and Finn O.J. Form and pattern of MUC1 expression on T cells activated in vivo or in vitro suggests a function in T-cell migration. Immunology, 108(1): 32-41, 2003.
5. **Vlad A** and Olivera J. Finn. **"**Glycoprotein tumor antigens in breast cancer". *Breast Disease* Vol. 20: 73-79, 2004. Book chapter.
6. **Vlad A,** Kettel J., Alajez N, Carlos C and Olivera J. Finn. **"**MUC1 Immunobiology: From discovery to clinical applications". *Advances in Immunology*, 82: 249-293, 2003. Review article.
7. **Vlad A** and Olivera J. Finn. Glycoprotein tumor antigens in breast cancer. In Breast Disease Vol. 20: 73-79, 2004. Book chapter.
8. **Vlad A** and Olivera J. Finn. "The cancer vaccine challenge". *Trends in Cancer Vaccine Research*. Nova Science Publishers, 2005. Book chapter.
9. **Anda Vlad** and Iulia Diaconu. "MUC1 in endometriosis and ovarian cancer". Immunologic Research. Humana Press 2006; 36/1-3: 229-236.
10. Budiu R, Diaconu I, Chrissluis R, Dricu A , Edwards RP, Finn OJ and **Vlad AM**. Mice progressing to human mucin 1 (MUC1)-positive ovarian endometriosis-like lesions develop anti-MUC1 antibodies and show increased Foxp3 positive T cells in regional lymph nodes. Dis Model Mech. Nov-Dec;2 (11-12):593-603, (2009).
11. **Vlad, AM,** Budiu R, Thaller J, Finn OJ and Edwards, RP. Immune monitoring of ovarian cancer patients treated with intraperitoneal IL-2 shows correlations of serum anti-MUC1 antibodies. Cancer Immunol Immunother. Aug 19. [Epub ahead of print], (2009)
12. Ryan SO, **Vlad AM**, Jean Gariépy, Finn OJ. Tumor-associated glycopeptides of the tumor antigen MUC1 are not subject to self-tolerance and improves immune responsiveness in the MUC1 transgenic mouse. Biol Chem. Jul;390 (7):611-8, 2009.
13. Modulation of human mucin 1 (MUC1) tumor-associated antigen in a syngeneic, transplantable, ovarian cancer transgenic mouse model. Budiu R, Diaconu I, Dricu A and **Vlad AM**. Biology and Therapy of Cancer Cell. Accepted. (2010)