

Award Number: W81XWH-10-1-0525

TITLE: Disease Heterogeneity and Immune Biomarkers in Preclinical Mouse Models of Ovarian Carcinogenesis

PRINCIPAL INVESTIGATOR: Anda M Vlad, MD PhD

CONTRACTING ORGANIZATION: Magee Womens Research Institute
Pittsburgh, PA 15213

REPORT DATE: October 2015

TYPE OF REPORT: Final report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE October 2015		2. REPORT TYPE Final Report	3. DATES COVERED 1Aug2010 - 31Jul2015
4. TITLE AND SUBTITLE Disease Heterogeneity and Immune Biomarkers in Preclinical Mouse Models of Ovarian Carcinogenesis		5a. CONTRACT NUMBER W81XWH-10-1-0525	
		5b. GRANT NUMBER	
		5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Anda M. Vlad E-Mail:vladam@upmc.edu		5d. PROJECT NUMBER	
		5e. TASK NUMBER	
		5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Magee Womens Research Institute 204 Craft Ave Pittsburgh PA 15213		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)	
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			
13. SUPPLEMENTARY NOTES			
14. ABSTRACT Human studies performed in yeas 3 and 4 led to the discovery of several immune genes that are differentially expressed in endometriosis, atypical endometriosis, endometriosis-associated ovarian cancer (EAOC, endometrial and clear cell). Of these genes, complement pathway genes were consistently present, suggesting that complement-induced immunity may be involved in the pathogenic events during the transition from endometriosis to EAOC. In year 4, we have focused on immune gene signatures associated with response to immune therapy. Using our new transplantable ovarian cancer model in completely syngeneic immune competent mice, we tested in vivo the efficacy of anti-PDL1 antibody administered intraperitoneal (IP). PD-L1 is a molecule in the immune checkpoint pathway. It binds to PD-1 receptor on T cells and induces inhibition of effector function of cytolytic T cells. Our results demonstrate that blocking the PD-1/PD-L1 interaction through IP administration of anti-PD-L1 antibody significantly increases survival and triggers upregulation of several immune genes associated with CD8 T cell function. Immune checkpoint blockade has been proven effective in recent clinical trials mostly in melanoma, lung and renal carcinomas. Our results provide strong support in its suitability in ovarian cancer.			
15. SUBJECT TERMS Ovarian cancer, preclinical animal model, MUC1, immune therapy			

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 19	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER <i>(include area code)</i>

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

	<u>Page</u>
1. Introduction.....	5
2. Keywords.....	5
3. Accomplishments.....	5
4. Impact.....	14
5. Changes/Problems.....	14
6. Products.....	14
7. Participants and Other Collaborating Organizations.....	17
8. Special Reporting Requirements.	19
9. Appendices.....	19

1. INTRODUCTION

The Ovarian Cancer Academy Career Development Award was designed to (i) support Principal Investigator career goals and (ii) advance ovarian cancer research.

The overarching goals were defined in the original proposal as follows:

1. Focus on premalignancy, cancer prevention and treatment.
2. Combine preclinical studies in mice with ex vivo findings in humans.
3. Promote a multidisciplinary approach to ovarian cancer research
4. Promote translation into the clinic of meaningful preclinical findings.
5. Generation of solid preliminary results for successful future R01 application(s).
6. Promotion to tenured Associate Professor.

The specific research aims are further detailed below. We are happy to report that we have been able to successfully reach all major goals and complete all milestones, as originally planned.

2. KEYWORDS: ovarian cancer, preclinical models, disease pathogenesis, Kras, Pten, cancer vaccines, MUC1, immune checkpoint.

3. ACCOMPLISHMENTS

3.1. Major research goals of the projects

Our work was conducted around the following three aims:

Aim 1: To investigate the müllerian tract versus the OSE as the potential originating sites for ovarian epithelial tumors in KrasPten mice

Aim 2: To profile disease heterogeneity and to identify immune biomarkers of natural and vaccine-induced immune responses in mice with either endometriosis, ovarian cancer or endometriosis progressing to ovarian cancer.

Aim 3: To identify human immune biomarkers of endometriosis and cancer and to validate in human specimens the disease biomarkers identified (in aim 2) in mice with endometriosis and ovarian tumors.

All aims have been completed and results have been published or are currently under review.

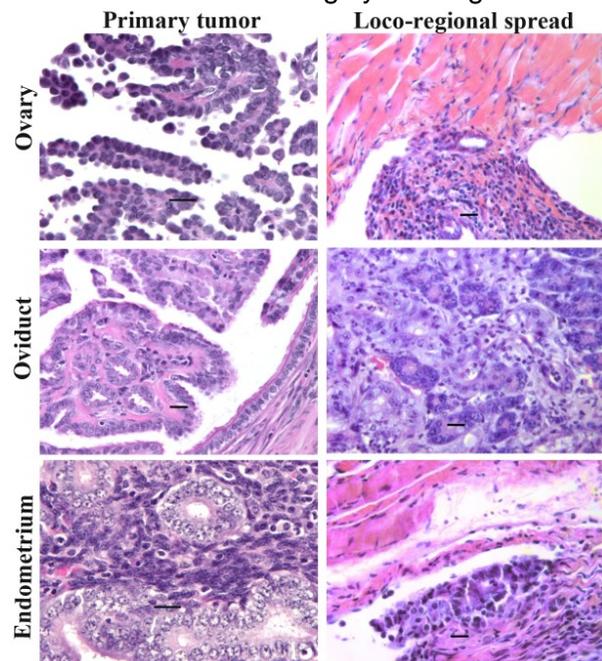
Accomplishments under each aim are further detailed below.

3.2 Key research accomplishments under each aim

Aim 1: All aim 1 tasks have been completed and results have been published (Tirodkar, Budiou et al, PLoS One, 2014 Jul 31;9(7):e102409.PMID 25078979, PDF attached):

Using complex genetic models with conditional mutations in Kras, Pten or both we have uncovered the following major findings:

1. Morphopathogenic characteristics of lesions triggered by AdCre injections via intrabursal, intraductal and intrauterine routes are highly heterogeneous and are accompanied by various immune microenvironments at



each location. Similarly to the ovarian tumors, tumors triggered in oviducts and endometrium have endometrioid histology (Fig. 1) and express increased levels of human MUC1 antigen, similar to MUC1 levels seen in human tumors (Fig. 2).

Fig. 1 Oviductal and endometrial tumors show endometrioid histology at both primary and satellite locations. Formalin fixed and paraffin embedded primary and metastatic tumor tissues were analyzed for histo-pathology. Representative images of H&E stained tumor sections are shown. Left column: primary tumors of the genital tract show endometrioid histology in the ovary, oviduct and endometrium. Right column: secondary tumors, including ovarian metastases to the diaphragm (upper), oviduct metastases to the pancreas (middle) and

endometrial metastases to the diaphragm (lower) also show endometrioid histology. Scale bar –20 μ m. (This figure appears as Fig. 3 in Tirodkar et al, PLoS One 2014, PMID: 25078979).

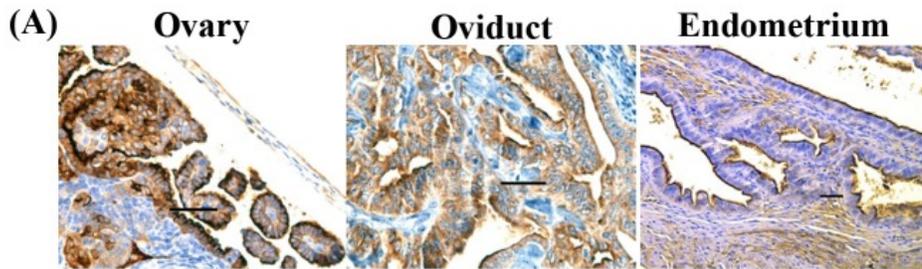
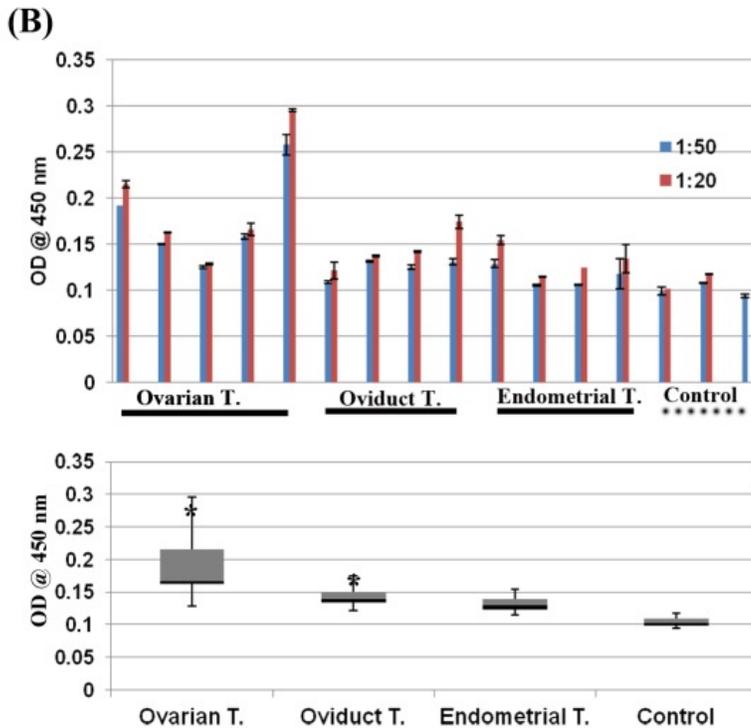


Fig. 2. Increased human MUC1 protein expression in Kras- and Pten- driven genital tract tumors of MUC1KrasPten triple transgenic mice triggers humoral immunity. (A) MUC1 immunohistochemistry staining of tumors occurring in the ovary (upper panel), oviduct (middle panel) or endometrium (lower panel). An antibody specific to the human MUC1 extracellular domain (clone HMPV, Representative immunohistochemical images shown. Scale bar –50 μ m). (B) ELISA measurement of human MUC1 peptide-specific IgG antibodies in sera from MUC1KrasPten mice with tumors (n=5 ovarian, n=4 oviductal and n=4 uterine). Upper panel: presence of antibodies at two different dilutions, using sing as target peptide a 100mer peptide comprising fie 20-aminoacid long peptide from the MUC1 extracellular domain of MUC1. Background levels were detected using sera from KrasPten mice with MUC1 negative tumors (i.e. wild -



type for MUC1). Vehicle only was also included as an additional negative control. The assay was run in duplicate and values were plotted as means with standard deviations. Lower panel: box and whisker diagrams (min, Q1, median, Q3, max) of readings at 1:20 dilution. Antibody levels are significantly higher (compared to control readings) in the ovarian and oviduct tumor group (one way ANOVA $p < 0.05$; *two tail t test; $p < 0.05$). Uterine tumors, $p = 0.052$. (This figure appears as Fig. 4 in Tirodkar et al, PLoS One 2014, PMID: 25078979).

2. Oviducts seem to favor the development of high grade tumors, providing preclinical evidence in support of the postulated role of fallopian tubes as the originating site for high grade human ovarian tumors (Fig. 3).

3. Survival for oviduct tumors was significantly lower than for endometrial tumors ($p = 0.0015$), yet similar to survival for ovarian cancer (Fig. 3).

4. An increased Foxp3 + T cell accumulation in the spleen of oviduct tumor-bearing mice and a higher ratio of Treg/CD8 in these mice compared to mice with uterine tumors (Fig. 3C, $p < 0.01$). No differences were noted between mice with ovarian and oviduct tumors, suggesting that both anatomical locations are similar in inducing an immune suppressive phenotype in the host, despite the high nuclear grade observed only in the latter.

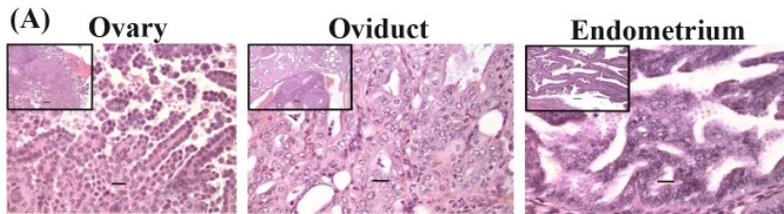
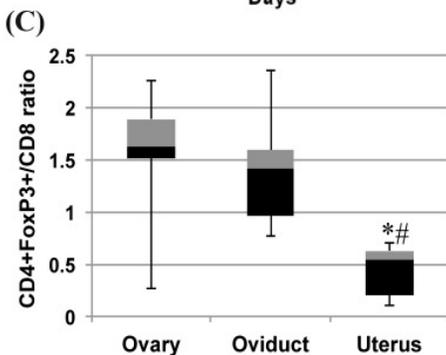
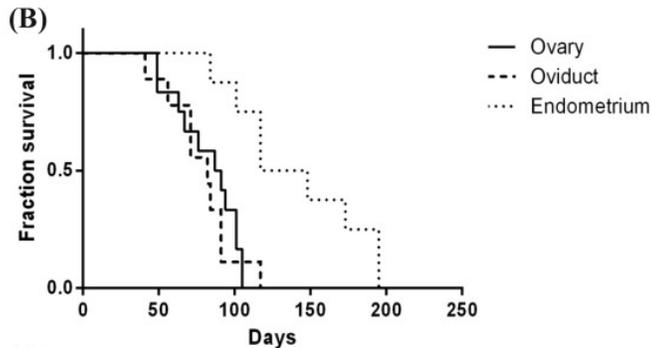


Fig. 3. Kras- and Pten- induced tumors differ in nuclear grade and survival based on the anatomical site of mutation activation.

(A) Nuclear grade of primary tumor tissues of the ovary, oviduct and the uterus.

Representative H&E images are shown. Scale bars: Main –20 μ m, Inset –100 μ m. (B) Kaplan Meyer curve shows that mice with uterine tumors survive significantly more than those with ovarian tumors (* $p=0.0015$) or those with ductal tumors (# $p=0.0016$). Individual group comparison after post ANOVA Bonferroni correction ($p<0.016$). The numbers of mice in each tumor group and median survival time for each tumor type are listed Table S1. Mice with premalignant lesions in the uterine tumor category were excluded from analyses.



(C) Splenic Treg/CD8 T cell ratios in mice with ovarian, oviduct or uterine tumors ($n=5$ mice/group), represented as box and whisker diagrams (min, Q1, median, Q3, max). CD4 and CD8 T lymphocytes were gated under the CD3 population. Foxp3 cells were gated under the CD4 population. One way ANOVA for comparison of all means ($p<0.03$) and two tail t tests between any two groups show significant differences between the ratios in uterine tumors

and any of the other two tumor types, ovarian and oviduct ($p<0.02$ and $p<0.01$, respectively). (This figure appears as Fig. 5 in Tirodkar et al, PLoS One 2014, PMID: 25078979).

Aim 2: All aim 2 tasks have been completed (Budiu et al, Oncogene. 2013 Aug; 32(32):3664-75. PMID: 22964632- PDF attached; Mony et al, Cancer Immunology Immunotherapy 2015 Sep;64(9):1095-108. PMID: 25998800-PDF attached).

Using triple transgenic mice with MUC1 expressing orthotopic ovarian tumors we were able to demonstrate how MUC1 influences biology of ovarian tumors and to explore the in vivo efficacy of MUC1 based vaccine (Budiu et al, Oncogene 2013 PMID: 22964632). Specifically, we showed that the triple Tg mice had an average of 2.6 metastatic sites versus 1.4 in double Tg mice ($P = 0.03$, Table 1), suggesting that the MUC1-expressing tumors migrate and metastasize more widely in the peritoneal cavity, a hypothesis further tested in cell lines (Fig. 4) .

Table 1. Tumor characteristics in double (KP, $n = 13$) and triple (MKP, $n = 10$) Tg mice

	KP; $n=13$ (56.52%)	MKP; $n=10$ (43.48%)	P value (two-sided)
Ascites_Yes, n (%)	7/13 (53.85)	9/10 (90.00)	0.09 †
Ascites Vol , mean (SD)*	719.23 (1281.69)	965.00 (1827.27)	0.34 ††
Nr Mets, mean (SD)**	1.46 (1.20)	2.60 (0.84)	0.03 ††
Tumor Score, mean (SD)***	2.69 (0.63)	2.10 (0.88)	0.08 ††

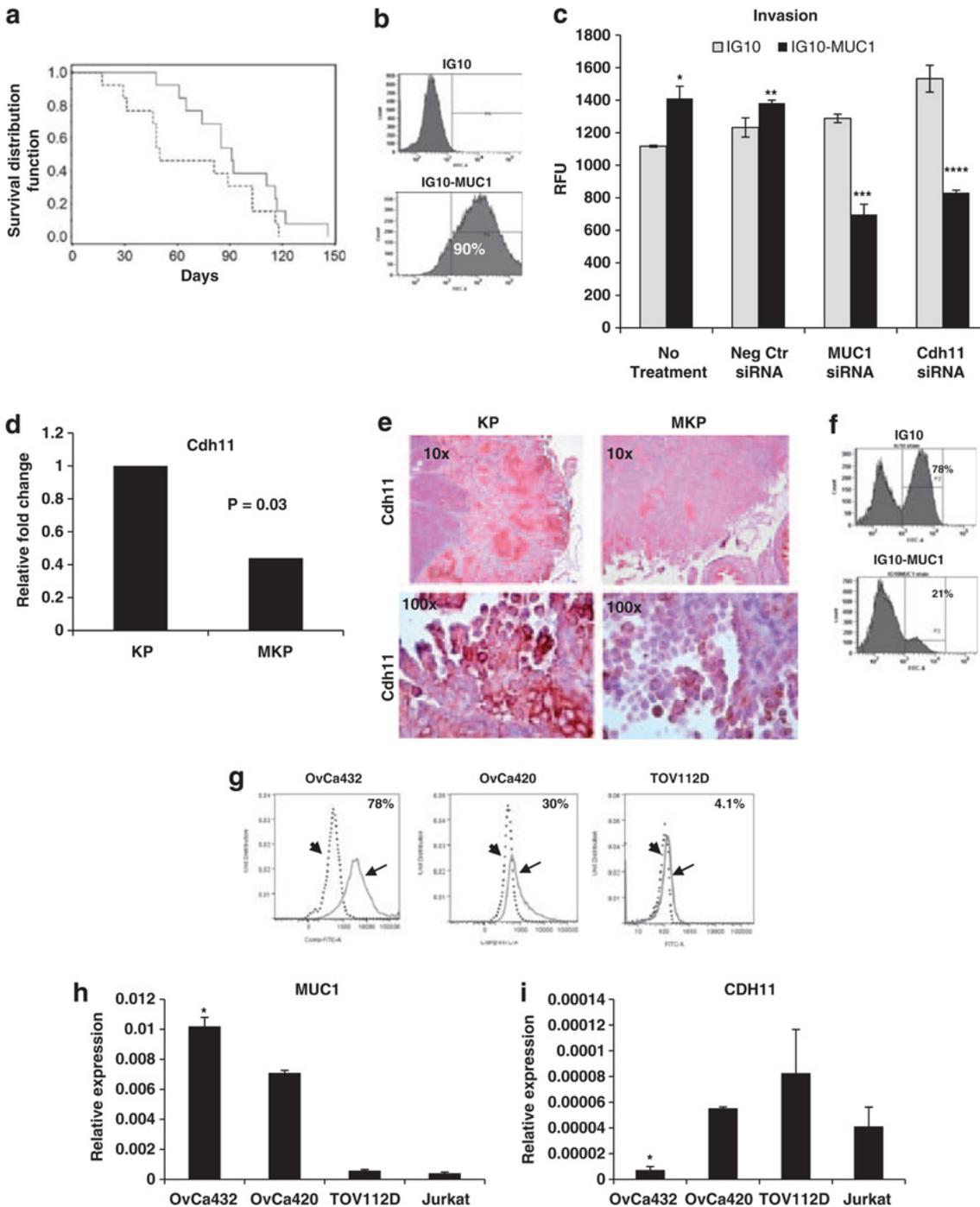


Fig. 4. MUC1 roles in survival and invasion. (a) Kaplan–Meier curve of overall survival comparing MUC1KrasPten ($n = 13$, solid line) and KrasPten ($n = 13$, dotted line) tumor-bearing mice, using log-rank test ($P = 0.128$). (b) MUC1 expression by flow cytometry in IG10 cells (parental controls) and IG10 cells transfected with a MUC1-encoding plasmid (IG10-MUC1). Percentages were obtained using FACSDiva software, after gating on single-cell population and subtracting background events from isotype control antibody. (c) Invasion of IG10 and IG10-MUC1 cell lines before and after transient knockdown of human MUC1 and mouse Cdh11 with specific siRNAs. The cells were grown *in vitro* in the presence of negative control siRNA, MUC1 or Cdh11 specific

siRNA, for 48 h and then subjected to invasion assay (described in Materials and methods). Each assay was run in triplicate and results from one of four independent assays are shown. The bars represent the averaged fluorescence readings. *IG10 compared with IG10-MUC1, no treatment ($P = 0.0068$); **IG10 compared with IG10-MUC1, cells treated with negative control siRNA ($P = 0.007$); ***IG10 compared with IG10-MUC1, cells treated with four different siRNA specific to human MUC1 ($P = 0.00007$); ****IG10 compared with IG10-MUC1, cells treated with four different siRNA specific to mouse Cdh11 ($P = 0.0034$). Student's *t*-test. (d) Cdh11 expression by qRT–PCR. RNA was extracted from frozen KrasPten ($n = 4$) and MUC1KrasPten ovarian tumors ($n = 4$). All results were normalized to a pool of three housekeeping genes (Gusb, Hsp90ab1 and Hprt1) and presented as relative fold change. *P*value was calculated using RT Profiler PCR Array Data Analysis Software (SABiosciences). (e) Detection of mouse Cdh11 protein by immunohistochemistry (rabbit polyclonal

anti-Cdh11). One KrasPten (left) and one MUC1KrasPten (right) tumor are shown. Lower panels represent higher magnification of framed fields from upper panels. (f) Detection of mouse Cdh11 protein by flow cytometry (rabbit polyclonal anti-Cdh11—Invitrogen). Histograms show Cdh11 staining pattern in the parental (IG10, upper panel) and MUC1-transfected cells (IG10-MUC1, lower panel). (g) Flow cytometry for cell surface MUC1 in three ovarian cancer cell lines. Percentages shown represent MUC1-positive cells (arrows), gated after staining with isotype control antibody (arrowheads). (h) MUC1 gene expression in ovarian cancer cell lines, by qRT-PCR. * $P < 0.01$, OvCa432 versus any of the others, Students' t -test. (i) CDH11 gene expression, by qRT-PCR, in triplicates. * $P < 0.01$, Student's t -test.). (This figure appears as Fig. 3 in Budiu et al, Oncogene. 2013 Aug; 32(32):3664-75. PMID: 22964632).

We also asked whether MUC1-targeted therapy can prolong survival and reverse immune dysregulation in MUC1KrasPten mice. To address this, we used a vaccine based on a synthetic 100mer MUC1 peptide (comprising five tandem repeats from the MUC1 extracellular domain), loaded onto dendritic cells (DC) matured under type 1 polarizing conditions (DC1). The DC1-MUC1 vaccine was administered subcutaneously (SC) in the right flank, at weeks 4, 6 and 8 after AdCre injection. A total of 3000 MUC1-loaded DC1 cells were administered in each vaccine. This low number represents the mouse mass-adjusted equivalent of an adult human vaccine of ~ 6.5 million DC. Our results show that the DC1-MUC1 vaccine significantly prolongs survival in vaccinated MUC1KrasPten mice ($n = 10$, $P = 0.033$) compared with MUC1KrasPten non-vaccinated mice ($n = 13$, Figure 5). No mice died before the scheduled time for the first vaccine dose.

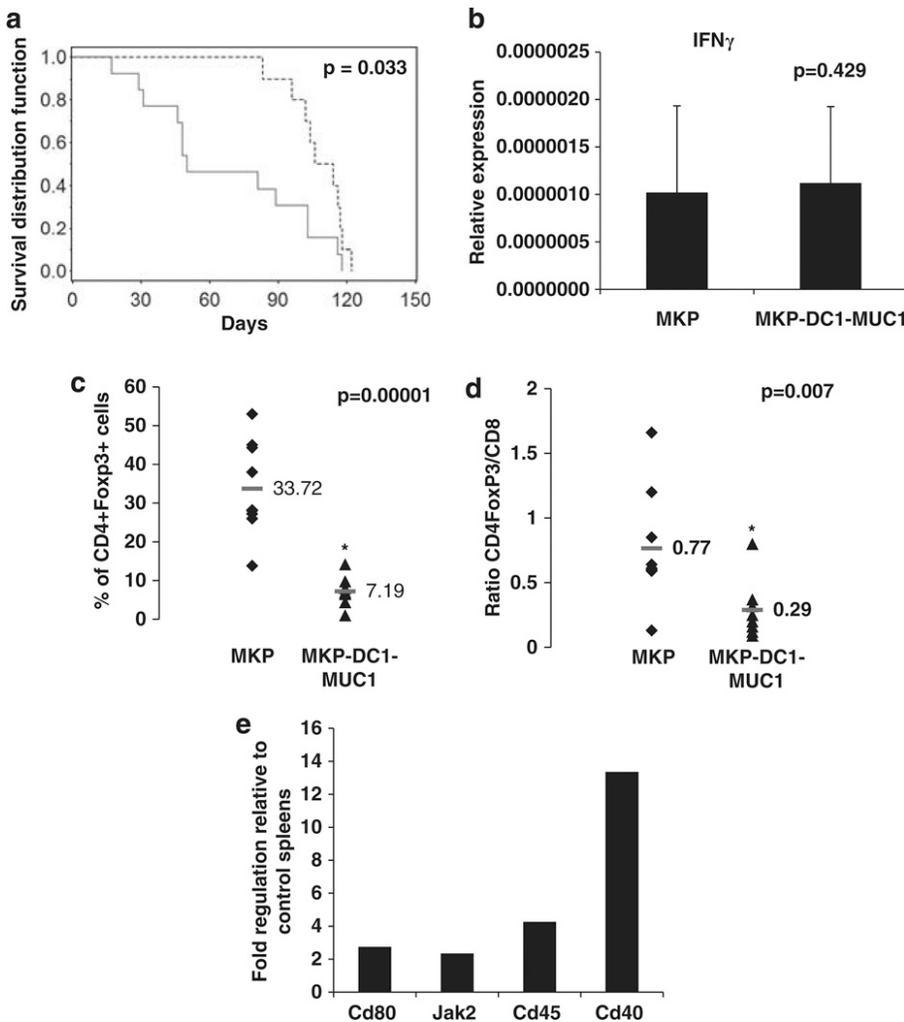


Fig. 5. DC1 characterization and vaccination protocol. (a) Flow-cytometry analysis of CD40, CD80, CD83 and CD86 expression on in vitro DCs matured with either Th1-inducing cocktail (granulocyte-macrophage colony-stimulating factor, IL-4, LPS, IFN γ and Poly I:C—dark line) or with IL-4 + LPS (gray line). All the analyses were based on the isotype control for each marker (dotted lines). (b) In vitro IL-12 (p70) secretion by immature, IL-4/LPS or Th1 polarized dendritic cells (DC1). DC1-MUC1 cells were loaded with MUC1 peptide before maturation, as described in Materials and methods. * $P < 0.01$, Student's t -test. (c) Vaccination diagram for DC1-MUC1 vaccine administration after intrabursal AdCre injection of 6–8-week-old female MUC1KrasPten mice. (This figure appears as Fig. 6 in Budiu et al, Oncogene. 2013 Aug; 32(32):3664-75. PMID: 22964632)

In follow up studies we used a novel transplantable ovarian cancer tumor model to test in vivo efficacy of a PD-L1 blockade. The therapeutic PD-L1 blockade employed three administrations of anti-PDL1 (200ug/dose/mouse) administered IP two weeks apart. Treatment was started 21 days after tumor induction, and administered intraperitoneal (IP) every two weeks for a total of 3 doses. Our results demonstrate that IP administration of anti-PD-L1 in a new preclinical model for aggressive ovarian cancer significantly increases survival (Fig. 6 and Mony et al, Cancer Immunology Immunotherapy 2015 Sep;64(9):1095-108. PMID: 25998800-PDF attached).

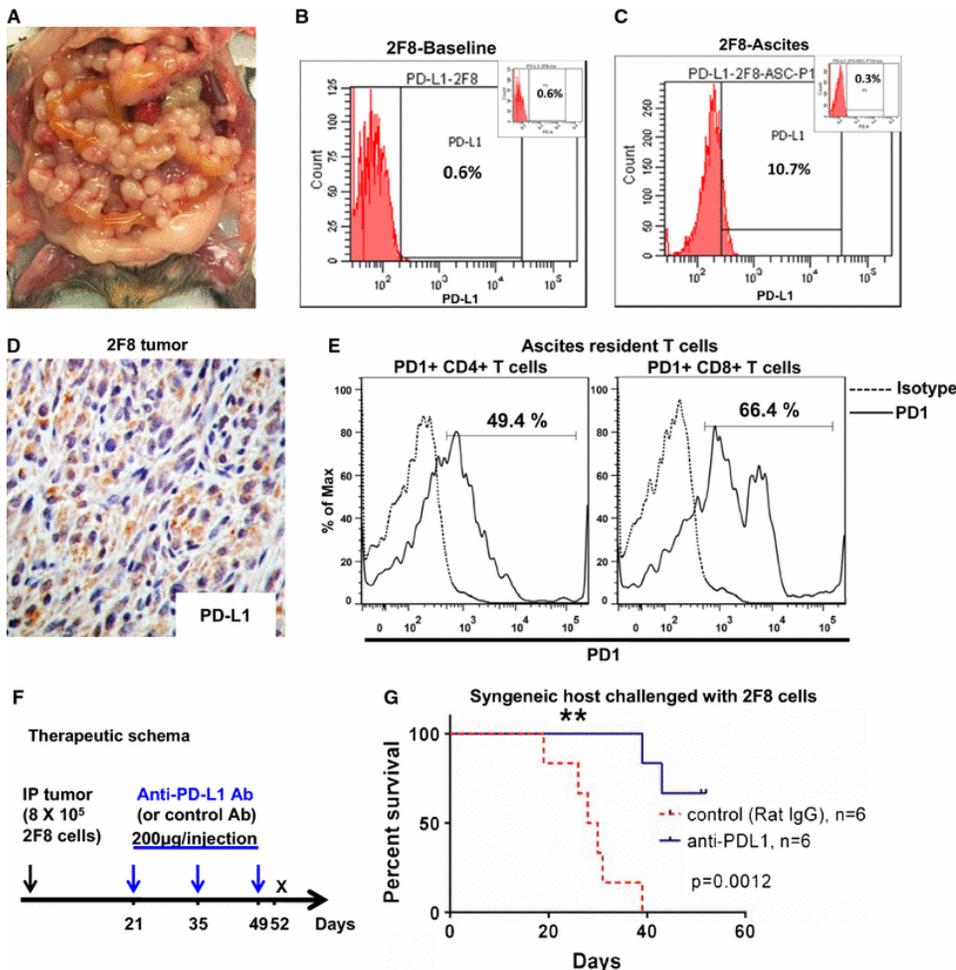


Fig. 6. Late treatment with low-dose anti-PD-L1 antibody significantly improves survival. a MUC1.Tg mice challenged IP with 8×10^5 syngeneic 2F8 cells. Image representative of tumor burden at day 29. b, c Flow cytometry staining for cell surface PD-L1 protein expression on 2F8 cells in culture at baseline (b) and after isolation from ascites, post in vivo growth (c). Percentages show positive cells measured outside the isotype control, shown as insets. d Tumor PD-L1 by IHC. e Flow cytometry detection of PD-1+ CD4+ and PD-1+ CD8+ T cells isolated from ascites of 2F8 tumor-bearing mice. Dotted histograms represent staining with isotype control antibody; solid histograms are representative of cells stained with anti-PD-1 antibody. Percentages represent PD-1 positive cells, gated under the CD4 (left) and CD8 (right) populations, respectively. f Therapeutic schema (n = 12 mice): Protocol was started 21 days after

IP tumor challenge with 8×10^5 2F8 cells. Half of the mice (n = 6) received IP 200 μ g of anti-PD-L1 antibody. The remaining (n = 6) received control rat IgG. All mice received a total of three doses, 2 weeks apart. g Kaplan–Meier survival curve of survival of mice that received anti-PD-L1 antibody (blue) and control IgG (red, p = 0.001). (This figure shows as Fig.1 in Mony et al, Cancer Immunology Immunotherapy 2015 PMID: 25998800).

Aim 3: All aim 3 experiments have been completed and results have been published (Suryawanshi S et al, Clinical Cancer Research 2013 Mar; 19(5):1213-24. PMID: 23362326- PDF attached; Suryawanshi S et al, Clinical Cancer Research Oct 2014, PMID: 25294912- PDF attached)

We profiled the miRNA and immune microenvironment in endometriosis and endometriosis-associated ovarian cancer (EAOC) using Nanostring and the nCounter® GX Human Immunology Kit, comprising probes for a total of 511 immune genes.

Our studies revealed several miRNA signatures that can differentiate different disease categories (controls, benign precursors and cancer) (Fig. 7)

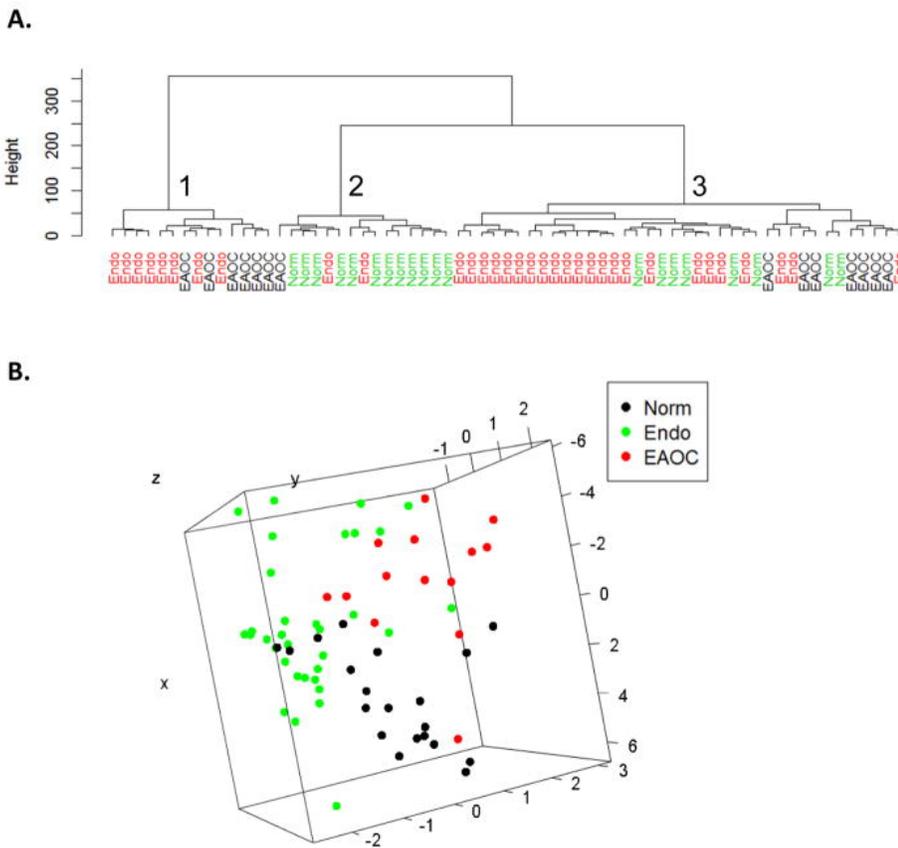


Fig.7 . Plasma miRNA expression profiles can distinguish different disease categories. A) Unsupervised hierarchical clustering was applied to miRNAs with < 30% missing values in healthy controls, endometriosis, and EAOC samples (n=20, 33, and 14, respectively). Different distance measure and link were explored. Samples are classified into three clusters based on the expression signature of 23 plasma miRNAs. B) Principal component analysis was applied to markers with adjusted p value < 0.2 in either one of the three groups' pair-wise comparisons. First three components were used for the three-dimensional plot. Norm, healthy controls; Endo, endometriosis. (This figure shows as Fig. 1 in Suryawanshi S et al, *Clinical Cancer Research* 2013 Mar; 19(5):1213-24. PMID: 23362326).

One third of the endometriosis patients revealed a tumor-like inflammation profile, suggesting that cancer –like immune signatures may develop earlier, in patients classified as clinically benign (Fig. 8). Gene expression analyses revealed the complement pathway as most prominently involved in both endometriosis and EAOC (Fig. 9). These findings reveal new characteristics of inflammation in precursor lesions and point to previously unknown roles of complement in endometriosis and EAOC. Most importantly, our findings reveal that chronic inflammation in endometriosis is dominated by complement, which remains active in EAOC but not tumors with serous histology, further demonstrating heterogeneity in the inflammatory milieu within this category commonly referred to as ovarian cancer. Pharmacologic inhibition of complement is currently tested in clinical trials and results from these studies will provide much needed clinical evidence to support (or refute) the recent paradigm shift on pro-tumor roles of complement in cancer. Profiling studies like the one presented here might aid in patient selection for a personalized approach (Suryawanshi S et al, *Clinical Cancer Research* 2013 Mar; 19(5):1213-24. PMID: 23362326).

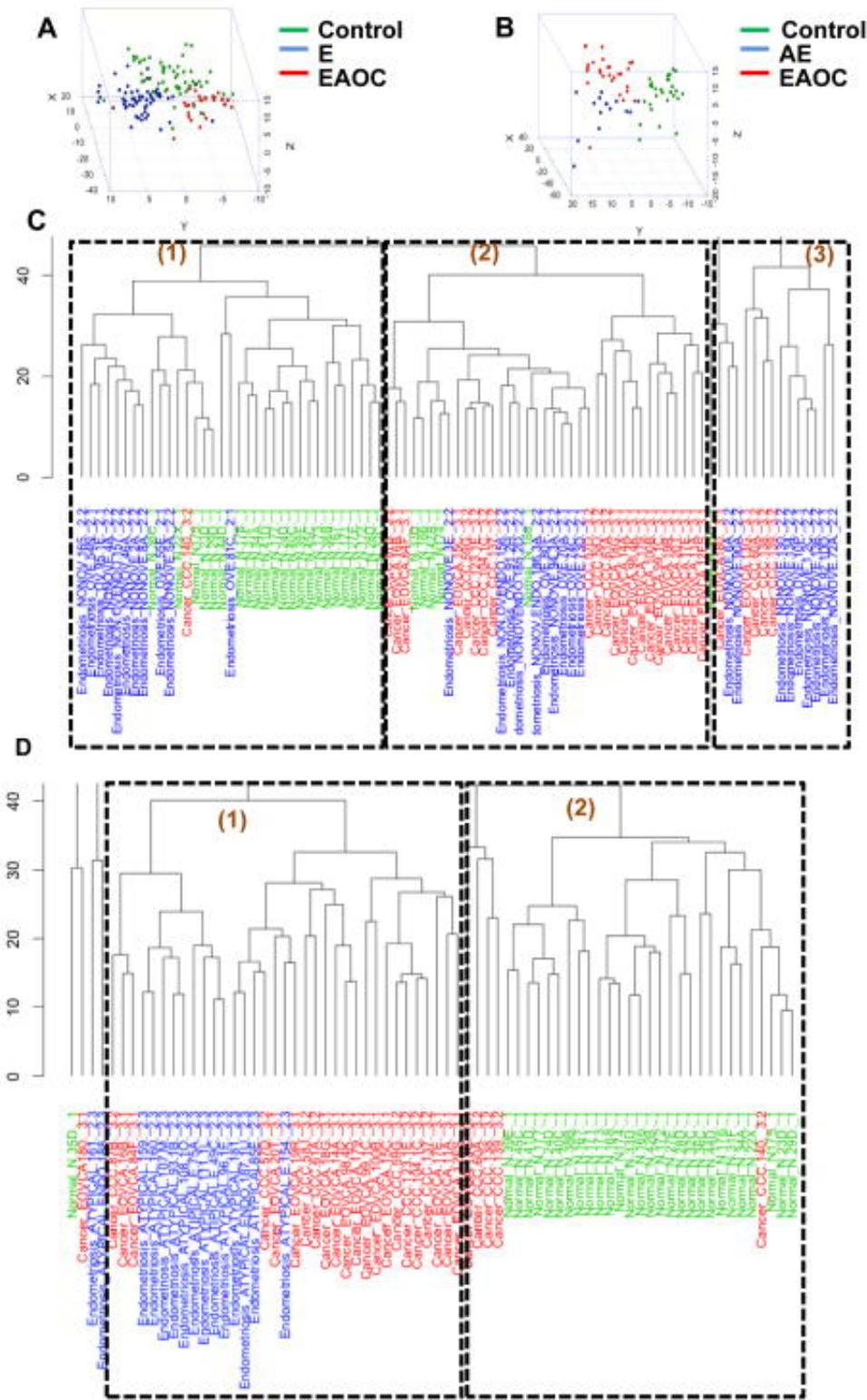


Fig. 8. Global gene expression across disease categories. A–B. Multidimensional scaling plot showing separation of disease categories with for differentially expressed (DE) genes filtered with 50-quantile cutoff of mean and standard deviation. Endometriosis-associated ovarian cancer (EAOC) cases are shown in red and controls in green. Endometriosis (E) and atypical endometriosis (AE) are shown in blue (panel A and B, respectively). C–D. Unsupervised clustering of 100 filtered genes. EAOC cases are listed in red and controls in green; E and AE are listed in blue (panel C and D, respectively). (This figure shows as Fig. 1 in Suryawanshi S et al, Oct 2014, PMID: 25294912).

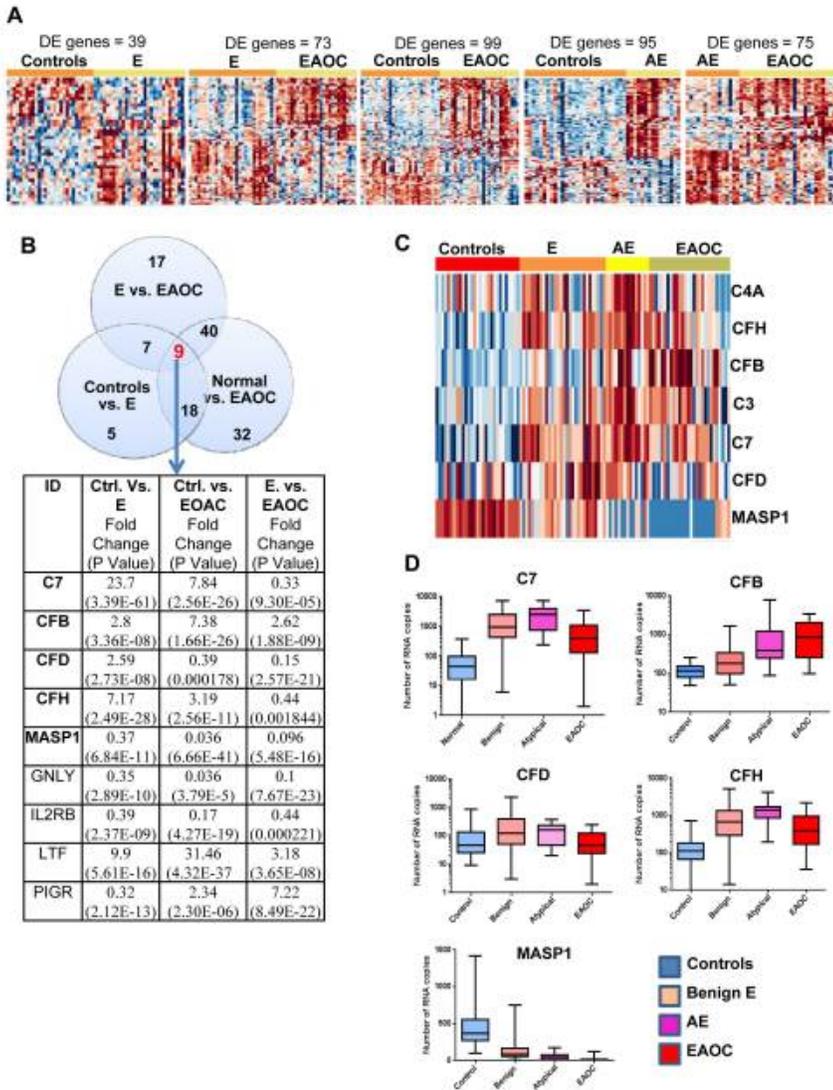


Fig. 9. Differentially expressed genes across disease categories. A. Heatmaps of DE genes in five different two-group comparisons: control vs. E (n=39), endometriosis vs. EAOC (n=73), normal vs. EAOC (n=99), control vs. AE (n=95), AE vs. EAOC (n=75). B. Venn diagram showing number DE genes that are differentially expressed in controls, E and EAOC. Accompanying table lists the nine common DE genes (common intersection, arrow), fold change and P value. Complement genes are in bold. C. Heat map of 7 complement genes (C4A, CFH, CFB, C3, C7, CFD, MASP1) shows a differentiating pattern among the four different disease categories (controls, E, AE and EAOC). D. Individual gene expression profile of five common complement DE genes (C7, CFB, CFD, CFH, MASP1) across the four cohorts (controls, E, AE and EAOC). All genes are significantly different (false discovery rate FDR < 0.05 and absolute log FC greater than 1) for any of the two group comparisons (normal, endometriosis and EAOC). (This figure shows as Fig. 2 in Suryawanshi S et al, Oct 2014, PMID: 25294912)

Timeline for milestones completed during the award period

Milestone #1: first round of publication submissions.

This milestone has been completed (1, 2).

Milestone # 2: second round of publication submissions.

This milestone has been completed (references 3,4 and 6)

Milestone # 3: first R01 submission. Originally planned for year 3, we report that we secured our first R01 earlier than proposed, in year 2.

Milestone #4: promotion to tenured Associate Professor. Completed in year 5 of the award.

3.3 Opportunities for training and professional development

Due to this award the PI has been able to travel to one large professional meeting (like AACR or AAI) where she attended workshops and/or presented her work either as oral or platform presentations. Titles of presented work are listed below.

3.4 Result dissemination to communities of interest

The PI has presented her work to meetings attended by ovarian cancer survivors (TRC3, AACR Marsha Rivkin).

3.5 Goals for next reporting period

Nothing to report.

4. IMPACT

4.1. Impact on principal discipline

Our studies have advanced our understanding of disease pathogenesis in ovarian cancer, particularly in endometriosis-associated ovarian cancer and our ability to design improved therapies via novel vaccines and immune biologics.

4.2 Impact on other disciplines

Our animal models have been used in radiology/imaging studies (Ocak et al, Molecular Pharmaceutics 2015 Feb 2;12(2):542-53. PMID: 25536192).

4.3 Impact in technology transfer

We have developed new preclinical models (mice, cell lines) for ovarian cancer research. These models are currently (and will continue to be) shared via Material Transfer Agreements between the University of Pittsburgh and the institutions of requesting investigators.

4.4 Impact on society beyond science and technology

Our studies can lead to new clinical trials and improved treatments for ovarian cancer. Reducing suffering due to cancer can have a positive impact not only on cancer patients and their families but the society as a whole.

5. CHANGES/PROBLEMS

Nothing to report.

6. PRODUCTS

6.1 Publications, conference papers and presentations

- **Journal Publications (Peer Reviewed)**

1. Budiu RA, Mantia-Smaldone G, Elishaev E, Chu T, Thaller J, McCabe K, Lenzner D, Edwards RP and **Vlad AM**. Soluble MUC1 and serum MUC1-specific antibodies are potential prognostic biomarkers for platinum-resistant ovarian cancer. *Cancer Immunol Immunother*. 2011 Jul; 60(7):975-84. PMID: 21461842.
2. Flint MS, Budiu RA, Teng PN, Sun M, Stolz DB, Lang M, Hood BL, **Vlad AM**, and Conrads T. Restraint stress and stress hormones significantly impact T lymphocyte migration and function through specific alterations of the actin cytoskeleton. *Brain Behav Immun*. 2011 Aug; 25(6):1187-96. PMID: 21426930.
3. Zhang L, Vlad AM, Milcarek C and Finn OJ. Human mucin MUC1 RNA undergoes different types of alternative splicing resulting in multiple isoforms. *Cancer Immunol Immunother*. 2013 Mar; 62(3):423-35. PMID: 22941036.
4. Budiu RA, Elishaev E, Brozick J, Lee M, Edwards RP, Kalinski P and **Vlad AM**. Immunobiology of human mucin 1 in a preclinical ovarian tumor model. *Oncogene*. 2013 Aug; 32(32):3664-75. doi: 10.1038/onc.2012.397. PMID: 22964632.
5. Suryawanshi S*, **Vlad AM***, Lin HM, Mantia-Smaldone G, Laskey R, Lee M, Lin Y, Donnellan N, Klein-Patel M, Lee T, Mansuria S, Elishaev E, Budiu R, Edwards RP, Huang X. (*Authors with equal contributions). Plasma microRNAs as novel biomarkers for endometriosis and endometriosis-associated

ovarian cancer. *Clinical Cancer Research* 2013 Mar; 19(5):1213-24. PMID: 23362326. Manuscript recommended to F1000Prime by Faculty of F1000 “as being of special significance in its field”.

6. Parikh RA, Appleman LJ, Bauman JE, Sankunny M, Lewis DW, **Vlad AM**, Gollin SM. Upregulation of the ATR-CHEK1 pathway in oral squamous cell carcinomas. *Genes Chromosomes Cancer*. 2014 Jan; 53(1):25-37. doi: 10.1002/gcc.22115. PMID: 24142626
7. Tirodkar TS, Budiu RA, Elishaev E, Zhang L, Mony JT, Brozick J, Edwards RP and **Vlad AM**. MUC1 positive, Kras and Pten driven mouse gynecologic tumors replicate human tumors and vary in survival and nuclear grade based on anatomical location. *PLoS One* 2014 Jul 31;9(7):e102409. doi: 10.137. PMID: 25078979
8. Suryawanshi S, Huang X, Elishaev E, Budiu RA, Zhang L, Kim S, Donnellan N, Mantia-Smaldone G, Ma T, Tseng G, Lee T, Mansuria S, Edwards RE and **Vlad AM**. Complement Pathway is Frequently Altered in Endometriosis and Endometriosis-Associated Ovarian Cancer. *Clinical Cancer Research* Oct 2014, PMID: 25294912
9. Ocak M, Gillman A, Bresee J, Zhang L, **Vlad AM**, Müller C, Schibli R, Edwards B, Carolyn J, Anderson CJ and Gach MJ. Folate Receptor-Targeted Multimodality Imaging of Ovarian Cancer in a Novel Syngeneic Mouse Model. *Molecular Pharmaceutics* 2015 Feb 2;12(2):542-53. PMID: 25536192.
10. Graw S, Meier R, Minn K, Bloomer C, Godwin AK, Fridley, **Vlad AM**, Beyerlein P and Chien J. Robust gene expression and mutation analyses of RNA-sequencing of formalin-fixed diagnostic tumor samples. *Scientific Reports*. 2015 Jul 23;5:12335; PMID: 26202458
11. Mony JT, Zhang L, Ma T, Grabosch S, Tirodkar T, Brozick J, Tseng G, Elishaev E, Edwards RP, Huang H and **Vlad AM**. Anti-PD-L1 prolongs survival and triggers T cell but not humoral anti-tumor immune responses in a human MUC1- expressing preclinical ovarian cancer model. *Cancer Immunology Immunotherapy* 2015 Sep;64(9):1095-108. PMID: 25998800.

- **Book Chapters, Reviews, Opinions**

1. Mantia-Smaldone G, Edwards R.P and **Vlad AM**. Review article. “Targeted treatment of refractory ovarian cancer: current and emerging therapies”. *Cancer Management and Research*. 3:25-38 (2011).
2. Dricu A, Purcaru SA, Budiu R, Ola R, Tache DE and **Vlad AM**. Book Chapter. “Epigenetic alteration of receptor tyrosine kinases in cancer”. *DNA Methylation*, InTech Publishing (2011).
3. Huang X, **Vlad AM**. Letter to the Editor. Plasma microRNAs in Ovarian Cancer. *Clin Cancer Res*. 2013 Jun; 19(12):3326. Doi: 10.1158/1078-0432.CCR-13-1022. PMID: 23616635.
4. Oesterreich S, Edwards R, **Vlad AM**. News and Views article. “Progestins: Pro-senescence therapy for ovarian cancer?” *Cell Cycle*. 2013 Jun; 12(11):1662-3. doi: 10.4161/cc.25072. PMID: 23713123.
5. Edwards RP, Huang X and **Vlad AM**. “Chronic inflammation in endometriosis and endometriosis-associated ovarian cancer: new roles for the “old” complement pathway”. *Oncoimmunology*. 2015 Feb 3;4(5):e1002732. PMID: 26155393.

- **Conference abstracts and presentations**

1. Budiu R, Elishaev E, Brozick J, Lee MJ, Edwards RP and Vlad AM. “Preclinical Testing of a MUC1 Vaccine in Triple Transgenic Mice with Ovarian Tumors”. Poster and oral presentation at the *14th Annual Meeting of the Translational Research Cancer Centers Consortium, Seven Springs Mountain Resort, Pennsylvania, USA. Febr. 24-26, 2011*
2. Budiu R, Elishaev E, Brozick J, Chu T, Predoiu S, Edwards RP and Vlad AM. “Vaccination with MUC1-pulsed dendritic cells prolongs survival in triple transgenic mice with orthotopic ovarian tumors”. Poster presentation at the *American Association for Cancer Research (AACR) 102nd Annual Meeting, Orange County Convention Center, Orlando, Florida, USA. April 2-6, 2011.*

3. Suryawanshi S, Budiu R, Mantia G, Kim, SH, Tseng, G, Elishaev, E, Bhargava, R, Huang X, Edwards RP and Vlad AM. "MicroRNA as Biomarkers of Endometriosis and Ovarian Cancer". *American Association for Cancer Research (AACR) Annual Meeting, Chicago, IL, March 31- April 4th, 2012.*
4. Suryawanshi S, Budiu R, Mantia G, Kim, SH, Tseng, G, Elishaev, E, Bhargava, R, Huang X, Edwards RP and Vlad AM. "Immune mechanisms involved in endometriosis and endometriosis-associated ovarian cancer". *AACR-Advances in Ovarian Cancer Research: From Concept to Clinic, Miami FL, September 18-21, 2013.*
5. Budiu R, Vlad AM, Bathula C, Nazario, L Cooper K, Elishaev EL, and Flint MS. Impact of psychological stress on the adaptive immune system and disease progression in a triple negative breast and a transgenic ovarian cancer mouse model. Poster presentation at *Psycho-Neuro-Immunology Research Society's 20th Annual Scientific Meeting, Stockholm, Sweden, June 5-8, 2013.*
6. Zhang L, Budiu R, Brozick J and Vlad AM. "Novel ovarian cancer transplantable models generated from MUC1KrasPten tumors show in vitro and in vivo heterogeneity". *American Association for Cancer Research (AACR) Annual Meeting, San Diego, CA, April 5th-9th, 2014.*
7. Suryawanshi S, Huang X, Budiu R, Elishaev E, Kim SH, Tseng G, Mantia-Smaldone G, Klein Patel M, Lee T, Mansuria S, Edwards RP and Vlad AM. "Complement roles in endometriosis and endometriosis-associated ovarian cancer". *American Association for Cancer Research (AACR) Annual Meeting, San Diego, CA, April 5th-9th, 2014.*
8. Zhang L, Tirodkar T, Elishaev E, Mony J, Brozick J, Edwards RP and Vlad AM. "Natural and anti-PD-L1 induced tumor immunity in a novel ovarian cancer mouse model for human mucin 1 (MUC1)". Poster and oral presentation, *American Association for Immunology (AAI) Annual Meeting, Pittsburgh PA, May 2-5, 2014.*
9. Berger J, Beck T, Elishaev E, Sukhwani M, Zhang L, Vlad AM, Krivak T, Kelley J and Orwig K." A novel immune-competent mouse model of early-stage epithelial ovarian carcinoma". *Proceedings of the Annual Meeting of the American Society of Clinical Oncology (ASCO) Chicago, IL, May 30- June 3, 2014.*
10. Gillman, A, Ocak, M, Bresee, J; Zhang, L; Mueller, C, Vlad, AM; Edwards, B, Anderson, C and Gach, M. "Multi-modal folate-targeted imaging of intraperitoneal ovarian tumors in mice". *Society for Nuclear Medicine and Molecular Imaging (SNMMI) Annual Meeting, St Louis, MO, June 7-11, 2014.*
11. Moni J, Zhang L, Tirodkar T, Elishaev E, Brozick J, Edwards RP and Vlad AM. "Intraperitoneal anti-PD-L1 increases survival in a novel ovarian cancer model and its in vivo efficacy is influenced by baseline anti-tumor immunity of the host. *Proceedings from the Marsha Rivkin-AACR Ovarian Cancer Research Symposium, Seattle, WA September 8-9, 2014.* Abstract POSTER-THER-1434: *Cancer Research* 08/2015; 21(16 Supplement): DOI:10.1158/1557-3265.
12. Zhang L, Ma, Brozick J, Babalola K, Budiu R, Tseng G and Vlad AM. Lung metastasis of ovarian cancer in a transplantable MUC1.Tg mouse model is accompanied by upregulation of MUC1 expression and epithelial to mesenchymal transition. *Annual Meeting of the American Association for Cancer Research, Philadelphia PA, April 18-21, 2015.* Abstract 4081: *Cancer Research* 08/2015; 75(15 Supplement):4081-4081. DOI:10.1158/1538-7445.AM2015-4081
13. Grabosch, S. Vlad AM, Ma T, Mony T, Strange M, Brozick J, Thaller J, Tseng G, Huang X, Moore K, Odunsi K and Edwards RP. Prospective profiling of systemic and loco-regional alterations during intraperitoneal chemotherapy for ovarian cancer, results from GOG 271. Shannon Grabosch. *Annual Meeting of the American Association for Cancer Research, Philadelphia PA, April 18-21, 2015.* Abstract 414: *Cancer Research* 08/2015; 75(15 Supplement):414-414. DOI:10.1158/1538-7445.AM2015-414.
14. Berger J, Beck TL, Mital P, Elishaev E, Sukhwani M, Oesterreich S, Vlad AM, T, Krivak TC, Kelley JL, Orwig K. A novel orthotopic mouse model of epithelial ovarian carcinoma demonstrating progression from

early stage disease to carcinomatosis in both immune-competent and immune-deficient models. *Annual Meeting of the Society of Gynecologic Oncology*. *Gynecologic Oncology* 04/2015; 137:132.
DOI:10.1016/j.ygyno.2015.01.329

- **Websites**

Results from our studies are disseminated via several websites:

MWRI website <http://mageewomens.org/>

Vlad Lab webpage (MWRI) <http://www.mwrif.org/directory/?url=directory>

Vlad Lab webpage (Dept of Immunology) <http://www.immunology.pitt.edu/person/anda-m-vlad-md-phd>

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications and/or licenses**

Nothing to report.

- **Other products**

Nothing to report

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

1. Name	Swati Suryawanshi , PhD
Project Role	Post-doctoral trainee
Nearest person month worked	3
Contribution to project:	Identified biomarkers of transition from endometriosis to endometriosis-associated ovarian cancer (EAOC)
Funding support	UPMC Research Fund
2. Name	Tejas Tirodkar , PhD
Project Role	Post-doctoral trainee
Nearest person month worked	6
Contribution to project:	Explored roles of Kras and Pten pathways in tumorigenesis along the genital tract
Funding support	
3. Name	Kathlene Babalola

Project Role	Medical student
Nearest person month worked	2
Contribution to project:	Explored roles of Kras and Pten on MUC1 protein expression in murine ovarian cancer cell lines
Funding support	University of Pittsburgh School of Medicine- Scholarly Project
4. Name	Alok Nimgaonkar
Project Role	High school student
Nearest person month worked	1
Contribution to project:	Explored immune signatures in endometriosis and EAO
Funding support	University of Pittsburgh Cancer Institute (UPCI)- Summer Research Academy- High School Program
5. Name	Anisha Reddy
Project Role	High school student
Nearest person month worked:	1
Contribution to project:	Explored anti-MUC1 antibody responses in immunized mice
Funding support	University of Pittsburgh Cancer Institute (UPCI)- Summer Research Academy- High School Program
6. Name	Jyothi Mony, PhD
Project Role	Post-doctoral trainee
Nearest person month worked	6
Contribution to project:	Explored in vivo efficacy of PDF-L1 blockade
Funding support	Developmental Research Project, part of the UPCI- Roswell Park Cancer Institute Ovarian Cancer SPORE

7. Name	Shannon Grabosch, MD
Project Role	Gynecology-Oncology Fellow
Nearest person month worked	12
Contribution to project:	Explored in vivo efficacy of PD-L1 blockade
Funding support	UPMC Research Fund
8. Name	Leah Konig
Project Role	Undergraduate student
Nearest person month worked:	1
Contribution to project:	Developed cisplatin resistant ovarian cancer cell line
Funding support	MWRI-Summer Undergraduate Research Program
9. Name	Anna Edwards
Project Role	High school student
Nearest person month worked:	1
Contribution to project:	Explored roles of Kras and Pten on PD-L1 biology in ovarian cancer cell lines
Funding support	MWRI-Summer High School Research Program

8. SPECIAL REPORTING REQUIREMENTS

9. APPENDICES