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14. ABSTRACT While much advancement has been made in breast cancer treatment, metastatic breast cancer remains an incurable disease. MUC1 is a glycoprotein expressed on normal glandular epithelial but is over-expressed and underglycosylated in over 90% of human breast tumors and 100% of metastatic lesions, which lead to its ranking by NCI as the second most targetable antigen. Vaccines against tumor antigens have several benefits, including the chance to eliminate metastatic lesions that express the vaccinating tumor antigen. To this end, we have proposed vaccinating with peptides from the MUC1 protein core, which is only visible to the immune system on the tumor-associated form of the protein. Previous work from our lab has demonstrated that this vaccine does elicit a MUC1-specific immune response that can only be functional if the immunosuppressive tumor microenvironment is altered to allow efficient killing of tumor cells. Thus, we investigated the effectiveness of MUC1 vaccination in combination with drugs known to inhibit immunosuppression to determine which drug is the most effective. Methods: Mice that are transgenic for human MUC1 (MUC1.Tg) mice were orthotopically injected with a syngenic breast cancer cell line expressing human MUC1 (Mtag.MUC1). Mice were vaccinated after palpable tumor formation with the vaccine cocktail, consisting of two MHC class I-restricted MUC1 tandem repeat peptides and a class II pan helper peptide mixed with GM-CSF and CpG ODN, in incomplete Freund's adjuvant. Previous work in our lab has shown that blocking the cyclooxygenase pathway (COX) resulted in an inhibition of immunosuppression. Thus we used the following drugs in combination with the MUC1-vaccine therapy: Indomethacin (COX1 and COX2 inhibitor), Celecoxib (COX2 inhibitor), 1-methyl tryptophan (indoleamine 2,3 dioxygenase inihibitor), and AH6809 (EP2 receptor antagonist). Mice were euthanized and tissue was collected post the final vaccination. MUC1 vaccine therapy alone caused a slight reduction in tumor burden, although not significant. The combinational therapy of Indomethacin + Vaccine resulted in a significant reduction in tumor burden, whereas all other treatments resulted in no significant reduction in tumor burden, as measured by caliper measurements. The combination treatment of Vacc+Indomethacin and Vacc+Celecoxib both reduced PGE2 levels compared to vaccine alone. In a repeat experiment, we found that the combination of Vacc+Indomethacin caused a significant reduction in tumor wet weight compared to vaccine alone as well as compared to control. However, Indomethacin alone did not significantly reduce tumor wet weight compared to control, indicating a synergistic effect of vaccine and indomethacin. Since Indomethacin but not Celecoxib reduced tumor burden when given in combination with the MUC1 vaccine, we are further investigated COX-independent pathways involved in this mechanism.

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INTRODUCTION:

Breast Cancer is diagnosed in 200,000 individuals in the United States each year and contributes to approximately 40,000 deaths annually. For tumors confined within the breast, surgical removal can result in a favorable outcome. However, tumors have the ability to metastasize to distant sites, such as lymph nodes, lungs, liver or brain. Complications from metastatic disease are the leading causes of cancer-related deaths. It is for this reason that research now focuses on the development of novel breast cancer-specific vaccines. MUC1 is a transmembrane mucin glycoprotein that is overexpressed in >90% of breast carcinomas [1-5]. Recently, MUC1 was listed as the second most targetable tumor antigen by the national cancer institute [6]. Our lab has demonstrated the effectiveness of MUC1-directed tumor vaccines in colorectal, pancreatic, and breast cancer models; however immunosuppression was observed at the tumor site, hindering the immune response to the vaccine [7-9]. Thus, combining immunotherapy with available adjuvant treatments may sufficiently alter the tumor microenvironment such that the effector cells can function properly. COX-2 is an enzyme that converts arachidonic acid to prostaglandins. COX-2 is induced in breast cancer during various pathologic conditions. Our lab previously found that Cyclooxygenase 2 (COX2) over-expression and subsequent Prostaglandin E2 (PGE2) production, in response to vaccination, are immunosuppressive [7, 10]. Further, COX-2 inhibition, via the use of Celecoxib, reduced breast tumor levels of indolamine 2, 3-dioxygenase (IDO). This project is focused on 1) understanding the role of IDO enzymatic activity on tumor development and immune function and 2) investigating the efficacy of a MUC1-based tumor vaccine in an IDO null environment, as well as, in an environment in which IDO enzymatic activity is suppressed by its competitive inhibitor, 1-methyl-D-tryptophan (1MT). Last year, we concluded that tumor burden does not differ between tumors that were injected into IDO null mice or blk6 mice, no matter whether they were IDO expressing or IDO null tumors. As far as tumor burden is concerned, the phenotype of the mouse does not matter, but the phenotype of the tumor does. This is demonstrated by IDO null tumors that have significantly lower tumor burden than either of the two IDO producing tumors. This year, our focus was Aim 3 of the study, in which we wanted to determine if combination with 1-methyl-D-tryptophan (a competitive inhibitor of IDO; 1-MT) will enhance efficacy of MUC-1 targeted immunotherapy. We tested the MUC1 specific tumor vaccine with targeted inhibition of immune suppression in an effort to achieve a maximum clinical response. We tested the MUC1 vaccine in combination with an indoleamine 2,3 dioxygenase (IDO) inihibitor (1-MT; 1-methyl tryptophan), and unfortunately found no significant difference in tumor burden. Therefore, we tested the vaccine in combination with a variety of targeted inhibition of immune suppression in an effort to achieve a maximum clinical response. This included testing the combination of vaccine in combination with an indoleamine 2,3 dioxygenase (IDO) inihibitor (1-MT; 1-methyl tryptophan), a COX1 and COX2 inhibitor (Indomethacin), a COX2 inhibitor (Celecoxib), as well as in combination a PGE2 antagonist (AH6809). Our results indicate that Indomethacin in combination with the MUC1 vaccine resulted in a significant reduction in tumor burden. All other drug combinations tested were unable to significantly reduce tumor burden at the dosages tested. While further studies are needed to better understand the molecular mechanisms of this reduction in tumor burden, this data clearly indicate that an enhanced clinical response can be achieved when the MUC1 vaccine is combined with the COX1 and COX2 inhibitor, Indomethacin. However, it appears as though indoleamine 2,3 dioxygenase (IDO) inihibitor, 1-MT is unsuccessful in improving vaccine efficacy. As the goal of this project is to improve MUC1 vaccine efficacy, we would like to further investigate COX-independent pathways involved in the mechanism of tumor reduction when tumors are treated with a combination of vaccine + indomethacin.

BODY:

The third aim of this project is to: Determine if combination with 1-methyl-D-tryptophan (a competitive inhibitor of IDO; 1-MT) will enhance efficacy of MUC-1 targeted immunotherapy. Immune function, tumor development, and MUC1-specific cellular and humoral immune responses will be assessed' In order to test the efficacy of the vaccine in combination with 1-MT, mice were orthotopically injected in the mammary fat pad, and treated with a combination of vaccine +1-MT. At the same time, we tested a variety of COX inhibitors, and inhibitors of downstream molecules.

Previously, the PyVMT (Polyoma virus Middle T Antigen) spontaneous breast cancer mouse model was tested in our lab. In that study, the spontaneous mouse model was tested in combination with a MUC1 specific vaccine in combination with the COX-2 inhibitor Celecoxib. PyVMT tumors from untreated mice were dissected and dissociated using collagenase IV. The cell line generated from these tumors was designated as MTAG cells. In order to test the MUC1 vaccine in vivo, in an injectable breast cancer model, we transfected the MTAG cells with the full length MUC1 plasmid. In order to insure a high purity of MUC1 expressing MTAG.MUC1 cells, the transfected cell line was sorted for MUC1 expression using FacsAria. Expression phenotype of the MUC1 cell line was analyzed using the HMFG2 antibody which targets sparsely glycosylated VNTR repeats of the human MUC1 extracellular domain. Using HMFG2 antibodies for flow cytometry, we confirmed that MTAG.MUC1 cells are positive for MUC1 (Figure 1).

In order to test the efficacy of the vaccine in combination with 1-MT, mice were orthotopically injected in the mammary fat pad. At the same time, we tested a variety of COX inhibitors, and inhibitors of downstream molecules. 24 female MUC1.Tg mice were orthotopically injected with MTAG.MUC1 cells in the mammary fat pad. When tumors were palpable, approximately day 8 post tumor cell injection (p.t.i.), mice were randomly assigned to five different treatment groups: vaccine alone, vaccine + 1-MT, vaccine + indomethacin, vaccine + celecoxib, vaccine + AH6809. Unfortunately, in this pilot experiment, we did not have MUC1.Tg female mice available to include all appropriate controls. In future experiments, this pilot experiment will be repeated with the appropriate controls included.

All treatment groups were administered the MUC1 vaccine subcutaneously on day 8 p.t.i. In addition to vaccine administration, mice were treated with either 1-MT (400mg/kg), indomethacin (3mg/kg), Celecoxib (10mg/kg), or AH6809 (200ug) on a five day on, two day off, schedule. All drugs were administered once per day with the exception of 1-MT which was administered twice per day. Mice were again administered the MUC1 vaccine on days 19, 34 and 35 p.t.i. Mice were monitored for signs of distress, and tumor burden was measured three times per week. Mice were sacrificed on day 35 p.t.i. Results demonstrate that MTAG.MUC1 tumors treated with a combination of vaccine + indomethacin significantly reduced tumor burden beginning on day 30 p.t.i. as compared to vaccine alone. This significance was maintained until mice were sacrificed (Figure 2). All other treatment combinations did not display a significant reduction in tumor burden compared to vaccine alone. Upon sacrifice, the tumors were weighed, prepared for lysates, and fixed for immunohistochemistry. Analysis of the tumor wet weight displayed similar trends, suggesting that the only group in which there was a reduced tumor wet weight was the vaccine + indomethacin group, however, this reduction was not significant (Figure 3).

COX-2 derived PGE-2 is the major prostaglandin produced by breast cancer cells. Production of PGE2 in the tumor lysate is an appropriate measure of COX-2 activity in this orthotopic mouse model of breast cancer; however, PGE2 is unstable in vivo. Therefore, we measured PGEM, the PGE2 metabolite (namely, 13,14-dihydro-15-keto-PGA2) in order to provide a reliable estimate of PGE2 production. PGEM levels were measured in the tumor lysates of all treatment groups by ELISA. A significant reduction in tumor PGEM was observed in mice treated with vaccine + celecoxib, as well as vaccine + indomethacin, as compared to vaccine alone ($p<0.05$, Figure 4). There was no significant reduction of PGEM levels of mice treated with the combination of vaccine + 1-MT.

As stated previously, COX-2, PGE2, and IDO have been linked with T regulator (T-regs) and myeloid-derived suppressor cells (MDSCs) presence in the tumor microenvironment. Tregs play a key role in the

maintenance of immune tolerance to both self-and foreign antigens and are reviewed in [11]. Upon antigen stimulation, Tregs potently suppresses the activation/proliferation of $CD4^+$ or $CD8^+$ cells *in vitro*. It is well established that Tregs are present in the tumor microenvironment and hamper efficient anti-tumor immune responses. Several reports have documented the potential role of Treg removal for the induction of tumor rejection. Although Tregs are well known as suppressor cells there are other types of suppressor cells like MDSCs, also known as immature myeloid cells [12-14]. MDSCs can suppress the activation of $CD4^+$ and $CD8^+$ T cells, inhibiting the generation of an antitumor response [15-19]. MDSCs are thought to be induced by a variety of cytokines and growth factors (TGF-β, VEGF) which are produced within the tumor microenvironment [20, 21]. MDSCs have poor antigen-presenting capability, and produce factors that suppress T cell proliferation and activity, and promote angiogenesis [22]. This phenotype contrasts markedly with the phenotype of classically activated type I or M1 macrophages that are efficient immune effector cells able to kill microorganisms and tumor cells, present antigens, and produce high levels of T cell stimulatory cytokines.

Therefore, in order to determine the underlying mechanism of the inefficacy of the vaccine + 1-MT treatment, we isolated splenocytes from MTAG.MUC1 tumors bearing mice, pooled the splenocytes, stained, and assessed a number of immune parameters. Levels of myeloid-derived suppressor cells were assessed, characterized by the co-expression of Gr1 and CD11b. There was no significant difference observed in MDSC levels in mice treated with any of the combinational treatments tested (Figure 5A). Helper T cells were defined as CD4+, whereas T regulatory cells (Tregs) were characterized by the coexpression of CD4 and FoxP3. No significant difference was observed in the percentage of helper T cells or Tregs in any of the combinational treatments tested (Figure 5 B, C). However, there was a slight increase in the percentage of Tregs in the mice treated with the combination of vaccine +AH6809, although this increase was not significant.

Functionally distinct phenotypes of CD8+ T cells spanning from naïve (CD8+CD62L+CD11b-CD44-) to an effector and/or memory stage of differentiation have been described [23] . Effector $CD8^+$ T cells (CD8+CD62L-CD11b+CD44+), are terminally differentiated and are known to release an array of cytokines upon stimulation (IFN-γ and TNF-α), as well as display strong cytolytic activity with high expression of perforin and granzyme. Memory T cells were defined as CD8+CD62L-CD11b-CD44+.Therefore, in order to determine the nature of the cells induced by this treatment, we assessed levels of naive, memory and effector T cells, as well as CD8+ T cells. No significant differences were observed among the different treatment groups in overall CD8+ T cells (Figure 6 A). The Naïve T cell population was significantly reduced in the vaccine + celecoxib treatment group (Figure 6 B). The combinational treatment of vaccine + AH6809 significantly reduced effector T cell populations (Figure 6 C), while there was no significant difference observed among any of the combinational treatment groups with respect to memory T cells (Figure 6 D).

In order to examine the growth inhibitory effect that these drugs have on the tumor cells in vitro, MTAG.MUC1 tumor cells were treated with each drug and its corresponding vehicle control. Cells were treated following 24 hours of serum starvation to achieve cell cycle synchronization. Cells were treated with doses of drug ranging from 0um to 400uM. Proliferation was measured by [^{3}H]-thymidine uptake at 24 and 48 hours post treatment. Celecoxib treatment resulted in a significant decrease in proliferation at all dosages tested at both 24 and 48 hours post treatment (Figure 32A, Figure 33A). MTAG.MUC1 cells treated with AH6809 showed no significant decrease in proliferation compared to vehicle control, irrespective of the dose given or time point tested (Figure 7B, Figure 7B). It appears as though the vehicle used for administering AH6809 may be toxic to the cells itself, and therefore needs to be optimized before conclusions can be drawn about the effect of AH6809 on MTAG.MUC1 cells. Indomethacin treatment resulted in a significant decrease in proliferation when treated with dosages ranging from 100-400uM,at both 24 and 48 hours post treatment (Figure 7 C, Figure 7 C). Additionally, at 24 hours post treatment, there was a significant decrease in proliferation when MTAG.MUC1 cells were treated with 50uM of Indomethacin (Figure 7 C). No significant difference was observed when cells were treated with varying doses of 1-MT, at both 24 and 48 hours post treatment (Figure 7 D, Figure 7D). Again, the variability in this data suggests that the vehicle needs to be optimized for 1-MT administration.

In order to further examine the enhanced efficacy of the combinational treatment vaccine + indomethacin, since we were not seeing any enhanced efficacy with the combinatorial 1-MT treatments, female

MUC1.Tg mice were orthotopically injected with MTAG.MUC1 cells in the mammary fat pad. By day 6 p.t.i. tumors were palpable, and mice were divided into four different treatment groups. One group served as a control, whereas the other three groups were treated with indomethacin alone, vaccine alone, or vaccine + indomethacin. The treatment groups receiving the MUC1 vaccine were vaccinated on days 6, 15, 24, 27, and 28 p.t.i. Mice receiving indomethacin treatment were gavaged three days per week (3mg/kg). Tumor burden was monitored three times per week, while body weight was measured twice weekly. Mice were sacrificed on days 27 and 28 p.t.i. Results demonstrate that MTAG.MUC1 tumors treated with the combination of vaccine + indomethacin resulted in a significantly reduced tumor burden beginning at day 17. This significant reduction in tumor burden was maintained until mice were sacrificed (Figure 9 A). Indomethacin alone, as well as vaccine alone, resulted in a significant reduction in tumor burden, as compared to control, beginning at 24 days p.t.i (Figure 9 B). Results also demonstrated that tumor burden of mice treated with vaccine + indomethacin was significantly lower than either indomethacin alone or vaccine alone. This significance was noted at day 20 p.t.i and remained until mice were sacrificed (Figure 9 B). This is suggestive of a synergistic effect between vaccine and indomethacin treatment.

Upon sacrifice, the tumors were weighed, prepared for lysates, and fixed for immunohistochemistry. Analysis of the tumor wet weight displayed similar trends, specifically, mice receiving the combination treatment of vaccine + indomethacin had significantly decreased tumor wet weight as compared to control ($p<0.01$). Moreover, the combination treatment also resulted in a significantly reduced tumor burden compared to vaccine alone ($p<0.05$, Figure 35). However, no significant difference was observed between mice treated with indomethacin alone and control mice (Figure 10). In order to insure that the treatment was indeed effective in reducing PGE2 levels, Prostaglandin E2 Metabolite (PGEM) was again measured in the tumor lysate of treated mice as a read out for PGE2 levels. The combination of vaccine + indomethacin as well as indomethacin alone, significantly decreased levels of PGEM in the tumor lysate of treated mice as compared to control mice (Figure 36). Additionally, the mice treated with the combination treatment of vaccine + indomethacin resulted in significantly decreased PGEM levels as compared to vaccine alone ($p<0.05$, Figure 11). Thus, we believe that this combinational treatment is immunologically relevant and warrants further investigation.

KEY RESEARCH ACCOMPLISHMENTS:

- Previous findings relevant to this report: Phenotype of the mouse does not affect tumor burden, ie. Tumor burden does not differ between tumors that were injected into IDO null mice or blk6 mice, no matter whether they were IDO expressing or IDO null tumors

Current Findings: We now have a better understanding of the role of IDO enzymatic activity on tumor development and immune functioning in MUC1 vaccinated mice:

- We have generated and characterized the MTAG.MUC1 cell line for future use with continued experiments
- We optimized an orthotopic injection animal model for use with the MTAG.MUC1 cell line, and now have an effective model to test vaccine combinations in.
- We found that there was no enhanced efficacy of the MUC1 vaccine when it was combined with IDO inhibitor, 1-MT
    - However, we did find that there was an enhanced efficacy of the MUC1 vaccine when it was combined with the COX-1, COX-2 non-selective inhibitor, Indomethacin
- We found that mice treated with vaccine + IDO inhibitor, 1-MT, did not have display decreased PGEM levels in the tumor lysate, as compared to vaccine alone
    - However, we did find that PGEM levels were reduced in the tumor lysate of mice treated with the vaccine + indomethacin combination
- We found no significant difference in the immune status of mice treated with vaccine + 1-MT as compared to vaccine alone
- We found that 1-MT administration did not cause a growth inhibitory effect on the MTAG.MUC1 tumor cells, as measured by tritiated thymidine uptake
    - However, we did find the MTAG.MUC1 tumor cells had significantly lower proliferative rates when treated with Indomethacin
- We found that Indomethacin + vaccine combinational treatment was the most effective treatment in reducing tumor burden, and enhancing vaccine

Milestones accomplished in the training program include:

- I have participated in the Tumor Immunology journal club
- I have attended the weekly seminars at the Breast Health Center Program in the The Blumenthal Cancer Center
- I passed my pre-qualifiers and qualifiers (March 2012, April 2012, respectively)
- My thesis proposal was approved (April 2012)

- I have completed the Advanced Immunology Course hosted by the American Association of Immunologists (July 2012)
- I have attended workshops on How to Write a Competitive Grant Proposal (April 2012, October 2102)
- I have set a defense date for February 21, 2013, and plan on graduating in May of 2013 (although I plan on continuing this work thereafter)

REPORTABLE OUTCOMES:

- The research conducted in the last year has resulted in a poster presentation at the American Association of Cancer Research, to be presented in April of this year.
- We have generated a breast cancer cell line that was transfected with MUC1, to expresses human MUC1; designated MTAG.MUC1
- We have optimized our orthotopic injection animal model for use with the MTAG.MUC1 cell line, and now have an effective model to test vaccine combinations in.
- We now have serum, tumor lysates, parafin embedded tissue sections, tumors sections in RNA later and OCT frozen sections from tumor bearing mice, treated with a combination of vaccine + 1-MT, vaccine + celecoxib, vaccine + indomethacin, vaccine + AH6809, as well as control mice, and mice treated with indomethacin alone. We will use these repositiories in the near future in a multiplex mouse cytokine array, as well as performing a microarray with these samples.
- I will obtain my PhD in February 2013, supported by this award

CONCLUSION:

Treatments that work by modulating the immune response are amongst the most widely used and accepted medical treatments. Most efforts thus far in cancer immunotherapy have focused only on enhancing immunity. However, tumors create an abnormal local microenvironment that allows them to escape immune detection and destruction. Thus, immune evasion is one major obstacle that has to be addressed prior to designing and delivering successful immunotherapy. A landmark study by Munn et al. demonstrated that tumor cells utilize a system that contributes to the immune suppression via expression of IDO. This project is focused on 1) understanding the role of IDO enzymatic activity on tumor development and immune function in the mice and 2) investigating the efficacy of a MUC1-based tumor vaccine in an IDO null environment as well as, in an environment in which IDO enzymatic activity is suppressed by its competitive inhibitor, 1-methyl-tryptophan (1MT). Thus far, we have concluded that the phenotype of the mouse does not affect tumor burden. We found that tumor burden does not differ between tumors that were injected into IDO null mice or blk6 mice, no matter whether they were injected with IDO null or IDO expressing tumors. In this study, we generated a breast cancer cell line from the tumors of PyVMT mice and retrovirally infected the cells with the full length MUC1 plasmid (Figure 26). With the use of an orthotopic injectable model of breast cancer, we tested the MUC1 specific tumor vaccine in combination with four different drugs, each with targeted inhibition of immune suppression in an effort to achieve maximal vaccine efficacy. Unfortunately, the combination of vaccine + 1-MT was unsuccessful in reducing tumor burden in these mice. The results clearly indicated that, compared to vaccine alone, the only combinational therapy that significantly reduced tumor burden, was the combination of indomethacin + vaccine (Figure 27). Interestingly, the previously effective COX-2 inhibitor, celecoxib, did not significantly reduce tumor burden in combination with the vaccine, as seen in the spontaneous model. Interestingly, IDO inhibition seemed to be ineffective treatment options in combination with the vaccine regimen. Mice treated with the combinational therapy of vaccine + indomethacin displayed a significant clinical response with significant reduction in tumor burden and tumor wet weight (Figure 34, Figure 35). This reduction in tumor burden was associated with a decrease in PGEM levels (Figure 36), indicating that indomethacin was indeed functional. While further studies are necessary to identify the molecular mechanisms underlying the reduced tumor burden associated with treatment, the data clearly indicate that an enhanced vaccine efficacy can be achieved with a combination of MUC1 peptide vaccine + non-selective, COX-1 and COX-2 inhibitor, indomethacin. Our preclinical studies offer us an opportunity to assess the feasibility of inhibition of COX pathway in combination with immunotherapy for the treatment of breast cancer.With this information in mind, we can more effectively design a breast cancer vaccine that specifically targets the immunosuppressive agent that is most inhibitory to our vaccine treatment.

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APPENDICES:

APPENDIX 1: AACR ABSTRACT SUBMISSION

AACR Annual Meeting 2013 in Washington DC

Combinational MUC1 vaccine therapy and Indomethacin treatment reduces breast tumor burden via a COX-independent pathway.

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*Both authors contributed equally

Abstract:
While much advancement has been made in breast cancer treatment, metastatic breast cancer remains an incurable disease. MUC1 is a glycoprotein expressed on normal glandular epithelial but is over-expressed and underglycosylated in over 90% of human breast tumors and 100% of metastatic lesions, which lead to its ranking by NCI as the second most targetable antigen. Vaccines against tumor antigens have several benefits, including the chance to eliminate metastatic lesions that express the vaccinating tumor antigen. To this end, we have proposed vaccinating with peptides from the MUC1 protein core, which is only visible to the immune system on the tumor-associated form of the protein. Previous work from our lab has demonstrated that this vaccine does elicit a MUC1-specific immune response that can only be functional if the immunosuppressive tumor microenvironment is altered to allow efficient killing of tumor cells. Thus, we investigated the effectiveness of MUC1 vaccination in combination with drugs known to inhibit immunosuppression to determine which drug is the most effective. Methods: Mice that are transgenic for human MUC1 (MUC1.Tg) mice were orthotopically injected with a syngenic breast cancer cell line expressing human MUC1 (Mtag.MUC1). Mice were vaccinated after palpable tumor formation with the vaccine cocktail, consisting of two MHC class I-restricted MUC1 tandem repeat peptides and a class II pan helper peptide mixed with GM-CSF and CpG ODN, in incomplete Freund's adjuvant. Previous work in our lab has shown that blocking the cyclooxygenase pathway (COX) resulted in an inhibition of immunosuppression. Thus we used the following drugs in combination with the MUC1-vaccine therapy: Indomethacin (COX1 and COX2 inhibitor), Celecoxib (COX2 inhibitor), 1-methyl tryptophan (indoleamine 2,3 dioxygenase inihibitor), and AH6809 (EP2 receptor antagonist). Mice were euthanized and tissue was collected post the final vaccination. MUC1 vaccine therapy alone caused a slight reduction in tumor burden, although not significant. The combinational therapy of Indomethacin + Vaccine resulted in a significant reduction in tumor burden, whereas all other treatments resulted in no significant reduction in tumor burden, as measured by caliper measurements. The combination treatment of Vacc+Indomethacin and Vacc+Celecoxib both reduced PGE2 levels compared to vaccine alone. In a repeat experiment, we found that the combination of Vacc+Indomethacin caused a significant reduction in tumor wet weight compared to vaccine alone as well as compared to control. However, Indomethacin alone did not significantly reduce tumor wet weight compared to control, indicating a synergistic effect of vaccine and indomethacin. Since Indomethacin but not Celecoxib reduced tumor burden when given in combination with the MUC1 vaccine, we are further investigated COX-independent pathways involved in this mechanism.

APPENDIX 2: CURRICULUM VITAE

Dahlia M. Besmer

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Raleigh, NC 27699
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Education

Doctoral Student • 2008 to present
Proposed Dissertation Topic: Role of MUC1 in Pancreatic and Breast Cancers
Major advisor: Dr. Pinku Mukherjee
GPA: 3.918
Expected date of graduation: May 2013
Department of Biology
University of North Carolina at Charlotte • Charlotte, North Carolina

Bachelor of Science in Biology • 2008
Graduated with Honors
Concentrations: Cell Biology/Physiology
Undergraduate research advisors: Dr. F. M. Hughes, Jr. and Dr. I. M. Sokolova
Honors research advisor: Dr. Yvette Huet
Department of Biology
University of North Carolina at Charlotte • Charlotte, North Carolina

Professional Experience

Individual Student Guidance • 2010-2012
Department of Biology
University of North Carolina at Charlotte • Charlotte, North Carolina

North Carolina DNA Day Volunteer • 2008-2010

Teaching Assistant • 2009
Biology 2130: General Biology II Laboratory • Department of Biology
University of North Carolina at Charlotte • Charlotte, North Carolina

Teaching Assistant • 2009
Biology 2120: General Biology I Laboratory • Department of Biology
University of North Carolina at Charlotte • Charlotte, North Carolina

Teaching Assistant • 2008-2009
Biology 3111: Cell Biology Laboratory • Department of Biology
University of North Carolina at Charlotte • Charlotte, North Carolina

Competitive Funding

Source: Department of Defense Breast Cancer Research Program: BCRP BC100361 Pre-Doctoral Traineeship Award (Besmer and Mukherjee)
Title: The Role of IDO in MUC1 Targeted Immunotherapy
Dates: 2011 – 2013

Source: Graduate and Professional Student Government
Title: Travel Award
Dates: 03/31/2012 – 04/04/2012

Source: Graduate and Professional Student Government
Title: Travel Award
Dates: 04/01/2011 – 04/07/2011

Source: Graduate Assistant Support Plan Award
Dates: 2009-2012

Source: NC Lottery Scholarship
Dates: 2007-2008

Source: Federal SMART Grant 3
Dates:2006-2007

Honors and Awards

Department of Defense Pre-Doctoral Traineeship Award• 2011-2013

Nominated to receive PEO Scholar Award • 2012

Nominated to attend the 61st annual Nobel Laureates Meeting • 2011
Lindau, Germany

Graduate Assistant Support Plan Award (GASP Award) • 2008-2010

Chancellor's List/Dean's List • 2005-2008

Institutional Honors: Cum Laude • 2008

Departmental Honors: Honors in Biology • 2008

Chancellor's/Dean's List• Spring 2005-Fall 2006

Tri Beta Honor Society• Fall 2006-May 2008

Professional Memberships And Service

President • 2010-2012
Association of Biology Graduate Students (ABGS)

Member • 2010-2012
Charlotte Biotechnology Conference Planning Committee (CBC)

Social Affairs Committee • 2010-2011
Graduate and Professional Student Government (GPSG)

Research Fair Planning Committee • 2010-2011
Graduate and Professional Student Government (GPSG)

Member • 2010-Present
American Association of Immunologists (AAI)

Member • 2010-Present
American Association of Cancer Research (AACR)

Member • 2010-Present
American Association for the Advancement of Science (AAAS)

Abstract Review Committee • 2009-2012
Graduate Research Fair (GRF)

Graduate Student Liaison • 2009-2010
Association of Biology Graduate Students (ABGS)

Member • 2008-2009
Association of Biology Graduate Students (ABGS)

Student Government Association- Judicial Branch• 2005-2008
-Hearing Panel Member- 2005
-Assistant Attorney General- 2005-2006
-Attorney General- 2006-2008

Leadershape -(not-for-profit organization that strives to enable college students to lead with integrity)- 2006

Member• 2005-2008
Student Court Orientation and Outreach Program

Chairperson• 2007-2008
Student Court Outreach Committee

Publications

1. Jorge Schettini, Amritha Kidiyoor*, **Dahlia M. Besmer***, Teresa L. Tinder, Lopamudra Das Roy, Joseph Lustgarten, Sandra J. Gendler, Pinku Mukherjee Intratumoral Delivery of CpG-Conjugated Anti-MUC1 Antibody Enhances NK Cell Anti-Tumor Activity. *Cancer Immunology Immunotherapy.* 2012 PMID: 22543528 2012 Apr 28. [Epub ahead of print]
*** Both authors contributed equally**

2. Andrea M. Murphy, **Dahlia M. Besmer**, Megan Moerdyk-Schauwecker, Natascha Moestl,David Ornelles, Pinku Mukherjee, and Valery Z. Grdzelishvili. Vesicular Stomatitis Virus as an Oncolytic Agent Against Pancreatic Ductal Adenocarcinoma. *Journal of Virology.* 2012 Mar;86(6):3073-87. Epub 2012 Jan 11. PMID: 22238308

3. Mahnaz Sahraei , Lopamudra Das Roy , Jennifer Curry , Teresa Tinder , Sritama Nath , **Dahlia M. Besmer** , Amritha Kidiyoor , Ritu Dalia , Sandra Gendler. MUC1 Regulates PDGFA Expression During Pancreatic Cancer Progression, *Oncogene.* 2012 Jan 23. doi: 10.1038/onc.2011.651

4. **Dahlia M. Besmer** , Dr. Jennifer M. Curry , Dr. Lopamudra D. Roy , Ms. Teresa L. Tinder , Ms. Mahnaz M. Sahraei , Dr. Jorge L. Schettini , Dr. Sun-Il Hwang , Dr. Yong Y. Lee , Dr. Sandra J. Gendler, Pinku Mukherjee (2011). Pancreatic Ductal Adenocarcinoma (PDA) mice lacking Mucin 1 have a profound defect in tumor growth and metastasis. *Cancer Research.* 71(13): 4432-42.

5. Lopamudra D. Roy, Mahnaz, M. Sahraei, Durai B. Subramani, **Dahlia M. Besmer**, Sritama Nath, Teresa L. Tinder, Kandavel Shanmugam, Ekta Bajaj, Sandra J. Gendler, and Pinku Mukherjee (2011). MUC1 enhances invasiveness of

pancreatic cancer cells by inducing epithelial to mesenchymal transition. *Oncogene.* 2011 Mar 24;30(12):1449-59. Epub 2010 Nov 22.PMID: 21102519

6. Jennifer M. Curry[1], Kyle J. Thompson[2], Shanti Rao[1], **Dahlia M. Besmer[1]**, Andrea Murphy[1], Valery Z. Grdzelishvili[1], William Ahrens[3], Iain H. McKillop[2], David Sindram[2], David Iannitti[2], John Martinie[2], and Pinku Mukherjee[1] The use of a novel MUC1 antibody to identify cancer stem cells and circulating MUC1 in mice and patients with pancreatic cancer. Accepted, Journal of Surgical Oncology

7. Das Roy, L, Sahraei, M., Schettini, J., **Besmer, D.,** Gruber, H.E., and Mukherjee, P. (2012). Treatment with anti-IL 17A significantly decreases breast cancer associated metastasis in models of induced and chronic arthritis. Accepted, Breast Cancer Research

8. Das Roy, L., Curry, J., Sahraei, M., **Besmer, D.,** Kidiyoor, A., Gruber, H.E and Mukherjee, P**.** Arthritis augments breast cancer metastasis: Role of mast cells and SCF/c-Kit signaling. Under Review, Breast Cancer Research.

9. Amritha Kidiyoor, Jorge Schettini*, **Dahlia M Besmer*,** Stephen Rego, Sritama Nath, Jennifer M Curry, Lopamudra Das Roy, Mahnaz Sahraei and Pinku Mukherjee. MUC1 in pancreatic cancer impacts the differentiation of myeloid derived suppressor cells. Manuscript in preparation, Immunology
*** Both authors contributed equally**

10. Eric Hastie*, **Dahlia M. Besmer*,** Nirav Shah, Andrea Murphy, Megan Moerdyk-Schauwecker, Natascha Moestl, Carlos Molestina, Pinku Mukherjee, and Valery Grdzelishvili "Development of a clinically relevant immunocompetent model for oncolytic virotherapy against pancreatic ductal adenocarcinoma" Manuscript in Preparation, Cancer Letters
*** Both authors contributed equally**

11. Jennifer M Curry*, **Dahlia M Besmer*,** Lopamudra D. Roy, Priyanka Grover, Sritama Nath, Shanti Rao, Pinku Mukherjee 'Combinational MUC1 vaccine therapy and Indomethacin treatment reduces breast tumor burden via a COX-independent pathway.' Manuscript in preparation, Breast Cancer Research

*** Both authors contributed equally**

Conference Presentations

1. Samantha R.L. Furr, **Dahlia M. Besmer**, Brittany N. Allen, Francis M. Hughes Jr. Translocation of aquaporin-9 during anoikis precedes downstream apoptotic events and is caspase-independent. UNC Charlotte Undergraduate Research Conference. Charlotte, North Carolina (2007).

2. Snimar Grewal, **Dahlia M. Besmer**, Francis M. Hughes, Jr., Inna M. Sokolova Apoptosis as a host defense mechanism in Crassostrea virginica and its modulation by Perkinsus marinus. The 25th Annual Conference on Managing Environmental Quality. Seattle, Washington (2007).

3. **Dahlia M. Besmer**, Yvette Huet. The effect of neonatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on C-myc and EGFR expression in adult congenic mice. UNC Charlotte Undergraduate Research Conference. Charlotte, North Carolina (2008).

4. **Dahlia M. Besmer** , Dr. Jennifer M. Curry , Dr. Lopamudra D. Roy , Ms. Teresa L. Tinder , Ms. Mahnaz M. Sahraei , Dr. Jorge L. Schettini , Dr. Sun-Il Hwang , Dr. Yong Y. Lee , Dr. Sandra J. Gendler, Pinku Mukherjee. Pancreatic Ductal Adenocarcinoma (PDA) mice lacking Mucin 1 have a profound defect in tumor growth and metastasis. American Association of Cancer Research. Orlando, Florida (2011).

5. Andrea M. Murphy, **Dahlia M. Besmer**, Megan Moerdyk-Schauwecker, Natascha Moestl, David Ornelles, Pinku Mukherjee, and Valery Z. Grdzelishvili. Vesicular Stomatitis Virus as an Oncolytic Agent Against Pancreatic Ductal Adenocarcinoma. Annual Biomedical Research Conference, UNC Charlotte. Charlotte, North Carolina (2011) *First Prize*

6. Amritha Kidiyoor, Jorge Schettini, Lopamudra Das Roy **Dahlia M. Besmer**, and Pinku Mukherjee. Pancreatic Tumor Cells that develop within MUC1 knockout mice generate less immunosuppressive MDSCs in vitro. American Association of Cancer Research. Orlando, Florida (2011).

7. Lopamudra Das Roy, Jennifer Curry, Mahnaz M. Sahraei, Amritha Kidiyoor**, Dahlia M. Besmer**, Helen E. Gruber and Pinku Mukherjee . Evaluate the mechanism of enhanced metastasis induced by arthritis. Department of Defense Era of Hope. Orlando, Florida (2011).

8. Amritha Kidiyoor, Jorge Schettini, Lopamudra Das Roy, **Dahlia M. Besmer**, Pinku Mukherjee Role of MUC1 on the ontogeny of Myeloid derived suppressor cells in pancreatic cancer. American Association of Cancer Research Conference. Orlando, Florida (2011)

9. Sritama Nath, Teresa L. Tinder, **Dahlia M. Besmer**, Amritha Kidiyoor, Jennifer Curry, Lopamudra Das Roy and Pinku Mukherjee MUC1 in chemoresistance in pancreatic cancer. Charlotte Biotechnology Conference (2011) *2nd place prize*

10. Murphy A., **Dahlia Besmer**, Pinku Mukherjee, and Grdzelishvili V.Z., 2011 "Vesicular stomatitis virus as an oncolytic agent against pancreatic ductal adenocarcinoma", *The 6th International Conference on Oncolytic Viruses as Cancer Therapeutics*, March 16-19, Las Vegas, Nevada, Abstracts, P28 *(poster)*

11. Murphy A., **Dahlia Besmer**, Pinku Mukherjee, and Grdzelishvili V.Z., 2011 "Vesicular stomatitis virus as an oncolytic agent against pancreatic ductal adenocarcinoma" *The 3rd international conference on Viral Oncology Research, Naples, Italy, October 4-6,* Abstracts, *(oral presentation)*

12. Lopamudra Das Roy, Jennifer M. Curry, Mahnaz Sahraei, Amritha Kidiyoor, **Dahlia M. Besmer**, Helen E. Gruber, Pinku Mukherjee. Arthritis augments breast cancer metastasis: Role of mast cells and SCF/c-Kit signaling. American Association of Cancer Research, Chicago (2012)

13. **Dahlia M. Besmer**, Amritha Kidiyoor, Sritama Nath, Lopamudra Das Roy, Jennifer Curry, Pinku Mukherjee. Investigating the Role of IDO in MUC1 Expressing Breast Cancers. American Association of Cancer Research, Chicago (2012)

14. Murphy A., Moerdyk-Schauwecker M., **Besmer D**., Shah N., Ornelles D., Pinku Mukherjee, and Grdzelishvili V.Z. 2012 "Evaluation of vesicular stomatitis virus as an oncolytic agent against pancreatic ductal adenocarcinoma" *12th Southeastern Regional Virology Conference*, March 9-11, Atlanta, Georgia, Abstracts, Section IIID *(oral presentation)*

15. Andrea M. Murphy[1], Megan J. Moerdyk-Schauwecker[1], **Dahlia M. Besmer**[1], Nirav R. Shah[1], David A. Ornelles[2], Pinku Mukherjee[1] and Valery Z. Grdzelishvili[1]Evaluation of vesicular stomatitis virus as an oncolytic agent against pancreatic ductal adenocarcinoma. American Association of Cancer Research, Chicago (2012)

16. Murphy A., Moerdyk-Schauwecker M., **Besmer D.,** Shah N., Ornelles D., Pinku Mukherjee, and Grdzelishvili V.Z. 2012 "Evaluation of vesicular stomatitis virus as an oncolytic agent against pancreatic ductal adenocarcinoma" *31th Annual Meeting of the American Society for Virology*, July 21-25, Madison, Wisconsin, Abstracts, W7-3 *(oral presentation)*

17. Jennifer M Curry*, **Dahlia M Besmer*,** Lopamudra D. Roy, Priyanka Grover, Sritama Nath, Shanti Rao, Pinku Mukherjee 'Combinational MUC1 vaccine therapy and Indomethacin treatment reduces breast tumor burden via a COX-independent pathway.'Abstract submitted to American Association of Cancer Research

*** Both authors contributed equally**

18. Eric Hastie*, **Dahlia M. Besmer*,** Nirav Shah, Andrea Murphy, Megan Moerdyk-Schauwecker, Natascha Moestl, Carlos Molestina, Pinku Mukherjee, and Valery Grdzelishvili "Development of a clinically relevant immunocompetent model for oncolytic virotherapy against pancreatic ductal adenocarcinoma" Graduate Research Fair, UNC Charlotte Spring 2013

*** Both authors contributed equally**

SUPPORTING DATA:

Figure 1

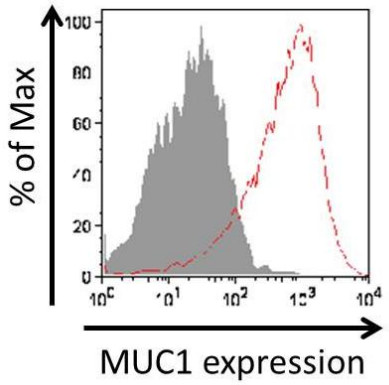


Figure 1: Characterization of the MTAG.MUC1 cell line. MUC1 expression was confirmed by flow cytometry. The gray histogram represents isotype control stained, and the red dashed line represents MUC1 staining.

Figure 2

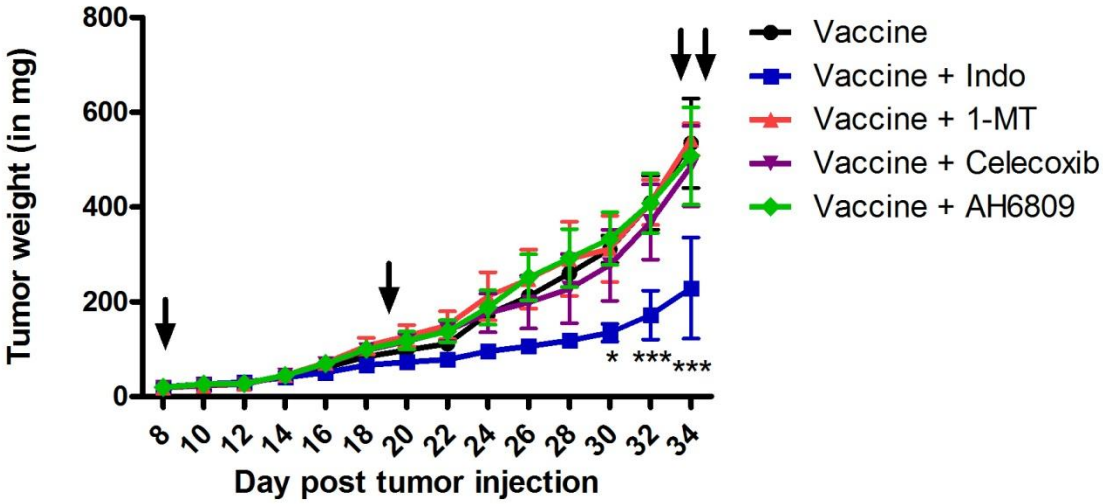


Figure 2: Indomethacin treatment with vaccination is the only combination that reduces tumor burden. Female MUC1.Tg mice, aged 8-12 weeks old were orthotopically injected with MTAG.MUC1 cells in the mammary fat pad (n=24). Tumors were palpable by day 8, and mice were randomly divided into 5 groups (n=5 per group, n=4 for vaccine). All mice were vaccinated on days 8, 19, 34, and 35 p.t.i.(as indicated by arrows) and treated with Celecoxib (10mg/kg), AH6809 (200ug), Indomethacin (3mg/kg) once daily, and 1-MT (400mg/kg) twice daily, five days a week. Tumor size was monitored by caliper measurements every other day until sacrifice. Body weight was measured every other day. Tumor weight was calculated according to the formula: grams = [(length in cm) x (width in cm)^2]/2. Mice were sacrificed 35 days p.t.i, at which time, mice were not yet presenting with clinical signs indicating severe morbidity. Comparison of groups was done using a two-way ANOVA with a bonferoni post-hoc test (*, p<.05; **p<0.01; ***p<0.001 compared to control).

Figure 3

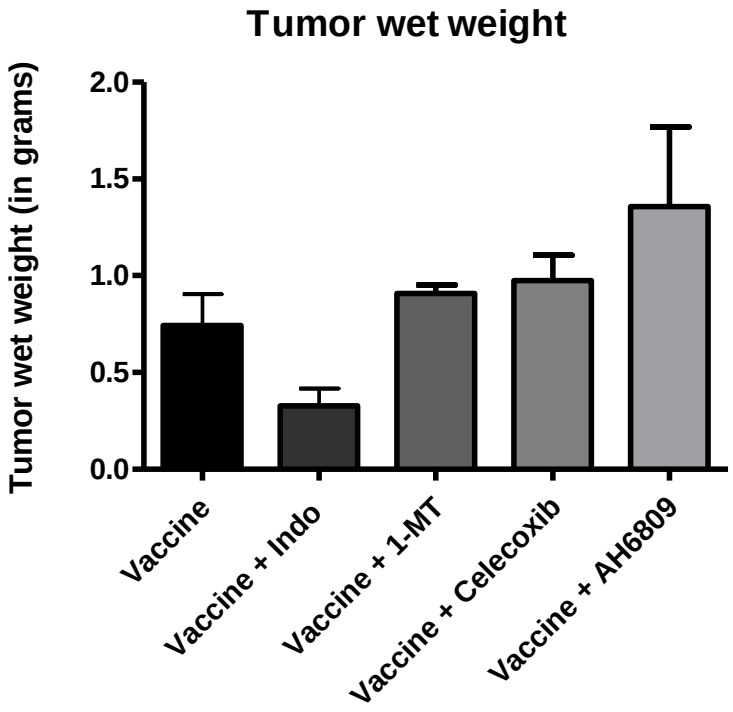


Figure 3: Indomethacin treatment with vaccination is the only combination that has a trend indicating reduced tumor burden. Female MUC1.Tg mice, aged 8-12 weeks old were orthotopically injected with MTAG.MUC1 cells in the mammary fat pad (n=24). Tumors were palpable by day 8, and mice were randomly divided into 5 groups (n=5 per group, n=4 for vaccine). All mice were vaccinated on days 8, 19, 34, and 35p.t.i. and treated with Celecoxib (10mg/kg), AH6809 (200ug), Indomethacin once daily (3mg/kg), and 1-MT (400mg/kg) twice daily, five days a week. Mice were sacrificed 35 days p.t.i, at which time tumors were excised and weighed. Comparison of groups was done using a one-way ANOVA with a Dunnetts multiple comparisons post hoc test. Although significance was not reached, there was a trend toward reduced tumor burden in the vaccine + indomethacin treatment group.

Figure 4

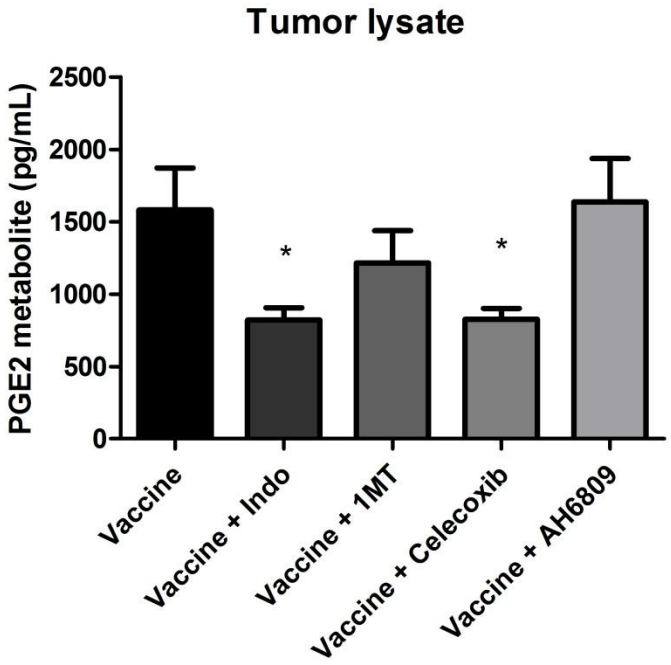


Figure 4: Celecoxib and Indomethacin both reduce PGE2 metabolite levels in combination with vaccination. Prostaglandin E2 Metabolite (PGEM) was measured in tumor lysate as a read out for PGE2 levels. Combinational treatment of vaccine + Indomethacin as well as vaccine + celecoxib significantly reduced tumor PGEM levels compared to vaccine treatment alone. Comparison of groups was done using a one-way ANOVA with a Dunnetts multiple comparisons post hoc test (*, $p<0.05$ vs.vaccine alone).

Figure 5

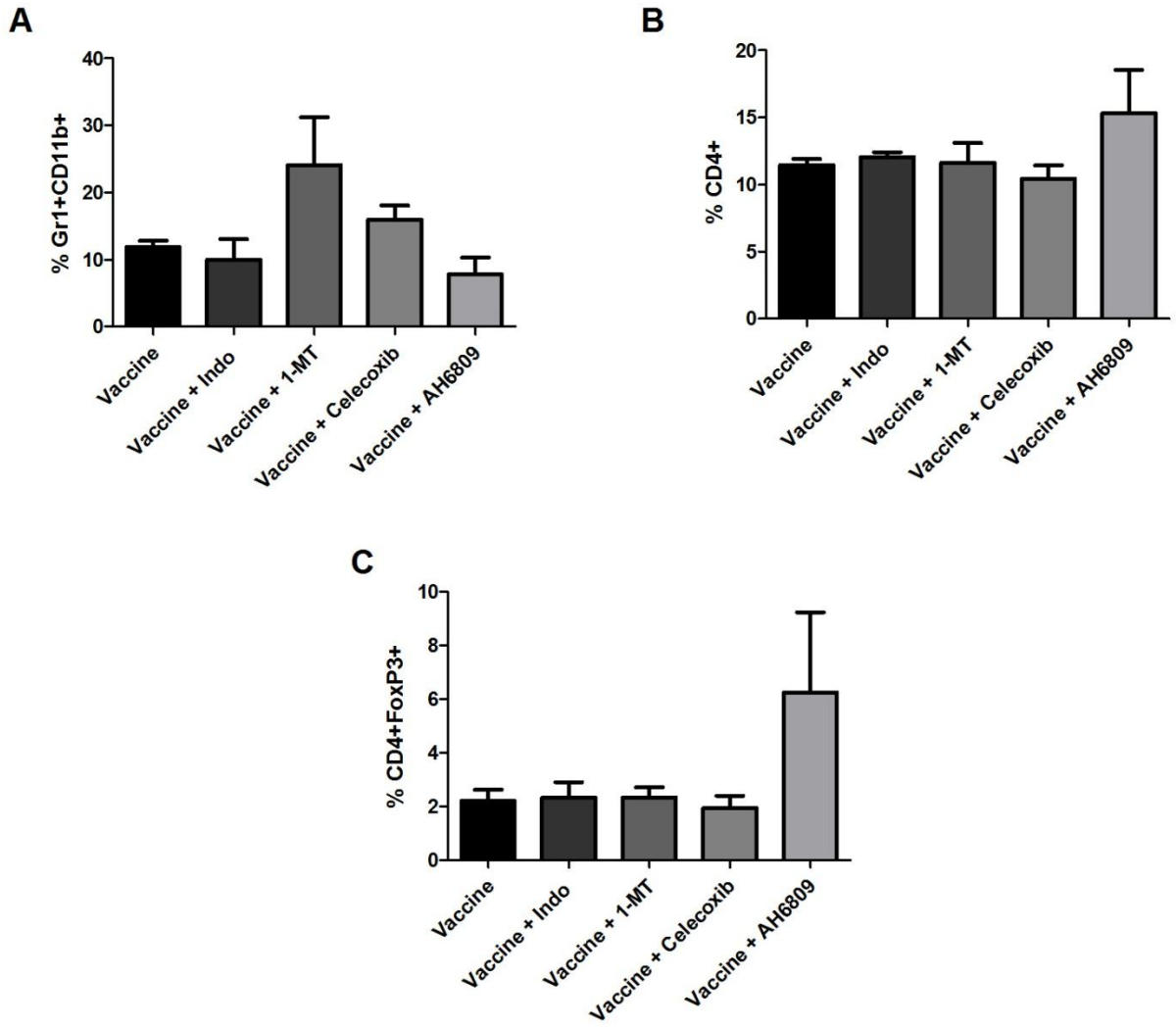


Figure 5: Immune analysis (MDSCs and Tregs) of combinational MUC1 vaccine therapy. Splenocytes from mice bearing MTAG.MUC1 tumors treated with vaccine therapy were assessed. A) Myeloid-derived suppressor cells (MDSCs) were characterized as Gr1+CD11b+ splenocytes. There was no significant difference in MDSC levels in mice treated with any of the combinational treatments. Vaccine in combination with 1-MT was the only group that seemed to increase MDSC levels, although the increase was not significant. B) Helper T cells were defined as CD4+ splenocytes. No significant difference was observed in the levels of T helper cells in any of the combinational treatment groups. C) Levels of T regulatory cells were measured in splenocytes, as defined by the co-expression of CD4 and FoxP3. No significant difference was observed in the levels of T regulatory cells in any of the treatment groups; however, the combination of Vaccine+AH6809 seems to increase percentage of T regulatory cells, although this increase was not significant. Comparison of groups was done using a one-way ANOVA with a Dunnetts multiple comparisons post hoc test (*, $p<0.05$ vs.vaccine alone).

Figure 6

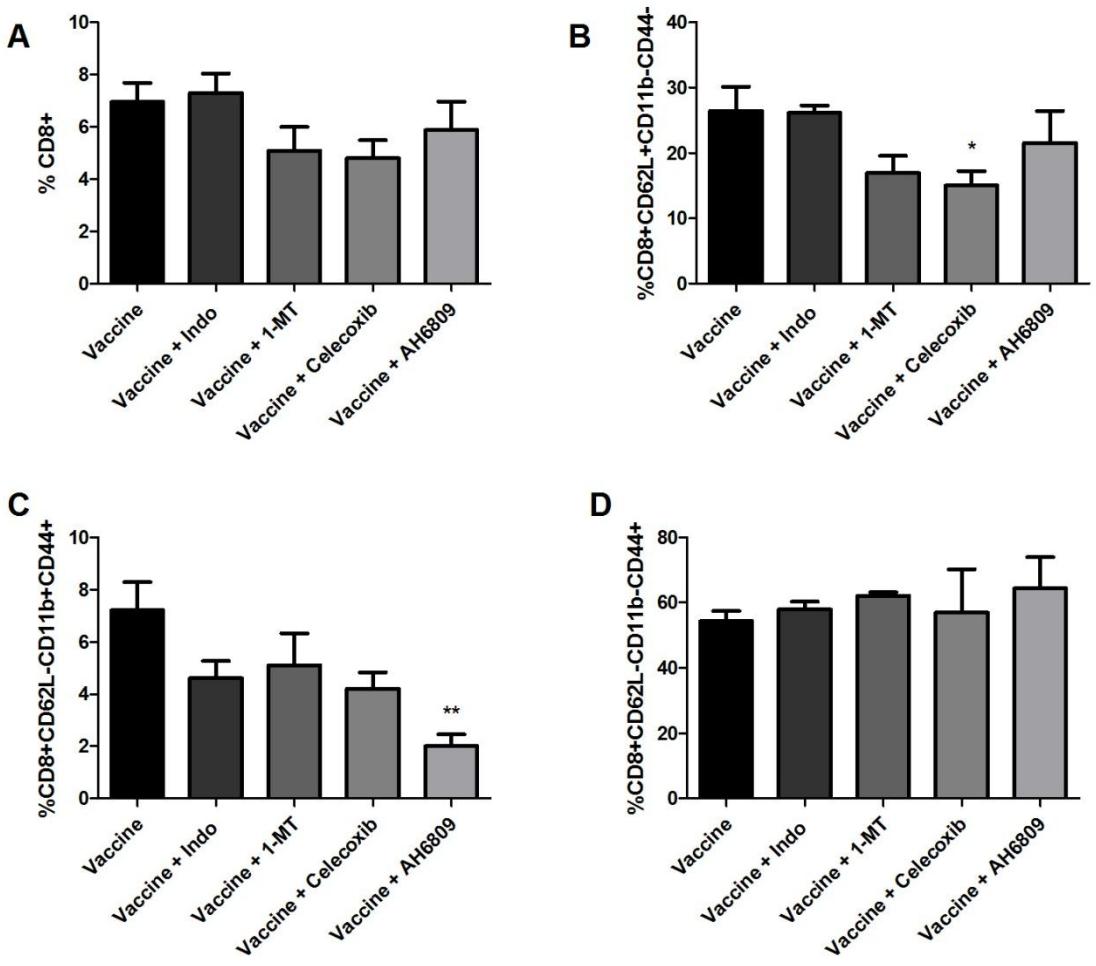


Figure 6: Immune analysis (T cells) of combinational MUC1 vaccine therapy.
Splenocytes from MTAG.MUC1 tumor bearing mice treated with MUC1 vaccine therapy were analyzed for T cell flow panels. For the T cell panel, Naïve T cells were defined as CD8+CD62L+CD11b-CD44-, Effector T cells were defined as CD8+CD62L-CD11b+CD44+ and Memory T cells were defined as CD8+CD62L-CD11b-CD44+.A) No significant changes were observed among the different treatment groups in overall CD8+ T cells. B) The combinational treatment of Vaccine+Celecoxib significantly reduced levels of Naïve T cell populations. C) The combinational treatment of Vaccine+AH6809 significantly decreased the percentage of effector T cells. D) No significant changes were observed among the different treatment groups in reference to memory T cells. Comparison of groups was done using a one-way ANOVA with a Dunnetts multiple comparisons post hoc test (*, $p<0.05$, **, $p>0.01$ vs.vaccine alone).

Figure 7

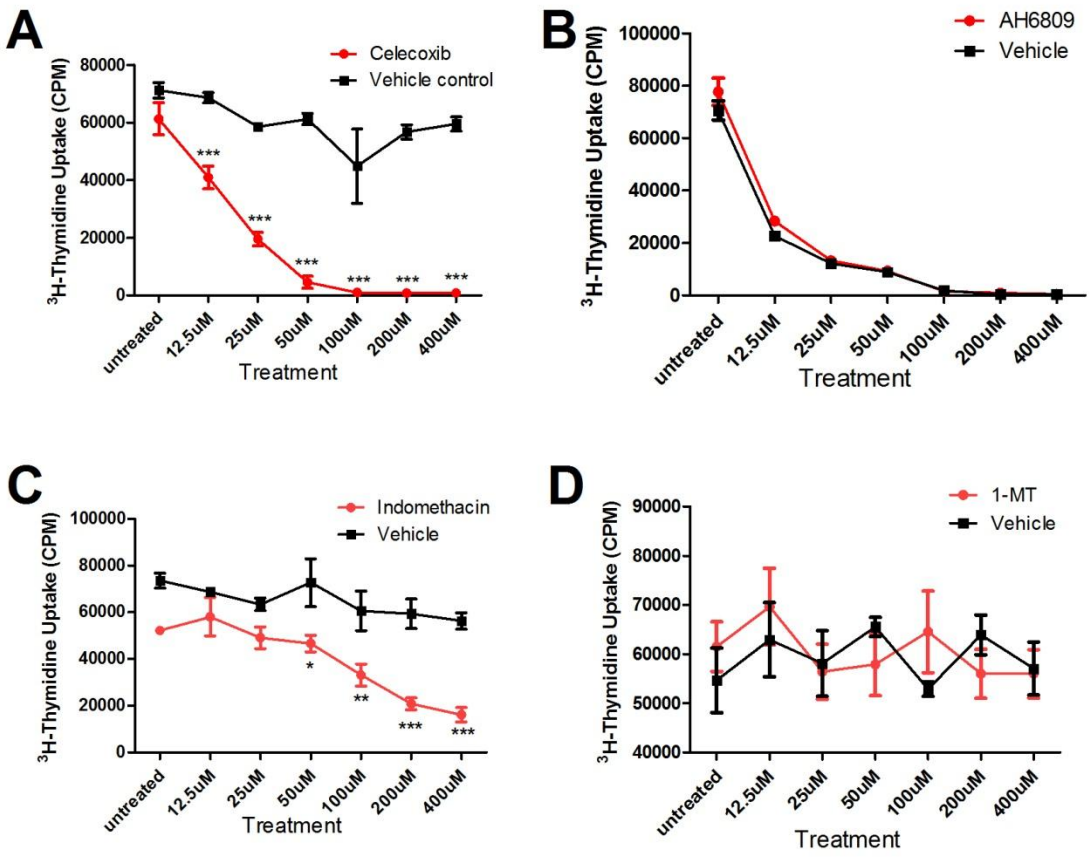


Figure 7: Proliferation assessed at 24 hours post treatment. Proliferation was measured by [^{3}H]-thymidine uptake. A) Treatment of MTAG.MUC1 cells with Celecoxib resulted in a significant decrease in proliferation at all dosages tested. B) There was no significant difference in proliferation of MTAG.MUC1 cells treated with AH6809. C) A significant decrease in proliferation of MTAG.MUC1 cells was noted when cells were treated with 50,100,200, and 400uM Indomethacin. D) No significant difference was observed when cells were treated with varying doses of 1-MT. Comparison of groups was done using a two-way ANOVA with a Bonferoni post hoc test (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$ vs.vehicle alone).

Figure 8

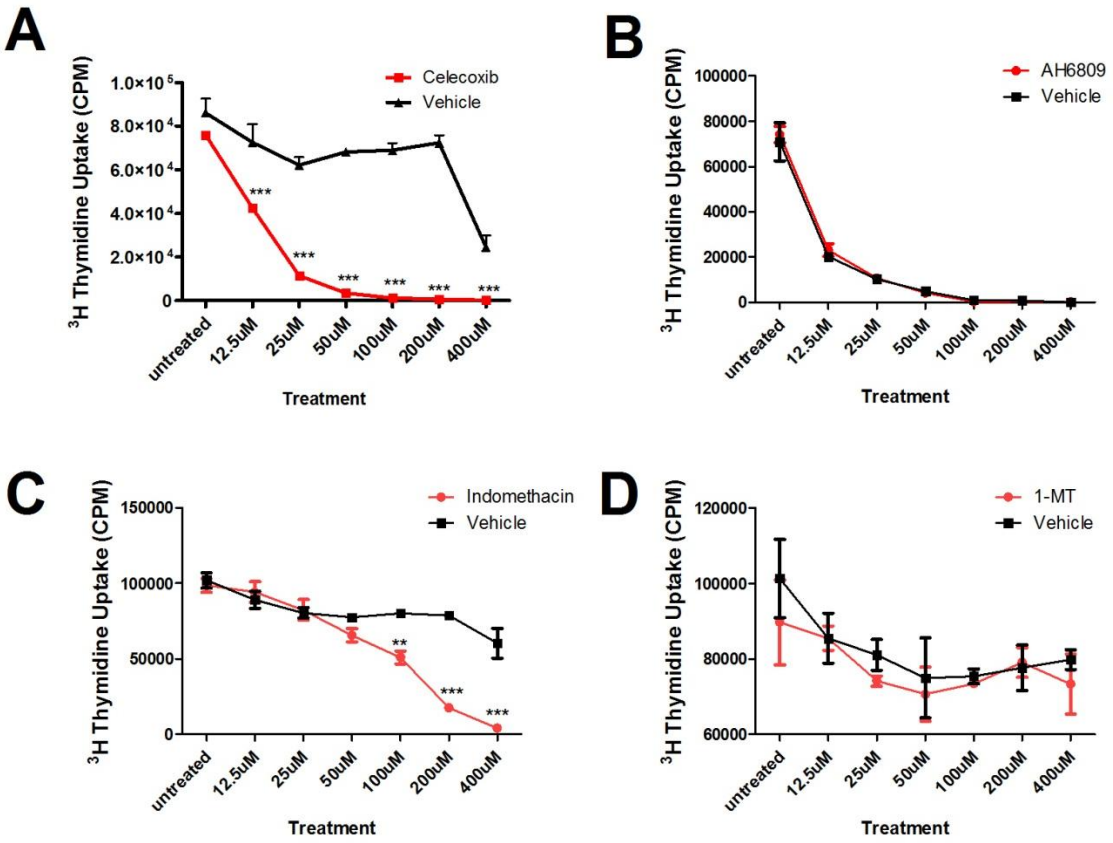
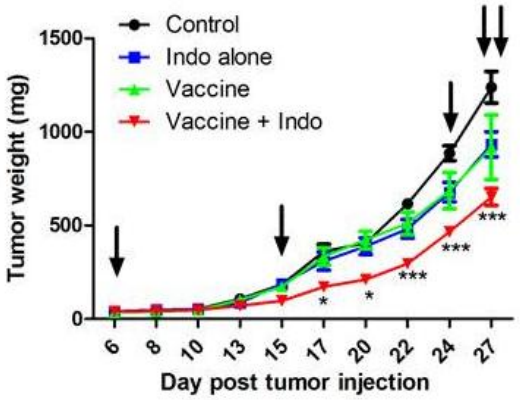


Figure 8: Proliferation assessed at 48 hours post treatment. Proliferation was measured by [^{3}H]-thymidine uptake. A) Treatment of MTAG.MUC1 cells with Celecoxib resulted in a significant decrease in proliferation at all dosages tested. B) There was no significant difference in proliferation of MTAG.MUC1 cells treated with AH6809. C) A significant decrease in proliferation of MTAG.MUC1 cells was noted when cells were treated with 100,200, and 400uM Indomethacin. D) No significant difference was observed when cells were treated with varying doses of 1-MT. Comparison of groups was done using a two-way ANOVA with a Bonferoni post hoc test (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$ vs. vehicle alone).

Figure 9

A



B

Treatment	Day post tumor cell injection				
	17	20	22	24	27
Control vs Indo				p<0.01	p<0.001
Control vs Vacc				p<0.05	p<0.001
Control vs Vacc + Indo	p<0.05	p<0.05	p<0.001	p<0.001	p<0.001
Indo vs Vacc + Indo		p<0.05	p<0.05	p<0.01	p<0.001
Vacc vs Vacc + Indo		p<0.01	p<0.01	p<0.01	p<0.001

Figure 9: Combinational treatment of Vaccine + Indomethacin significantly reduces tumor burden. Female MUC1.Tg mice, aged 8-12 weeks old were orthotopically injected with MTAG.MUC1 cells in the mammary fat pad (n=23). Tumors were palpable by day 6, and mice were randomly divided into 4 groups (n=6 per group, n=5 for indomethacin alone). One group served as a control, the indomethacin group was gavaged daily with 3mg/kg. The vaccine groups were vaccinated on days 6, 15, 24, 27, and 28 (as indicated by arrows). The combinational treatment group received both vaccination as well as three times a week treatment of indomethacin (3mg/kg) by gavage. Tumor size was monitored by caliper measurements three times a week, and body weight was measured twice weekly. Tumor weight was calculated according to the formula: grams = [(length in cm) x $(\text{width in cm})^2$]/2. Mice were sacrificed on day 27 and 28 days p.t.i. A) Treatment with vaccine + indomethacin resulted in a significant decrease in tumor burden vs. control beginning at day 17. B) Table displaying significant decreases in tumor burden. Data were analyzed using GraphPad software and are expressed as mean ± standard error mean. Comparison of groups was done by two-way ANOVA (*p<0.05, **p<0.01, ***p<0.001).

Figure 10

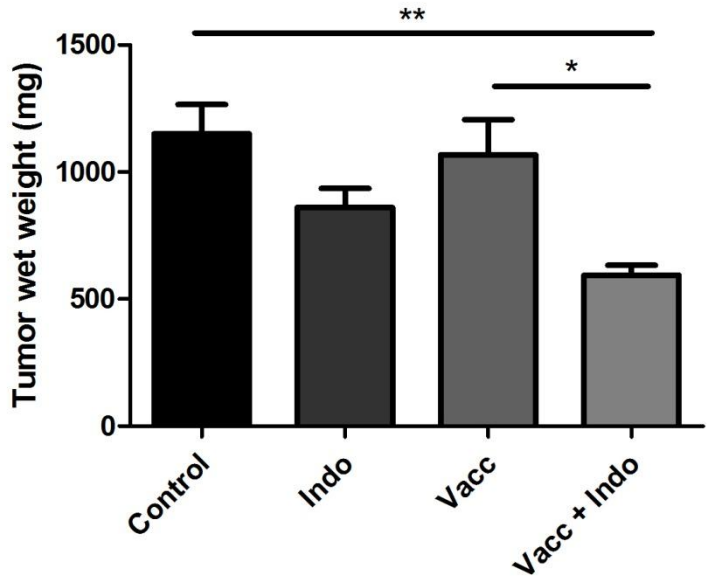


Figure 10: Combinational treatment of Vaccine + Indomethacin significantly reduces tumor wet weight. Female MUC1.Tg mice, aged 8-12 weeks old were orthotopically injected with MTAG.MUC1 cells in the mammary fat pad (n=23). Tumors were palpable by day 6, and mice were randomly divided into 4 groups (n=6 per group, n=5 for indomethacin alone). One group served as a control, the indomethacin group was gavaged daily with 3mg/kg. The vaccine groups were vaccinated on days 6, 15, 24, 27, and 28. The combinational treatment group received both vaccination as well as three times a week treatment of indomethacin (3mg/kg) by gavage. Mice were sacrificed on day 27 and 28 days p.t.i. Tumors were excised and weighed. Mice receiving the combinational treatment vaccine+indomethacin had significantly reduced tumor wet weight as compared to vaccine alone as well as control. Data were analyzed using GraphPad software and are expressed as mean ± standard error mean. Comparison of groups was done by one-way ANOVA with Tukey's post hoc test (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

Figure 11

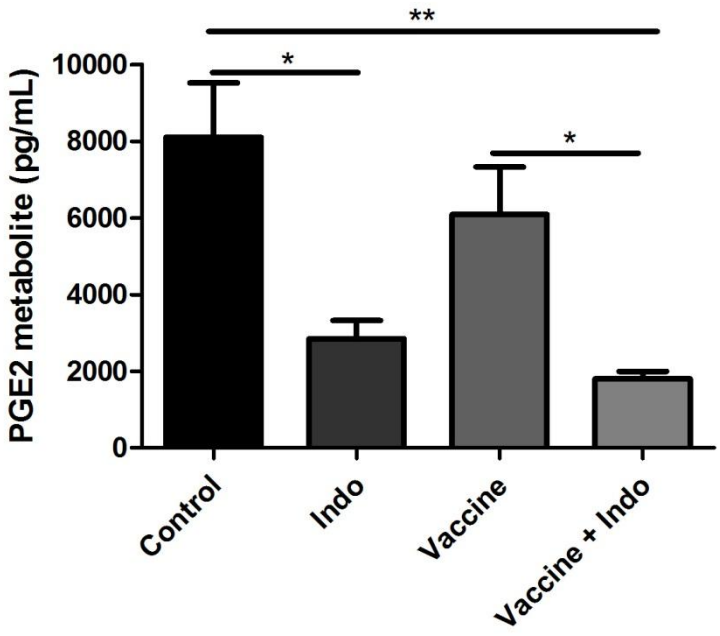


Figure 11: Indomethacin reduces PGE2 metabolite levels alone and in combination with vaccination. Prostaglandin E2 Metabolite (PGEM) was measured in tumor lysate as a read out for PGE2 levels. Indomethacine alone as well as the combinational treatment of vaccine + Indomethacin significantly reduced tumor PGEM levels compared to control. Additionally, the combinational treatment resulted in significantly reduced tumor PGEM levels as compared to vaccine alone. Comparison of groups was done using a one-way ANOVA with a Tukey's multiple comparisons post hoc test (*, $p<0.05$ vs.vaccine alone)